National Significant Events – *March–May 2020*

Selected U.S. Significant Climate Anomalies and Events for May and Spring

Late-season snowfall across the Northeast brought 6 to 12 in. of snow on May 8–9 from VT to ME.

March

Seasonal snowfall from Washington, D.C., to Boston remains 1-2 ft. below average.

April

Lowest seasonal snowfall total on record for Harrisburg, PA. Second lowest for Allentown, PA; Philadelphia, Wilmington, DE; and Atlantic City, NJ.

The average spring temperature for the contiguous U.S. was 52.6°F, 1.7°F above the 20th-century average. Average temperatures for March, April, and May were 4.6°F above average (10th warmest), 0.2°F below average, and 0.6°F above average, respectively. Globally, it was the second warmest March, the second warmest April, the warmest May, and the second warmest spring. The contiguous U.S. spring precipitation total was 8.40 inches, 0.46 inches above average. March, April, and May precipitation was 0.32 inches above average, 0.05 inches below average, and 0.13 inches above average, respectively.

Highlights for the Northeast

- **March** was **mild**, ranking among the 10 warmest Marches for some sites. **April** was **cool**. For example, Newark, NJ, **failed to reach 70°F** in April for the **first time on record**. Early **May** brought **record cold temperatures**, while late **May** had **record warmth.** See Regional Impacts for details.
- **Snowfall** was **below normal** this **spring**. Some sites including Baltimore, Philadelphia, and Boston set/tied their record for **least snowy March**. It was the **first time** with **no measurable snow in February and March** for Bridgeport, CT; Islip and Kennedy Airport, NY; and Allentown and Harrisburg, PA. On **May 9**, the New York City area climate sites had their **latest occurrence of snow** on record. See Regional Impacts for details.
- The region experienced two **strong storm systems** during April and some **severe weather** during spring. See Regional Impacts for details.
- Several **temperature and precipitation records** were set during spring:
  - *Warmest March day*: Beckley (WV)
  - *Greatest number of March days with measurable (0.01") precipitation*: Huntington, Charleston, Martinsburg (WV) and Wilmington (DE)
  - *Greatest number of April days with measurable precipitation*: Erie and State College (PA), Dulles Airport (VA), Morgantown (WV)
  - *Fewest number of 70°F days in April*: Newark (NJ), Hartford (CT), JFK and LaGuardia Airports (NY), Altoona and State College (PA)
  - *Coldest May temperature*: JFK and LaGuardia Airports and Binghamton (NY), Harrisburg (PA)
  - *Hottest May temperature*: Scranton (PA) and Burlington (VT)
  - *Wettest May and spring*: Charleston (WV)

Regional Climate Overview – *March–May 2020*

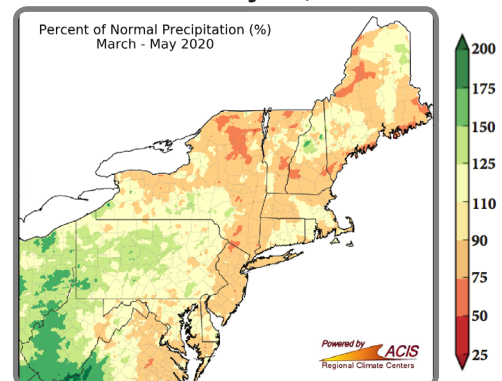### Temperature
Departure from Normal (°F)
March 1–May 31, 2020

Average Temperature Departure (°F)
March 1-May 31, 2020
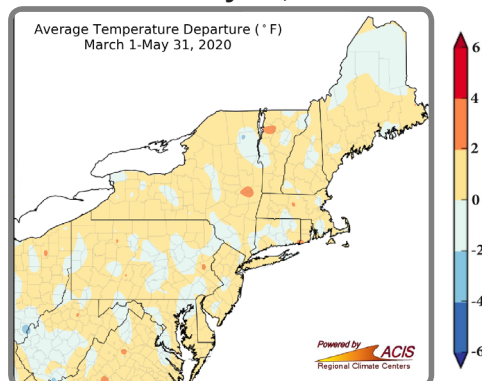
Climate normals based on 1981–2010 data; Rankings based on 1895–2020.

Precipitation
Percent of Normal (%)
March 1–May 31, 2020

Percent of Normal Precipitation (%)
March - May 2020

The Northeast's **spring** average temperature was 0.4°F above normal, ranking in the **warmest third** of all years. The region had its **10th warmest March** at 5.0°F above normal. It ranked among the 15 warmest Marches on record for 11 of the 12 Northeast states. **April** was 2.7°F below normal, ranking in the **coldest third** of all years. **May** was 1.1°F **below normal**, ranking in the middle third of all years.
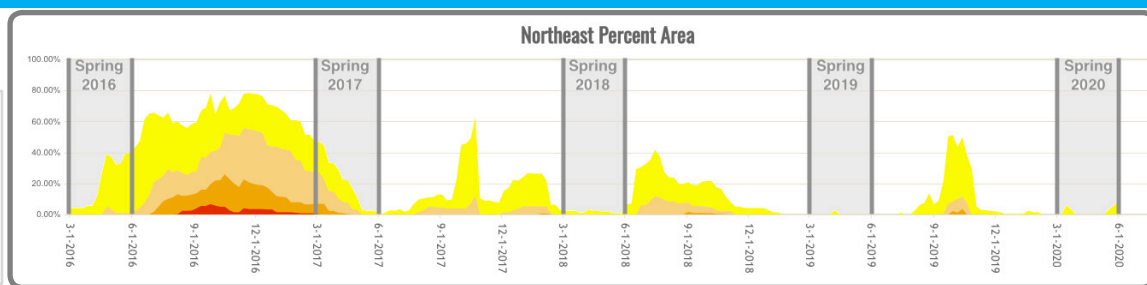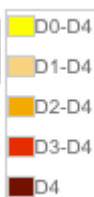
The Northeast saw 98% of normal precipitation during **spring**, ranking in the **middle third** of all years. West Virginia had its 11th wettest spring on record. **March** precipitation was 97% of normal, ranking in the **middle third** of all years. **April** precipitation was 119% of normal, ranking in the **wettest third** of all years. It was the fifth wettest April for West Virginia and the 19th wettest for Maryland and Massachusetts. **May** precipitation was 80% of normal, ranking in the **middle third** of all years.

Drought in the Northeast

D0	Abnormally Dry
D1	Drought - Moderate
D2	Drought - Severe
D3	Drought - Extreme
D4	Drought - Exceptional

- D0–D4
- D1–D4
- D2–D4
- D3–D4
- D4

Northeast Percent Area

In **early March**, the U.S. Drought Monitor showed **no abnormal dryness or drought** in the Northeast. Precipitation deficits, low streamflow, and below-normal groundwater levels led to **abnormal dryness** being introduced in New Jersey, New York, and southern New England in mid-March. The **March 17** U.S. Drought Monitor showed 6% of the Northeast was abnormally dry. This dryness eased by late March. The region was **free of abnormal dryness and drought** in **April** and the first half of **May**. Precipitation deficits, low streamflow, and other indicators led to the introduction of **abnormal dryness** in New York and New England by mid-May. The **May 26** U.S. Drought Monitor showed 5% of the Northeast was abnormally dry. Ipswich and Georgetown, MA, enacted **mandatory water restrictions**, while as of June 2, Maine had seen **600 wildfires**, more than in all of 2019. In early June, **dryness expanded** to include part of every Northeast state except New Jersey, with the **June 16** U.S. Drought Monitor showing 36% of the region as abnormally dry.

Regional Impacts and Updates – *March–May 2020*

Spring Temperatures and Snowfall

The weather pattern that brought the Northeast a **mild** winter persisted through **March**. In fact, Portland, ME, recorded its earliest 70°F day on March 9, five days earlier than the previous record. Most areas saw **below-normal snowfall**, with the largest deficits of more than 12 inches in northwestern Pennsylvania, New York, northern Vermont, and northern New Hampshire.

Snowfall Departure (inches) March 1-May 31, 2020

A pattern shift in **April** brought **colder, stormier conditions** to the Northeast. The highest temperature reached during April ranked as the **coldest on record** for April at some sites including Boston, MA; Bridgeport, CT; and Newark, NJ. Sites such as Hartford, CT, and JFK and LaGuardia Airports, NY, **did not reach 70°F** this April, tying the April record. Around 25% of New Jersey's blueberry crop was damaged by cold conditions. April **snowfall** ranged from 6 inches below normal in parts of New York and Vermont to more than 6 inches above normal in parts of Maine, New Hampshire, Pennsylvania, and New York.
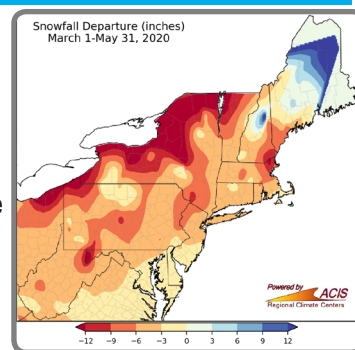
From **May 8–11**, **Arctic air** spilled into the Northeast. Low temperatures were in the 20s and 30s, as much as 30°F colder than normal, with a few sites recording their **coldest May temperature** and others ranking it among their 10 coldest. High temperatures on May 9 struggled to make it to 50°F, as much as 30°F below normal. Binghamton, NY, had its **coldest max temperature for May**, while other sites ranked their high temperatures among their 10 coldest. Strong winds and cold temperatures damaged vegetable crops in Delaware. Much of the region also saw snowfall on May 8 and/or 9. Elkins, WV, saw an inch of snow for only the second time in May, having its **snowiest May day** and **snowiest May**. Concord, NH, saw measurable snow in May for the **first time in over 50 years**, and Burlington, VT, observed its third latest measurable snow. In coastal/southern locations such as eastern Massachusetts, the New York City metro area, and Delaware, snow fell several times during the day, which is **unusual for May**. While these areas only saw a trace, it made **May a snowier month than February** for Islip, NY. May snowfall was near or above normal for most areas. Just over two weeks later, from **May 26 and 29**, parts of the region, particularly New York, New England, and northern Pennsylvania, experienced **unusually**

Snowfall in Plainview, NY, on May 9, 2020. Credit: John Murray, NWS OKX.

warm temperatures. The warmest locations had highs in the 80s and 90s, around 20°F above normal, with a few sites having their **hottest May day** and others having one of their three hottest. It was the third earliest occurrence of a day above 90°F in Buffalo, NY. Low temperatures ranked as the **warmest for May** at Caribou, ME, and Burlington and among the 10 warmest for May at other sites. Caribou also had a dewpoint of 70°F for the **first time in May**.

Most of the Northeast saw **below-normal snowfall** during **spring** and the **snow season** (October–May). Sites such as Philadelphia, PA; Baltimore, MD; and Atlantic City, NJ, tied their record for **least snowy spring**, while others had one of their three least snowy. It was only the second time since 1885 that Philadelphia recorded **no snowfall during spring**. Allentown and Harrisburg, PA, had their **least snowy seasons**, while other sites ranked this season among their 10 least snowy. Philadelphia and Washington, D.C., saw less than an inch of snow for the season for only the third time since 1885. The snowy exception was northern Maine, where Caribou recorded its 10th snowiest spring and its sixth snowiest season.

Spring Storms
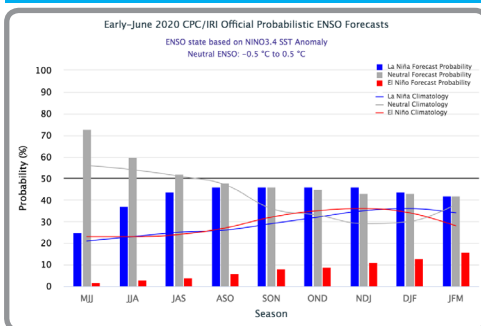

Damage in Maryland from a tornado on April 13, 2020. Credit: NWS Mount Holly

From **April 7–9**, southern areas saw **severe weather**. In western Pennsylvania, there were three weak **tornadoes** and the Pittsburgh International Airport recorded its **second highest thunderstorm wind gust** at 75 mph. Straight-line winds caused significant damage in western West Virginia. The storm rapidly strengthened over the Gulf of Maine to a near-record pressure level for April for Maine, resulting in strong wind gusts for the Northeast and a **major late season snowstorm** for parts of New England from **April 9–10**. Snow totals were up to 21 inches, with Caribou, ME, having its **second snowiest April day**. The heavy, wet snow and strong winds caused more than 266,000 customers in Maine, around a third of the state, to lose power. From **April 12–13**, **wind gusts** of 40–60 mph were common across the Northeast, with the highest gusts reaching 82 mph near Lanoka Harbor, NJ, and 80 mph in Milton, MA. The strong winds downed trees, damaged roofs, and caused power outages. **Two weak tornadoes** also touched down in northern Maryland. On **April 21**, severe thunderstorms produced several **waterspouts**, one of which came onshore, and **straight-line winds** of up to 80 mph, causing damage in New Jersey.

Parts of West Virginia saw several rounds of **heavy rain** during **May**. A slow-moving storm from **May 18–21** dropped up to 5 inches of rain, with Charleston having its third wettest May day. The remnants of **Tropical Storm Bertha** brought up to 3 inches of rain on May 28. Both events caused flooding, which led to road closures. There were two notable days in May with **severe weather** in northern parts of the region. On **May 15**, severe thunderstorms in New York and New England produced an **EF-1 tornado** and straight-line winds of up to 100 mph. An EF-1 tornado damaged trees in eastern New York on **May 29**. Southern parts of the region did not see much severe weather in May. This trend was also noted across the U.S. which had its **fewest number of tornado watches** on record.

Early–June 2020 CPC/IRI Official Probabilistic ENSO Forecasts
ENSO state based on NINO3.4 SST Anomaly
Neutral ENSO: –0.5 °C to 0.5 °C

ENSO

During May, El Niño-Southern Oscillation **(ENSO)-neutral conditions** were observed in the equatorial Pacific Ocean. NOAA's Climate Prediction Center indicates there is a 60% chance ENSO-neutral conditions will continue through summer and nearly equal chances (40–50%) of ENSO-neutral or La Niña conditions during autumn and winter.

Northeast Partners

National Oceanic and Atmospheric Administration offices including:

NESDIS/National Centers for Environmental Information

NWS, Eastern Region

NWS, Climate Prediction Center

NWS, National Operational Hydrologic Remote Sensing Center

NMFS, Fisheries Science Centers and Regional Office, Atlantic

NOS, Office for Coastal Management

NOS, National Centers for Coastal Ocean Science

OAR, Climate Program Office and Geophysical Fluid Dynamics Lab

OAR, National Sea Grant Office

NOAA's North Atlantic and Great Lakes Regional Collaboration Teams

And the following other offices:

Northeast Regional Climate Center
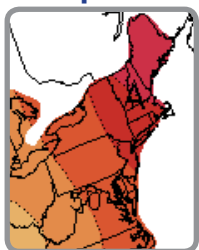
National Integrated Drought Information System

Consortium of Climate Risk in the Urban Northeast

Cooperative Institute for the North Atlantic Research

Northeast Region State Climatologists

Mid-Atlantic RISA

Temperature and Precipitation

Probability of Above: 33% 40% 50% 60% 70%

Normal July–September average temperatures range from the 50s in northern New England to the 70s in the Mid-Atlantic. NOAA's Climate Prediction Center favors **above-normal temperatures** for **July–September** in the Northeast (map above).

Above-normal precipitation is favored for **July–September** for southern areas, with **equal chances** of below-, near-, or above-normal precipitation for New York and New England (map right). Normal July–September precipitation ranges from less than 10 inches in parts of New York to more than 15 inches in northern New England.

Probability of Above: 33% 40% 50% 60% 70%
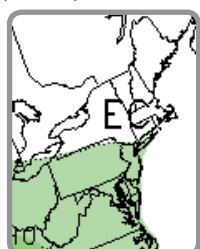
Atlantic Hurricane Season

	2020 Atlantic Season Outlook	Average Season
Number of Named Storms	13-19	12
Number of Hurricanes	6-10	6
Number of Major Hurricanes	3-6	3

NOAA's 2020 Atlantic hurricane season outlook indicates an **above-normal season** is most likely, with "a likely range of 13–19 named storms (winds of 39+ mph), of which 6–10 could become hurricanes (winds of 74+ mph), including 3–6 major hurricanes (Category 3, 4, or 5; winds of 111+ mph)." Several factors including a lack of El Niño conditions, warmer-than-normal sea surface temperatures, reduced wind shear, weaker trade winds, and an enhanced west African monsoon favor increased storm activity. For the sixth consecutive year the **season started early**, with the first storm forming on May 16, the second storm on May 27, and already the third storm on June 2. The season runs from June 1–November 30, peaking from mid-August–late October.

Connecticut

Drought Preparedness and Response Plan

Adopted by the Connecticut Water Planning Council
November 6, 2018

The Interagency Drought Workgroup is responsible for administering the Connecticut Drought Preparedness and Response Plan and consists of representatives from:

CT Department of Agriculture

CT Office of Policy & Management

CT Department of Public Health

CT Department of Energy and Environmental Protection, including the Public Utilities Regulatory Authority

CT Department of Emergency Services and Public Protection, Division of Emergency Management and Homeland Security

With technical assistance from:

U.S. National Weather Service

U.S. Geological Survey

Public information and drought updates can be found at www.ct.gov/waterstatus

Cover Photo: Colebrook River Lake in Colebrook, CT, as seen on September 22, 2017.
Credit: CT Department of Energy and Environmental Protection, Bureau of Water Protection and Land Reuse

Glossary of Acronyms:

CGS	Connecticut General Statutes
CIRCA	Connecticut Institute for Resilience and Climate Adaptation
CLEAR	Center for Land Use Education and Research
COG	Council of Governments
DCP	Department of Consumer Protection
DEEP	Department of Energy and Environmental Protection
DEMHS	Division of Emergency Management and Homeland Security
DESPP	Department of Emergency Services and Public Protection
DEWS	Drought Early Warning System
DoAg	Department of Agriculture
DPH	Department of Public Health
IDW	Interagency Drought Workgroup
NIDIS	National Integrated Drought Information System
NOAA	National Oceanographic and Atmospheric Administration
NWS	National Weather Service
OPM	Office of Policy and Management
PURA	Public Utilities Regulatory Authority
USDA	United States Department of Agriculture
USGS	United States Geological Survey
WPC	Connecticut Water Planning Council
WUCC	Water Utility Coordination Committee

Table of Contents

EXECUTIVE SUMMARY

The *Connecticut Drought Preparedness and Response Plan* ("Drought Plan") provides state and local decision-makers and public water suppliers with a set of formal operating procedures and administrative guidance for proactive drought planning and response. The Drought Plan is designated as a "support plan" within the State Response Framework, Connecticut's umbrella emergency management operations document.

The State Interagency Drought Workgroup (IDW), consisting of representatives from five state agencies, is responsible for facilitating the planning and response activities of the Drought Plan. Subject matter experts from two federal agencies provide technical assistance to the IDW.

The Drought Plan has the following objectives:
1. Describe what preparations should be in place prior to a drought declaration in order for the state and local municipalities to act when it becomes necessary;
2. Define criteria and guidelines for the IDW to assess the severity of drought conditions in a given area and recommend appropriate actions.
3. Identify the state, regional, local, federal, and private sector entities that are primarily responsible for managing drought-related activities;
4. Provide a guide for entities to undertake drought-related activities for which they are responsible, in the areas of coordination, public outreach, assessment, and preparedness;
5. Define communication strategies to integrate activities of the responsible parties;
6. Identify a progression of water use restrictions that may be implemented at the state or local level; and
7. Promote effective mobilization of public and private resources to manage drought mitigation efforts.

There are several long-term planning and preparedness strategies that should be in place in anticipation of droughts before they occur. Such strategies fall under the categories of coordination and management, public outreach and education, and data collection and monitoring. Section 4.2 summarizes these strategies by category. Some fundamental strategies include: designating a water coordinator in each municipality, promoting industry and public awareness of appropriate conservation activities, expecting all water withdrawers to prepare water supply contingency plans, and ensuring a clear understanding of authority for mitigating drought conditions and enforcing water use restrictions. It is critical that each municipality adopt a water use restriction ordinance in order to establish such authority.

In order to accommodate a tiered response appropriate for the severity of the drought, Section V of the Drought Plan identifies five stages of increasingly dry conditions:

> Stage 1: Below Normal Conditions
> Stage 2: Incipient Drought (formerly Drought Advisory)
> Stage 3: Moderate Drought (formerly Drought Watch)
> Stage 4: Severe Drought (formerly Drought Warning)
> Stage 5: Extreme Drought (formerly Drought Emergency)

In contrast to the 2003 edition of the State Drought Plan, the nomenclature used for the five drought stages differs from the drought stage names that are required to be used in water supply plans prepared by public water suppliers. This change is intended to better distinguish the State Drought Plan — intended to be a comprehensive, statewide plan — from the more targeted water supply plans prepared for specific public water suppliers. It is possible for a public water supplier to suffer from a drought-related water deficit without the State having declared a drought under the Drought Plan, and vice-versa. Individuals served by public water suppliers must be cognizant of the context of messages coming from their public water suppliers, and from municipal and state officials, as they may not always be uniform. Conversely, public

water suppliers and public officials have a duty to be clear of the context of their messages and how they relate to each other via well-constructed press releases and other means.

For each of the five stages, the Drought Plan lists criteria, also called indicators, which the IDW consider when identifying the intensity and geographic scope of drought. Criteria include precipitation, groundwater levels, streamflow, reservoir levels, Palmer Drought Severity Index, Crop Moisture Index, Vegetation Drought Response Index (only available during growing season), fire danger, United States Drought Monitor intensity level, and weather forecasts. The IDW makes recommendations for drought designations in a particular region(s) of the state guided by these criteria, but because droughts are not an exact science, professional judgment is crucial in making such recommendations. Several criteria are specific to a certain type of drought (e.g. agricultural versus hydrological) or time of year. The criteria are re-assessed periodically by the IDW as conditions warrant. The IDW may choose to consult other qualitative information beyond the listed criteria.

Section V lists suggested mitigation actions and preparations appropriate for each drought stage, categorized by type. The actions necessary and the recommended mitigation measures increase in magnitude as drought conditions worsen. It should be noted that it may not be appropriate to take every action listed in the table for a particular stage of drought. Actions listed under a particular drought stage may be appropriate at a later stage, or vice-versa.

At such time when the drought criteria, or other qualitative information when warranted, signal that drought conditions are improving, the IDW considers whether to scale back the state's drought declaration(s) to a lower stage of drought. Section VI describes post-drought activities to assist in the drought recovery and restoration of resources affected by drought, taking into consideration resource maintenance and long-term sustainability.

I. PURPOSE AND SCOPE

1.1. Background

Droughts are known to occur in Connecticut, despite the fact that the state typically receives plentiful precipitation. The most severe drought in the state's recorded history occurred during a multi-year period from 1961—1969, when much of the northeastern United States grappled with devastated agricultural crops, dry river beds, and depleted drinking water supplies. In the time since then, expanding population and increasing water demands have made the state even more vulnerable to the effects of drought.

Following a historically significant drought in 1980—1982, an independent drought task force was created under executive power to actively monitor conditions and issue recommendations. In 1999, the state recognized a need to develop a set of formal operating procedures and administrative guidance to improve the function, effectiveness, and predictability of future drought response. Consequently, the drought task force was re-established as the Interagency Drought Workgroup (IDW), a collection of agencies assigned with developing and administering a Connecticut Drought Preparedness and Response Plan ("State Drought Plan" or "Drought Plan").

State agencies represented on the IDW are:
- [Office of Policy and Management](#) (OPM) (lead agency)
- [Department of Agriculture](#) (DoAg)
- [Department of Emergency Services and Public Protection](#) (DESPP)
- [Department of Energy and Environmental Protection](#), including the [Public Utilities Regulatory Authority](#) (DEEP/PURA)
- [Department of Public Health](#) (DPH)

The United States Geological Survey and the National Weather Service provide technical assistance to the IDW. Appendix A contains a listing of drought-related agency functions and responsibilities.

1.2 Overview

The purpose of the State Drought Plan is to:
- Recommend a framework for a coordinated approach to the assessment of, and response to, drought conditions as they develop;
- Set forth guidance on drought action levels and the appropriate response actions that should occur as drought conditions change; and
- Preserve a balance between essential water uses during a period of water supply deficiency (e.g. water used to satisfy federal, state, and municipal public health and safety requirements, water used for firefighting, and water needed to maintain natural ecosystems).

The Drought Plan provides guidance to assess and to minimize the impacts of a drought for all water users in Connecticut. The Drought Plan is applicable even if a declaration of drought is restricted to a local area. To accomplish the above-stated purpose, the Drought Plan:
1. Describes what preparations should be in place prior to a drought declaration in order for the state and local municipalities to act when it becomes necessary;
2. Defines criteria and guidelines for the IDW to assess the severity of drought conditions in a given area and recommend appropriate actions.
3. Identifies the state, regional, local, federal, and private sector entities that are primarily responsible for managing drought-related activities;

4. Provides a guide for entities to undertake drought-related activities for which they are responsible, in the areas of coordination, public outreach, assessment, and preparedness;
5. Defines communication strategies to integrate activities of the responsible parties;
6. Identifies a progression of water use restrictions that may be implemented at the state or local level; and
7. Promotes effective mobilization of public and private resources to manage drought mitigation efforts.

The IDW is responsible for determining what level of concern is warranted based on observed and forecasted conditions, communicating concerns to the Governor and agency commissioners, and facilitating implementation of the Drought Plan.

Appropriate mitigation actions vary depending on the type of drought (hydrologic, agricultural, or meteorological), the resources most affected, the variability in the relative severity of impacts experienced in different areas of the state or by different water suppliers, and the time of year. Because every drought situation is unique, the Drought Plan allows for maximum flexibility by the IDW in responding to a drought, serving as a menu of possible actions rather than a regimented sequence of events. Conditions are evaluated as soon as data become available so that the IDW may recommend appropriate mitigate actions or adapt them to address specific needs.

In the face of changing climate, technological advancements, and increasing development pressures, the most prudent measures to address drought, and the agencies or individuals responsible for implementing those measures, will likely change over time. As new data and approaches for drought management become available, they should be incorporated in future updates to the Drought Plan. The IDW meets at least biennially to review the Drought Plan, evaluating long-term preparedness strategies and reviewing agency functions as needed. Additionally, the IDW may consider and recommend amendments to the Drought Plan.

1.3 Statutory Authority

Although there is no legislation specifically authorizing the adoption of a State Drought Plan, it has historically functioned under the existing authority of the executive branch. The Connecticut Water Planning Council (WPC), established in 2002 by Public Act 01-177 (CGS Sec. 25-33o), oversees development and adoption of the Drought Plan. The WPC first adopted the Drought Plan in 2003, followed by this revised version in 2018. Future revisions to the Drought Plan will be undertaken in coordination with the State Water Plan to be adopted pursuant to CGS Sec. 22a-352 as amended by Public Act 14-163 and Executive Order No. 66 as issued by Governor Malloy.

The IDW implements the Drought Plan independently from the WPC, within existing resources from its member agencies. State agencies, public water suppliers, and municipalities are expected to redirect resources from non-critical programs in order to prioritize drought planning and response, when appropriate. In more advanced stages of drought, emergency resources may be called upon. However, a robust implementation of every long-term preparedness recommendation included in the Drought Plan is unattainable without resources dedicated specifically for such implementation.

When a drought occurs, the Drought Plan calls upon the authority of various existing statutes and agency regulations pertaining to drought, drinking water quantity and quality, environmental protection, and utility operations, as well as the powers granted to the Governor during emergency declarations. Collectively, these authorities provide state agencies and others with the regulatory and enforcement capabilities to effectively manage and respond to water shortages and environmental and public health hazards as they develop. Municipalities are encouraged to enhance the state's drought mitigation capacity by adopting water use restriction ordinances that would provide a conservation enforcement mechanism in the event of a water supply shortage. A model water use restriction ordinance is featured in Appendix B.

II. INTRODUCTION

2.1 Definition of Drought

A drought is a prolonged period of abnormally low precipitation, often combined with abnormally high evaporation, that adversely affects the water resources of a given geographic area. It is not typically a distinct event that has a clearly defined beginning and end (such as a storm); nor does it affect all water resources or users equally. According to Victor Miguel Ponce of San Diego University, "drought is more than a physical phenomenon or natural event. Its impact results from the relationship between a natural event and demands on the water supply, and it is often exacerbated by human activities."[1]

As mentioned in the [2011 Connecticut Climate Change Preparedness Plan](#), recent climate change studies predict that drought—as well as flooding rains—will become increasingly frequent and severe in the future. Although much less sudden than a hurricane or earthquake, droughts can have similar widespread social, economic, and environmental consequences, requiring the response of numerous parties.

The duration of a drought, from onset to recovery, can occur over any time period from weeks to years, and the severity level can fluctuate over time. There are three distinct types of drought: meteorological, hydrologic, and agricultural. It is possible to experience more than one type of drought at the same time.

- A meteorological drought occurs when measurable precipitation is below normal and may or may not result in a hydrologic or agricultural drought, or both, depending on the time of year it occurs and other weather factors. Depending on the season, it may affect fire danger.
- A hydrologic drought is characterized by low stream flow and by low reservoir and groundwater levels. Depending on the season and water demand, it may or may not affect water supplies.
- An agricultural drought occurs when inadequate precipitation and excessive evapotranspiration deplete the soil moisture necessary to sustain crops and vegetation, resulting in serious damage and economic loss to agriculture. An agricultural drought occurs during the growing season and may worsen and recover more quickly than a hydrological drought. It is characterized by the Palmer Drought Severity Index, the Vegetative Drought Response Index, or the Soil Moisture Index. It may affect the terrestrial ecosystem. As with a hydrologic drought, it may or may not affect water supplies, although it can significantly increase demands on water systems for outdoor water uses.

The consequences of a drought depend on the severity of the water deficiency, the season in which it occurs, and the demand for water. Drought conditions can affect the availability of water, obtained from either a reservoir or from groundwater wells, including private wells. Low streamflow can affect aquatic life because of reduced habitat or water quality issues, such as warmer water temperatures. In addition, drought conditions can increase the risk of wildfire danger.

It is not unusual for a given period of water deficiency to represent a more severe drought of one type than another type. For example, a prolonged dry period during the early summer may substantially lower the yield of crops due to a shortage of soil moisture in the plant root zone (agricultural drought), but have little effect on groundwater storage that was replenished the previous spring (hydrologic drought). In addition, it is not unusual for the severity of drought to differ by geographic location within the state.

The demand for water in a particular area can affect the severity of drought impacts in that area. Typically, water use is highest during the summer due to outdoor domestic, agricultural, and commercial water use. Summer is also when stream flows and groundwater levels typically are lowest because of evaporation and transpiration by plants. Conversely, if it is very dry at a time of year when relatively little water is used, the effects are not as noticeable. Because drought impacts are most prevalent when there is an imbalance of water availability and demand, both must be considered.

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[1] http://ponce.sdsu.edu/three_issues_droughtfacts01.html

2.2 Stages of Drought

The State Drought Plan identifies five drought stages of increasingly dry conditions:

- **Stage 1**: Below Normal Conditions
  Stage 1 is a preliminary preparedness stage intended to advise state, regional, and local officials and public water suppliers of potentially worsening drought conditions and to re-establish lines of communication. Typically, this stage is activated in response to early signals of abnormally dry conditions and serves as a "heads up" for the possibility of a developing drought. There is no expectation for a broad public notice of a Stage 1 declaration. Specific criteria thresholds are not defined for Stage 1 as the decision to begin focusing on a possible developing drought is based on the IDW's professional judgment.

- **Stage 2**: Incipient Drought
  Stage 2 represents an emerging drought event, potentially impacting water supplies, agriculture, or natural ecosystems. Impacts from a Stage 2 drought are typically limited or isolated in nature, and are likely to be felt first in the most water-constrained settings. At Stage 2, government officials and public water suppliers in affected areas should be engaged and preparing to undertake mitigation activities should they become necessary. Data collection and reporting activities are increased, where appropriate, and communication and coordination becomes a priority. Stage 2 is the first publicly-announced stage of drought.

- **Stage 3**: Moderate Drought
  At Stage 3, a drought event is well-established across a significant area, with impacts increasing in extent and intensity and potentially including isolated severe impacts. At Stage 3, the tone of public messaging is intended to convey the more serious nature of conditions, and preparations are made for the possibility of widespread emergency response, should conditions continue to worsen.

- **Stage 4**: Severe Drought
  At Stage 4, widespread severe impacts to water supplies are imminent or already occurring, with negative economic and ecological impacts likely. Depending on the time of year, there might be a complete loss of some crops. Mandatory water use restrictions are likely, particularly regarding non-essential outdoor uses. In the most severely impacted areas, public officials and public water suppliers can be expected to undertake emergency measures. The Governor is likely to consider convening the Unified Command to coordinate the drought response and an emergency declaration is possible.

- **Stage 5**: Extreme Drought
  A Stage 5 drought declaration corresponds to an emergency situation for part or all of the State. Public water and firefighting supplies are depleted to a point threatening public health and safety. The Governor is likely to declare a state of emergency and activate the State Emergency Operations Center.

The names of the stages differ from those used in utility water supply plans so as not to confuse the public when public water suppliers declare a particular drought stage representing their unique water supply and demand conditions.

2.3 Categories of Water Supply

Water users in Connecticut obtain their water from a variety of sources:
- municipal/public suppliers
- regional/quasi-public suppliers
- investor-owned suppliers
- privately-owned suppliers
- on-site private wells
- stream, lake, and pond withdrawals (non-potable water)

The Drought Plan uses the term "public water" for any supplied water (not from a private well) in accordance with Department of Public Health (DPH) definitions. To assure a safe and adequate supply of drinking water, DPH regulates any public water system supplying groundwater or surface water, or both, to 15 or more connections or 25 or more persons daily at least 60 days of the year. This regulation is accomplished through numerous state laws and regulations that address public drinking water quality and quantity.

Each public water supplier has a unique water system that may include one or more reservoirs, one or more groundwater wells, or both. These sources vary in capacity. In addition, the geographical area of the watershed and the local geologic and hydrologic conditions affect the water available and how each water system responds to a drought.

Any public water supplier defined in Section 25-32d of the Connecticut General Statutes (CGS) that supplies water to at least 1,000 people or at least 250 customers is required to prepare and submit to DPH a water supply plan that includes a "water supply emergency contingency plan."[2] Such plan identifies responses for four drought stages — Drought Advisory, Drought Watch, Drought Warning, and Drought Emergency — and includes identification of trigger levels which initiate each stage based on the projected water supply availability and demand situation.

With this update of the State Drought Plan, the plan uses stage names that differ from those used in water supply plans. In part, this is in order to avoid confusion between broad drought declarations made under the State Drought Plan and targeted drought declarations that a public water supplier issues specifically for its customers within a defined supply area. For this reason, all water users must pay attention to information issued by the State (based on this plan), their municipality, and their public water supplier, as applicable.

Private well owners are encompassed by the State Drought Plan, but might also be subject to municipal ordinances, where adopted. Private wells vary greatly in design and hydrogeological characteristics. As there is no practical method to continually monitor the amount of available groundwater everywhere in the state, assessing the potential impact of drought on private well users is extremely challenging. Historically, droughts have resulted in increased work for well drillers and public health officials, who receive requests to hydrofrack or deepen existing wells that have run dry. This is an example of qualitative, ancillary information that may be collected by the IDW to more fully understand the impact of a drought. Private well owners should always use water conservatively during a drought and heed any recommendations or instructions issued by local authorities or by the State in accordance with the Drought Plan.

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[2] For further information on Water Supply Plans, see Sections 25-32d-1a through 25-32d-6 of the Regulations of Connecticut State Agencies (https://eregulations.ct.gov)

III. IMPLEMENTATION

3.1 Data Resources and Decision-Making Criteria

The following criteria are routinely monitored by the IDW for the purposes of analyzing conditions leading up to and during a drought and used to recommend appropriate mitigation actions:

Quantitative criteria include:
- Cumulative precipitation
- Groundwater levels
- Streamflow
- Drinking water reservoir levels
- Palmer Drought Severity Index
- Crop Moisture Index
- Vegetation Drought Response Index (only available during growing season),
- Fire danger
- U.S. Drought Monitor

Qualitative or auxiliary criteria include, but are not limited to:
- Groundwater trends
- Streamflow trends
- Overall public water supply status, including reservoir and wellfield trends
- Number of public water suppliers that have issued voluntary and mandatory water conservation orders
- Number of public water suppliers with unique water supply situations
- Number of requests for private well deepening or fracking
- Agricultural reports
- Weather forecasts
- Northeast Drought Early Warning System (DEWS)

Much of the quantitative criteria are publicly available data from state and federal agencies that are mandated to collect, analyze, and distribute these data. For example, the U.S. Geological Survey continuously monitors streamflow and groundwater levels, the National Weather Service continuously monitors cumulative rainfall and weather conditions, and the Connecticut Department of Public Health routinely receives and compiles reservoir and production well conditions from public water suppliers.

The data used to analyze drought conditions may represent a single location (such as a streamflow gage) or a broad land area (such as Palmer Drought Severity Index). The IDW assesses data on a regional basis to determine if a drought declaration will be recommended for one or more areas of the state.

It is expected that agency members of the IDW will determine what additional information may be obtained by each respective agency to create a complete snapshot of conditions across the state. Such information may include qualitative data and field observations (such as fisheries and forestry reports) to inform the decisions made by the IDW. Any person with pertinent drought impact information is encouraged to contact the IDW via the contact information listed on the drought information website, www.ct.gov/waterstatus.

The National Integrated Drought Information System (NIDIS) was authorized by Congress in 2006 for the purpose of interstate and interagency coordination and integrated drought research that builds upon existing federal, tribal, state, and local partnerships to create drought early warning systems (DEWS) across

the nation. As defined by NIDIS, a DEWS utilizes new and existing networks of federal, tribal, state, local and academic partners to make climate and drought science accessible and useful for decision makers; and to improve the capacity of stakeholders to monitor, forecast, plan for, and cope with the impacts of drought. Following an intense drought in parts of the Northeast in 2016, NIDIS hosted collaborations with stakeholder agencies throughout the region, including representatives from Connecticut, to develop the first ever strategic plan for the northeast region DEWS. The 2018-2019 Northeast DEWS strategic plan was released on June 26, 2018.

The IDW recognizes the need to be an active participant in the Northeast DEWS and Strategic Plan. With a goal of future integration of the Northeast DEWS into the State Drought Plan, the IDW will collaborate with NIDIS and Connecticut's strategic partners in surrounding states to develop activities and work towards outcomes which improve Connecticut's early warning capacity, understanding of drought-related impacts, and long-term drought resilience.

3.2 Application of the Drought Plan

The IDW is the entity responsible, under the authority of the WPC, for actively monitoring water conditions and, as guided by the Drought Plan, recommending drought declarations and mitigation actions to the Office of the Governor and state agency commissioners. Whenever any member agency of the IDW becomes aware of considerably dry conditions via routine monitoring of internal or external sources, the following sequence of events shall take place:

1. The alerting agency shall contact OPM, which, as the lead agency, shall convene a meeting of the IDW. A public meeting notice and agenda shall be circulated.

2. The IDW shall assess and discuss the drought criteria and any other appropriate data to determine whether dry conditions warrant a declaration(s) of drought conditions for any portion(s) of the state, consistent with the thresholds specified in Section V of this plan. The IDW may cite professional judgement in its determination, if not consistent with the thresholds specified in Section V. A determination to recommend such declaration(s) shall be supported unanimously by all member agencies on the IDW.

3. In the event that the IDW cannot reach unanimous agreement to recommend a drought declaration(s), as guided by the Drought Plan, the IDW shall take no action. A follow-up meeting of the IDW shall be scheduled within 14 business days to re-evaluate conditions. At such follow-up meeting, any determination made by the IDW to recommend a drought declaration(s) shall be by simple majority of the member agencies.

4. Any such recommendations of the IDW shall be transmitted by OPM to the Office of the Governor. The Office of the Governor may accept, reject, or modify the recommendations of the IDW. The Office of the Governor may defer to the secretary of OPM or the commissioner of any IDW member agency to accept, reject, or modify the recommendations of the IDW. State agencies shall commit the resources necessary to undertake mitigation actions outlined in Section V.

5. Whenever a drought declaration is active for any portion of the state, OPM shall schedule regular meetings of the IDW as deemed necessary to re-evaluate conditions and implement mitigation actions associated with each drought stage, as guided by Section V. Responsibilities that are not already clearly assigned shall be delegated to appropriate member agencies based on resource availability, subject matter expertise, and statutory and regulatory authorities. The lead agency of the IDW shall oversee such delegation of responsibilities.

6. The IDW shall determine whether to recommend changes to any active drought declarations (scaling up or scaling back), following the same decision-making protocols in 2-4 above.

Under the National Incident Management System (NIMS) Emergency Support Function System, the Office of Policy and Management is designated the primary state agency in a drought event. When conditions reach Stage 3 (Moderate Drought), the Governor may consider convening his/her Unified Command in accordance with the State Response Framework (SRF) prepared by the Department of Emergency Services and Public Protection, Division of Emergency Management and Homeland Security.   The Governor's Unified Command is comprised of the key state agencies and public sector partners relevant to a particular potential or actual emergency. If conditions reach Stage 5 (Extreme Drought), many necessary response functions may be coordinated under the SRF, including activation of the State Emergency Operations Center to centralize planning and response operations.

During times of no drought, the IDW shall meet not less than biennially for the purpose of reviewing and implementing long-term drought preparedness activities in Section IV, and to review any changes in agency staffing or resource demands that affect the IDW. The IDW may, at any time, create and oversee one or more independent working groups consisting of staff from state, local, and/or private entities for the purpose of conducting drought preparedness exercises or activities, or for other drought preparedness needs as the IDW may determine.

IV. LONG-TERM PLANNING AND PREPAREDNESS

In addition to ongoing water conservation practices, proactive drought planning will help Connecticut's residents prepare for and mitigate future droughts.

Droughts can vary widely in duration, severity, location, and local impact. Their effects will vary depending on the time of year; summer droughts typically will have a greater impact than winter droughts. However, a winter drought may limit groundwater recharge and reservoir refilling, which may affect water availability the following spring and summer if conditions do not improve.

Adequate drought response is predicated on long-term, continued planning, preparation, and well-thought policy that becomes part of the overall management of the water resources of the state, well before any declaration of a drought. The State Water Plan to be adopted by the Connecticut General Assembly in accordance with CGS Sec. 22a-352, and as required to be implemented pursuant to Executive Order No. 66, will assist the state achieve its vision of maintaining sustainable, healthy, and balanced water resources for all water users, and in doing so will make the state better prepared for times of drought.

4.1 Long-term Planning & Preparedness: Fundamental Strategies

Water Supply Plans
Each major public water supplier must prepare a water supply plan that indicates ongoing water conservation actions as well as an emergency contingency planning component. The plan includes triggers for water conservation during any water supply concern, including various stages of drought. In addition, Water Utility Coordinating Committees (WUCCs), in the preparation of their regional water supply plans, are required to evaluate water conservation. The State Drought Plan supplements these planning requirements and covers water users (private well owners) that would not fall under an individual public water supply plan.

Conservation Activities
Ongoing water conservation activities are critical to the long-term management of water resources in the state and should be a primary consideration in all water management decisions and strategies. During a water emergency, there may be insufficient lead-time to undertake major water-saving improvements. Water conservation is more important than ever due to increasing demands on water supplies, increased runoff and less infiltration from urbanized watersheds, and the high costs and difficulties in developing new water supplies. In addition, anticipated precipitation, extreme heat, drought, and runoff impacts associated with climate change will add to the concern. For these reasons, the general public should adopt water conservation practices.

Connecticut has a longstanding history of engaging in water conservation planning activities. There have been a number of accomplishments that have resulted in the state being better prepared to conserve water and mitigate drought impacts:

- Public water suppliers that serve over 1,000 people or 250 customers have prepared Water Supply Plans, as mandated by CGS Sec. 25-32d. These plans have a water conservation component and an emergency contingency plan component and outline actions to be taken in response to local public water supply conditions. (In 2016 there are approximately 80 water suppliers required to have prepared Water Supply Plans; this number fluctuates due to mergers and/or sales of companies.);
- Water conservation efforts are an important consideration in water supply diversion permitting under CGS Section 22a-369.
- Public Act 94-144 requires certain public water suppliers to distribute water conservation educational information to their customers annually. DPH has also published guidelines on water conservation;

- Public Act 89-303 requires the sale of only low flow devices, such as shower heads and toilets;
- Public Act 89-266 requires certain public water suppliers to make available, free of charge, many low flow devices to encourage customers to retrofit their residences with water conserving devices; and
- Public Act 13-78 requires that PURA and the WPC identify and recommend water conservation programs, and establishes conservation-related principles that DEEP and municipal legislative bodies must consider when setting water company rates.

Enforcement

The State can enforce water use restrictions only when the governor orders an emergency declaration (Stage 5 Drought). Therefore, municipal authority is necessary to enforce local conservation measures at earlier stages of drought. Most municipalities do not have water use restriction ordinances in place that would allow them to enforce mandatory water conservation measures during droughts and other water emergencies.

Legislation passed in 2014 (Public Act 14-168) encourages municipalities to adopt a water use restriction ordinance. As an aid to municipalities, the IDW provided a model ordinance as seen in Appendix B. There is a need to develop multiple model ordinances to address differing water systems, supply needs, and governmental structures in each community; however, doing so is outside the scope of the Drought Plan. In addition to the model ordinance, municipalities may use elements of this plan, including Appendix C, for guidance when developing a local water use ordinance tailored to their specific needs. In municipalities that have private wells, the ordinance should also include homeowner responsibilities. An important component of such an ordinance would be the requirement for adequate coordination between the municipality and the public water supplier(s) that serves the community. Representatives of several local departments or boards, including, but not limited to, selectmen, health district, police and fire, land use, and conservation, as well as the business community, should be parties to drafting the ordinance, along with the local public water supplier. The local ordinance should be included in the municipality's hazard mitigation and emergency preparedness plans. The State will continue to promote and encourage the use of local water conservation and drought management ordinances.

Coordination & Management

A platform for efficient and adequate coordination and management is arguably the most important aspect of drought preparedness. There are multiple entities (public and private) responsible for managing water resources in the state; therefore, coordination hinges on communication among state, regional, and local agencies and public water providers, and the timely dissemination of clear and succinct information to the public. It is essential that each municipality designate a "water coordinator" who acts as the municipality's primary point of contact. Every municipality already has an Emergency Management Director (EMD). The designated EMD may be a good fit for the role of water coordinator based on experience and funding offered from the state through the federally matching Emergency Management Performance Grant. If the role of municipal water coordinator is filled by another municipal official (e.g., public works director), that individual will maintain a regular communications flow with the municipal EMD.

Each municipality also has a Local Emergency Operations Plan, which includes the identification of a local Unified Command to address a particular emergency. In addition, the state is divided into five state Division of Emergency Management and Homeland Security Emergency Planning Regions, which each convene a Regional Emergency Planning Team (REPT) made up of all the municipalities in the region, and draft and implement a Regional Emergency Support Plan following the National Incident Management System (NIMS). This existing emergency management structure can provide the platform for drought coordination and situation management.

Connecticut residents get their water from a variety of sources; some have public water and some are self-supplied. In addition to information disseminated from the State, individuals and municipalities who have public water should look to their water supplier for information pertinent to their supply area.

In order to coordinate messages, each public water supplier should communicate with DPH when its drought triggers have been reached or a water supply emergency has occurred. This communication should occur as soon as possible and include a list of the mitigating steps being taken by the public water supplier.

Each municipality has access to the State's Emergency Notification System, commonly referred to by the vendor's proprietary name, Everbridge, which allows a municipality to send emergency messaging to the public free of charge.

Public Outreach & Education

Public outreach and education are critical components of long-term drought preparedness. Public water suppliers should periodically provide customers with information about their water—where it comes from, how to ensure its quality, and how to use it wisely. Conservation of water should be part of this education. Public water suppliers should identify large users and encourage them to adopt water conservation practices. Outdoor water use is the largest component of water demand during the summer. Education should include discussion of best management practices (BMPs) for various industries and for homeowners. A directory of BMPs is available in Appendix D.

Data Collection & Monitoring

Accurate, geographically distributed, readily accessible data are crucial to enable scientifically based recommendations for drought declarations. Existing data should be assessed to identify gaps and plans should be made to fill those gaps over time. In the absence of actual data, modeling may be utilized to fill some of the gaps. This augmented network of data-gathering sites, operated by various state, federal and local agencies and organizations must be funded, maintained, and utilized in order for the IDW to obtain the necessary information. These data are used in conjunction with qualitative information obtained from each member agency's contacts and the professional judgment of its staff. When conditions warrant, increased monitoring will be needed to make informed recommendations on the progression of the drought.

The ability to accurately assess current conditions and to predict the future status of a drought depends upon extensive long-term monitoring and data collection, as well as reliable forecasts of hydrology and weather. Drought management requires continuous monitoring of factors indicating the onset, extent, and duration of water deficiency conditions. The IDW needs real-time weather, streamflow, groundwater, reservoir, and soil moisture information to compare with a reliable historical record in order to inform recommendations.

4.2 Long-Term Planning & Preparedness: Ongoing Activities

Droughts can have widespread social and economic significance that require the response of numerous parties. As a result, drought response requires long-term, continued planning and preparation that becomes part of the overall management of the water resources of the state, well before any declaration of drought. The chart on the following page lists long-term planning and preparedness activities that are critical for effective implementation of the Drought Plan. The IDW shall meet at least once biennially to review progress with long-term planning and preparedness actions. After a drought occurs, this section should be amended to reflect lessons learned.

Long-Term Planning & Preparedness Activities		

Coordination & Management	*State Agencies coordinated through the Interagency Drought Work Group*	Routinely evaluate the adequacy of current executive branch legislative authorities and propose any necessary changes.
		Develop and maintain mechanisms to assess opportunities for proactive drought mitigation working with significant environmental, public health, and economic interests.
		Develop and maintain a database of water suppliers to assess needed assistance in an emergency and communicate vital information.
		Maintain a chart of non-essential water uses relative to the stages of drought and time of year.
		Identify or develop and promote drought mitigation strategies and best management practices for use in the planning, development, and operation of water-intensive facilities.
		Promote use of WebEOC, a web-based emergency communication resource that can be used when a utility has reached its drought criteria. Develop and regularly update a guidance document for using the WebEOC.
		Emergency preparedness officials should maintain familiarity with federal agencies, regional, municipal, and water utility emergency officials and their capacity for assistance.
		Maintain a process for public water systems to report any changes in status such as triggers identified in water supply plans or asset management plans.
		Develop a communication plan with small water suppliers.
		Provide outreach to municipal officials to educate them on the importance of local participation in preparing for a drought; integrate drought education into existing presentations.
	Municipalities/ Local Officials	Designate an official Water Coordinator, a single individual who is the primary point of contact for a municipality. The Water Coordinator should be identified in a municipal water ordinance and contact information should be sent annually (or whenever there is a change) to DPH, DEMHS, and other state agencies, as well as to local water utilities, as appropriate. The Water Coordinator should be familiar with the water supply plans serving his/her community.
		Involve water utilities in the development, review, and adoption of local water ordinances to ensure that enforcement of water conservation is a coordinated effort. Work with regional Councils of Governments (COGs) as appropriate to assist with identifying and eliminating barriers to implementing model water use ordinances.
		Work collaboratively with water utilities and heavy water users to implement drought mitigation strategies and best management practices for use in the planning, development, and operation of water-intensive facilities.
	Water Suppliers	Ensure contact information is sent annually (or whenever there is a change) to DPH and to municipal Water Coordinators.
		Water systems that are not required to have a water supply plan should develop an alternative plan that outlines procedures for a drought or water supply emergency.
		Work collaboratively with municipalities and heavy water users to implement drought mitigation strategies and best management practices for use in the planning, development, and operation of water-intensive facilities.
		Work with municipal officials when developing or updating water supply plans. Such plans should be coordinated with any municipal water ordinances and lay out an organized process for communicating with all water users — public and private — during a drought.
		Prepare an asset management program over the short-term and long-term to assess the systems and plan for improvements, including water sources, infrastructure, and operation; such information should be provided to DPH in order to allow for assessments in an emergency.
		Repair or improve infrastructure to reduce the amount of unaccounted for water. Assess dam leakage at reservoirs and, if necessary, undertake repairs where feasible.
Chart continues on next page.		

Public Outreach & Communication	*State Agencies coordinated through the Interagency Drought Work Group*	Promote the use of water-conserving devices and appliances and seek building code changes as appropriate.
		Prepare educational materials (e.g. flyers, websites, ads, etc.) so that they are quickly and readily available for distribution during a drought.
	Municipalities/ Local Officials	Encourage low-impact design (LID) for new development and existing infrastructure; these practices may include stormwater infiltration systems to recharge aquifers.
	Water Suppliers	Routinely promote water conservation and distribute educational materials to customers; e.g. promoting "water-sense" fixtures.
		Provide assistance such as water-use audits to large customers in detecting and fixing water leaks, and in the installation of additional water conservation devices.
		Implement smart-metering where practical, which will provide more frequent water usage data to customers and the ability to adjust rates during a drought.
Data Collection, Monitoring, & Preparedness	*State Agencies coordinated through the Interagency Drought Work Group*	Continuously identify new or better sources of information to use for drought monitoring.
		Periodically assess validity of historical drought monitoring benchmarks in light of anticipated climate change impact on drought length and severity.
		Develop guidelines, using statistical analysis as appropriate, for the most appropriate and reliable drought indices by user categories, including: water utilities, agriculture, ground water, and surface water. Such guidelines should address both seasonal variations and long-term trends.
		Identify emergency sources of water that can be made available as temporary water supplies during a water emergency.
		Develop and maintain a database incorporating all relevant data from all relevant sources.
		Maintain up-to-date lists of approved water haulers, approved bottled water purveyors, and licensed well drillers.
	Municipalities/ Local Officials	Collect and monitor data and information on local conditions and the impacts on affected critical services and water demands on a continuous basis.
	Water Suppliers	Regularly monitor and track drinking water supplies and demand, especially when supplies are below normal and/or demand is strong.
		Use historic data to inform the decision-making process and predict likely outcomes.
		Periodically assess the benefits of new interconnections and underutilized water supplies as a means to ensure a stable and resilient supply network.

V. Drought Declaration & Response

This section identifies a list of criteria thresholds intended to guide the IDW's identification of drought stages. The tables in this section provide a selection of possible drought mitigation actions appropriate for each stage of drought. The mitigation actions necessary and the restrictions implemented increase in frequency and significance as drought conditions worsen. The IDW may use professional judgement in determining whether or not it is appropriate or necessary to take all mitigation actions listed for a particular stage. As a drought worsens, all actions corresponding to earlier stages of drought should continue to be considered.

It is not possible to specify numerical thresholds for all potential drought criteria because of the many factors, both natural and man-made, that can affect or be affected by the availability of water and water-use patterns. Therefore, qualitative and other auxiliary data and sound subject matter expertise are crucial for guiding the IDW's recommendations.

Each drought stage is described in the colored boxes on the following pages, in order of increasing severity, and includes defining thresholds for drought criteria used in assessing conditions. It should be understood that the specified thresholds are not absolute, but are intended to guide the IDW in determining the severity of the drought being experienced. For example, because each drought is unique, it is possible that a particular drought stage could be triggered by less intense conditions experienced over a longer duration, or by more intense conditions experienced over a shorter duration.

Stage 1: Below Normal Conditions

Defining Criteria:

Stage 1 is a preliminary preparedness stage that serves to alert the parties who should be prepared to respond to potentially worsening drought conditions. The primary target audience includes state, regional, and local officials and public water suppliers. Typically, this stage is activated upon the first signals of impacts from abnormally dry conditions. There is no expectation for a broad public notice of a Stage 1 declaration.

Specific criteria thresholds are not defined for Stage 1 as the decision to begin focusing on a possible developing drought is based on the IDW's professional judgment.

Stage 1 Recommended Mitigation Actions		
Coordination & Management	*State Agencies coordinated through the IDW*	Pay attention to all aspects of agency operations that could indicate impending drought conditions; communicate and meet as needed.
		Delegate duties and responsibilities as necessary to assure information flow among state agencies.
		Designate agency spokesperson(s) to coordinate interaction with the public and expedite information referrals.
		Submit drought assessment reports as necessary to agency heads.
		Designate an individual to be the contact person for receiving and compiling drought-related information.
	Municipalities / Local Officials	Municipal water coordinators provide DPH with up-to-date municipal water coordinator contact information. If no municipal water coordinator exists, designate a local official competent in water supply issues as the municipal water coordinator and provide contact information. Municipal water coordinator maintains regular communications flow with local emergency management director.
	Water Suppliers	Designate a point contact person for communication with municipalities and the state. Provide up-to-date contact information to DPH to ensure the communication of vital information and assess needed technical and financial assistance in an emergency.
Public Outreach & Education		The Below Normal Conditions stage is intended to initiate internal communication and awareness among the IDW and other decision makers, in response to observations or reports that warrant heightened awareness of conditions. Communication with the public is not planned at this time.
Data collection, monitoring, & preparedness	*State Agencies coordinated through the IDW*	Continue to regularly monitor the primary indicators of drought; systematically collect, analyze, and disseminate real-time drought-related information.
		Identify geographic extent of dry conditions and determine affected regions.
		Plan what staff and/or funding could be made available, if necessary, to support increased monitoring activities.
		Verify that all monitoring networks and drought information websites are functioning and include relevant, up-to-date information.
		Review database of contact information for public water suppliers and municipal water coordinators and update as needed.
		Update database/map of public water suppliers that that have requested voluntary conservation and/or have placed mandatory water restrictions.

Stage 2: Incipient Drought

<table>
<tr><td colspan="2">Defining Criteria:

A decision to issue a Stage 2 declaration regarding incipient drought is guided by the following drought criteria thresholds, as well as any other ancillary data:</td></tr>
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Precipitation	Two-month total below 65% of average
Groundwater	Two out of three months below the 25th percentile
Streamflow	Two out of three months below the 25th percentile
Reservoirs	Average levels less than 80% of normal
Palmer Drought Severity Index	-2.0 to -2.99
Crop Moisture Index	-1.0 to −1.99, abnormally dry
VegDRI (seasonal)	Pre-drought conditions
Fire Danger	Moderate
U.S. Drought Monitor	Intensity level D1-D2

This stage was formerly called: Drought Advisory

Stage 2 Recommended Mitigation Actions		
Coordination & Management	*State Agencies coordinated through the IDW*	Alert municipal water coordinators and water suppliers of conditions.
		Coordinate with municipal water coordinators, local health directors, and water suppliers to promote water conservation, monitor local situations, and report problems.
		Offer technical assistance to water utilities experiencing problems to assist with system management and promotion of water conservation with specific measures tailored to each water utility. Assist water utilities in strengthening supply-side and demand-side conservation measures.
		Survey local Water Coordinators and assess municipal drought preparedness. • Advise municipalities to review appropriate ordinances to enable the enforcement of water conservation if needed in the future and to coordinate with water utilities, when pertinent. • Advise water utilities to implement their coordination plans with their municipalities.
	Municipalities / Local Officials	Water coordinator and water suppliers should review communications protocol and coordinate on any public announcements (this could involve multiple communities).
		Alert key town officials (police & fire chiefs, health director, chief executive officer, emergency management director, public works, parks & recreation, superintendent of schools) about conditions.
		Notify municipal public works departments and fire responders to consider suspending all unnecessary exercises that require fire hydrants to be opened.
	Water Suppliers	Communicate with DPH and municipal water coordinators about local conditions, concerns, and any changes to the status of water supply.
		Consider postponing discretionary water consuming maintenance, repair work, and shutdowns.
Public Outreach & Education	*State Agencies coordinated through the IDW*	Provide information to weather forecasters and other media to encourage public interest stories and facilitate dissemination of drought information to the public.
		Compile information on water conservation tips to homeowners, e.g., "Water Efficiency Measures for Residents," and "Water Efficiency Measures for Landscaping," in preparation for distribution through the Internet, public service announcements, and other timely mailings should the drought worsen.
		Increase awareness of the state's drought information website.
	Municipalities / Local Officials	Work with state agencies to prepare information on water conservation tips for future dissemination to water users through the Internet, newspapers, public service announcements, and other timely mailings.
		Issue guidance document for private well users who may require assistance with well repairs or enhancement and make this available via the Internet.
	Water Suppliers	Consider issuing voluntary conservation appeals to all customers.

		Respond to customer complaints and problems related to drought conditions.
Data collection, monitoring, & preparedness	*State Agencies coordinated through the IDW*	Continue to monitor the primary indicators of drought, increasing the frequency as needed. Include qualitative data. Prepare new assessment reports as conditions change.
		Review activities of neighboring states through websites, and in coordination with National Weather Service and
		Survey local health departments, well drillers, and the Department of Consumer Protection concerning well drilling activity related to dry conditions.
		Monitor WebEOC for activities related to dry conditions.
		Review and implement, as needed, the plan for managing potential forest fire hazards and threats.
		Review water supply systems that have historically had adequacy problems, "target systems," and provide technical assistance as needed.
		Review reservoir storage reports of the systems that use surface water supplies and consider more frequent reservoir level reporting for selected systems.
		Verify database accuracy of approved water haulers, approved bottled water purveyors, licensed well drillers, and upload lists to the state drought management website.
	Municipalities / Local Officials	Water coordinator should review any local sources of data on wells, dry hydrants, fire conditions, etc., and communicate to the appropriate state agencies.
	Water Suppliers	Monitor local water supplies and collect data more frequently as needed.
		Begin preparing for the possibility of bringing alternative/secondary supply systems online.
		Investigate any deviation from normal use registered on production meters.
		Review water supply emergency contingency plan triggers and mitigation activities; update if necessary.

Stage 3: Moderate Drought

<table>
<tr><td colspan="2">Defining Criteria:

A decision to issue a Stage 3 declaration regarding moderate drought is guided by the following drought criteria thresholds, as well as any other ancillary data:</td></tr>
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Precipitation	Three-month total below 65% of average
Groundwater	Four consecutive months below the 25th percentile
Streamflow	Four out of five months below the 25th percentile
Reservoirs	Average levels less than 70% of normal
Palmer Drought Severity Index	-3.0 to -3.99
Crop Moisture Index	-2.0 to −2.99, excessively dry
VegDRI (seasonal)	Moderate drought conditions
Fire Danger	High
U.S. Drought Monitor	Intensity level D2-D3

This stage was formerly called: Drought Watch

**Stage 3: Moderate Drought**		
Coordination & Management	*State Agencies coordinated through the IDW*	Declare a Stage 3 Drought and notify municipal water coordinators.
		Governor to consider convening Unified Command, including key state agencies and ESF 12 water companies, to review emergency plans and coordinate messaging. Consider establishing a Web EOC incident in order to track water issues.
		Contact each municipal Water Coordinator to ensure understanding of the required role and responsibilities of a municipal Water Coordinator.
		Commissioners require all state-owned facilities to enact water conservation measures and to review and update any specific drought/emergency plans.
		Communicate with the Army Corps of Engineers or other dam operators on possible use of impoundments for streamflow augmentation in locations where existing streamflow regulations are not adequately meeting the needs of fish and wildlife downstream.
		Initiate contact with federal agencies (FEMA/EPA/USGS/USDA/Corps) in order to identify federal assistance capabilities.
		Provide technical assistance to utilities on managing systems during dry conditions, including (a) administering expedited reviews of proposed system upgrades and alternative water supplies for drought-impacted community water systems; and (b) assist in the identification of emergency connections.
		Evaluate unused or underutilized high yield aquifers developable as temporary emergency water supplies including for non-potable uses.
		Disseminate generic press releases (DEMHS/OPM) and notification letters to water systems, local health directors, well drillers, etc.
		Direct state agencies to conserve water and repair leaks at state facilities.
	Municipalities / Local Officials	Municipal water supply coordinators should provide input to DPH on local conditions; for example, any change in status such as triggers identified in water supply plans or as defined by asset management plans, if any.
	Water Suppliers	Review operations to ensure that conservation efforts are maximized. Non-critical utility uses such as routine flushing, clearwell, clarifier or storage tank cleaning, meter testing and bleeders should be reviewed to eliminate, reduce or delay water use, where feasible.
		Preparation for mandatory conservation, including necessary enforcement mechanisms, will be initiated.
		Determine where temporary interconnections between water utilities are needed and coordinate with DEEP/DPH for expedited permitting.

		Consider preparations to activate "emergency" and "inactive" sources of water supply for potential use and coordinate with DPH.
Public Outreach & Education	*State Agencies coordinated through the IDW*	Send letters to municipal officials requesting they urge residents to curtail outdoor watering.
		Hold news conference to announce activation of the Water Status website and information line to get information on water status and conservation measures.
		Target heavy water users and evaluate mechanisms for water use reduction. Use guidance provided in "Industrial/Commercial/Institutional Water Users – Planning Guidance for Water Conservation and Emergency Contingency Plans" and "Agricultural Water Users - Planning Guidance for Water Conservation and Emergency Contingency Plans".
		Assist agricultural industry by determining possible issues, prospective situations, and remedial steps that can be taken, including the dissemination of information and technical assistance for irrigation improvements available under federal emergency programs to agricultural growers.
		Remind holders of registered water diversions of their legal responsibilities and conditions that are prerequisite to a suspension of minimum stream flow standards pursuant to CGS Sec. 22a-6 and RCSA 26-141a-4(b).
		Use the Internet, public service announcements and radio station broadcasts to urge residents and businesses to conserve water (provide conservation tips such as "Water Efficiency Measures for Residents," and "Water Efficiency Measures for Landscaping"). Encourage water users to cooperate with local officials and utilities as conditions may be worse in specific areas, requiring greater efforts in accordance with adopted utility plan.
	Municipalities / Local Officials	Set a voluntary outdoor water use reduction for all residents and businesses.
	Water Suppliers	Voluntary conservation will be promoted in residential, commercial and industrial facilities to reduce demand from previous non-drought projected usage for the appropriate month.
Data collection, monitoring, & preparedness	*State Agencies coordinated through the IDW*	Monitor implementation of individual water supply plans (through WebEOC or other means).
		Ensure municipal preparedness: • Obtain feedback from large water systems concerning adequacy of municipal authorities in place for water emergencies. • Follow-up and provide technical assistance to towns regarding local ordinances (recommend model ordinances, authorities, and fines.)
		Identify non-essential water uses during the Severe Drought Stage relative to time of year.
		Determine where temporary interconnections between water utilities may be needed, in accordance with CGS Sec. 22a-378.
		Initiate process for drafting Emergency Executive Order for the Office of the Governor.
		Assess and report agricultural impacts of worsening drought.
	Municipalities / Local Officials	Track and report problems related to the drought for both deep and shallow wells.
	Water Suppliers	Review adequacy of water monitoring and consumption records and invest in increased monitoring capabilities where needed.
		Evaluate potential funding needs for actions required under severe or extreme drought conditions to ensure the availability of adequate funding through budgets or emergency measures.
		Initiate increased reservoir level monitoring and reporting as directed by DPH.

Stage 4: Severe Drought

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Defining Criteria:

A decision to issue a Stage 4 declaration regarding severe drought is guided by the following drought criteria thresholds, as well as any other ancillary data:

Precipitation	Five-month total below 65% of average
Groundwater	Six consecutive months below the 25th percentile
Streamflow	Six out of seven months below the 25th percentile
Reservoirs	Average levels less than 60% of normal
Palmer Drought Severity Index	-4 or less
Crop Moisture Index	-3 or less, severely dry
VegDRI (seasonal)	Severe drought conditions
Fire Danger	Very high
U.S. Drought Monitor	Intensity level D3-D4

This stage was formerly called: Drought Warning

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Stage 4: Severe Drought		
Coordination & Management	*State Agencies coordinated through the IDW*	Declare a Stage 4 Drought and notify municipal water coordinators.
		Governor to consider convening Unified Command, including key state agencies and Emergency Support Function (ESF) 12 water companies, to review emergency plans and coordinate messaging. Coordinate communications strategy. Consider establishing a Web EOC incident in order to track water issues. Governor considers activating State Response Framework (SRF) upon consultation with State Emergency Management Director, DEEP, DPH, and OPM, including the ESF 12 Energy and Utility Annex. Governor considers declaring a civil preparedness emergency under Title 28. Consider activation of ESF12 Task Force and/or State Emergency Operations Center. Review state statutes and regulations for potential waiver under Conn. Gen. Stat. Section 28-9.
		Consider requesting a Presidential Emergency Disaster Declaration if direct federal assistance is required. Consider other sources of federal assistance.
		DEMHS Regional Coordinators to work with Emergency Regional Planning Teams (REPTs) in each emergency planning region to review plans including Regional Emergency Support Plan.
		If State EOC is activated, provide regular situation status reports to state and municipal partners, the Federal Emergency Management Agency and the US Army Corps of Engineers regarding drought impacts and response measures being taken by state and local officials.
		Issue emergency and temporary permits, as appropriate, and expedite drought-related emergency requests for water utility interconnections and access to alternative water sources in accordance with CGS Sec. 22a-378.
		DoAg should coordinate with USDA to assess agricultural impacts of worsening drought and provide federal relief/emergency assistance for farmers.
		Coordinate with water suppliers to bring "emergency" and "inactive" sources of water supply into production, including accessible and developable high yield aquifers.
		Encourage the activation of unused or underutilized water sources as temporary emergency water supplies for non-potable uses.
		Facility managers should implement water efficiency improvements at facilities.
		Prohibit aquifer pumping tests unless a) the test is associated with a groundwater remediation project, or b) the test is associated with a replacement well for a previously approved, allocated diversion source, or c) the test is associated with a drinking water supply well necessary to ensure uninterrupted water supply during a water supply emergency.
		Make recommendations to Governor on communications strategy.

		Expedite drought-related diversion permit applications and requests for temporary and/or emergency authorization.
	Municipalities / Local Officials	Direct municipal Water Coordinator to provide situation reports periodically. Identify to which agency these reports should be made, including to DEMHS Regional Coordinators if State Response Framework is activated.
	Water Suppliers	Initiate first phase of mandatory conservation. At this level, all unnecessary water usage will be banned. No outside hose usage will be allowed, nor are in-ground sprinkler systems to be used. A 20 percent reduction in usage from previous non-drought projections for the appropriate month will be targeted.
		A plan will be formulated in concert with state and local officials for strict rationing of water if a drought emergency should be reached. The needs of high priority customers, homes, commerce, and fire protection will be established and prioritized. Plans will be made for emergency service of drinking and cooking water by tanker to any areas where normal water service must be terminated.
		Coordinate with water suppliers to bring "emergency" and "inactive" sources of water supply into production, including accessible and developable high yield aquifers.
		Encourage the activation of unused or underutilized water sources as temporary emergency water supplies for non-potable uses.
		All possible supplementary water sources will be prepared for use. Coordination with local officials concerning alternative facilities for obtaining water will be initiated, as required.
Public Outreach & Education	*State Agencies coordinated through the IDW*	Prohibit all outdoor watering and curtail other water uses as appropriate.
		Assist community water systems in exploring alternative sources of water for non-potable uses.
		Increase the degree of public education and information; increase the tone of seriousness in public service announcements, press releases, etc.
		Coordinate with Governor's Infoline (United Way 211) to respond to public inquiries, through State EOC if activated.
	Municipalities / Local Officials	Increase the degree of public education and information; increase the tone of seriousness in public service announcements, press releases, etc. Coordinate messaging with local, regional, and state partners.
		Enforce or assist with enforcement of water use restrictions.
		Assist owners of residents with dry wells with obtaining permits to construct wells or evaluate the feasibility of connecting to a public water supply.
	Water Suppliers	Meet with individual, large, water-intensive industries to discuss water use cutbacks.
		Increase the degree of public education and information; increase the tone of seriousness in public service announcements, press releases, etc.
		Request municipalities to assist with enforcement of water use restrictions.
		Synchronize messaging with the State by notifying all customers, through business communication channels and the media, of drought declarations made by State officials, actions being taken by the State and by the water supplier, and on the implementation of mandatory conservation.
Data collection, monitoring, & preparedness	*State Agencies coordinated through the IDW*	Determine, on a service area or broader geographic basis, the need to declare a public water supply emergency pursuant to Section 25-32b and direct appropriate water utility actions.
		Establish an event on WebEOC.
		Develop plans to deliver drinking water to key distribution stations within each municipality. Assess capability and finalize readiness plans for the mobilization of water distribution and storage equipment to armories and other designated locations. Explore alternative means of water delivery during outages.
		Identify and communicate with authorities to implement a ban of non-essential water uses during the Extreme Drought Stage (see Appendix C for guidance).
		Draft an Emergency Executive Order for the Office of the Governor.
		Prepare to request a Presidential Disaster Declaration.

		Determine the terms of the sale of any water pursuant to an order by DPH for the sale, supply or taking of any waters or the temporary interconnection of water mains for the transfer of water among water utilities.
		Expedite permitting of temporary interconnections in accordance with CGS Sec. 22a-378
	Municipalities / Local Officials	Increase public education and information as appropriate for this stage.
	Water Suppliers	Prepare timely water supply status reports for distribution to state and local officials as ordered/requested.

Stage 5: Extreme Drought

Defining Criteria:

A decision to issue a Stage 5 declaration regarding extreme drought is guided by the following drought criteria thresholds, as well as any other ancillary data:

Precipitation	Seven-month total below 65% of average
Groundwater	Eight consecutive months below the 25th percentile
Streamflow	Seven consecutive months below the 25th percentile
Reservoirs	Average levels less than 50% of normal or less than 50 days of supply
Palmer Drought Severity Index	-4 or less
Crop Moisture Index	-3 or less, severely dry
VegDRI (seasonal)	Extreme drought conditions
Fire Danger	Extreme
U.S. Drought Monitor	Intensity level D4

This stage was formerly called: Drought Emergency

Stage 5: Extreme Drought		
Coordination & Management	*State Agencies coordinated through the State Emergency Operations Center, Governor's Unified Command*	Governor likely to activate the State Response Framework prepared by the Department of Emergency Services and Public Protection, Divison of Emergency Management and Homeland Security, operating under the National Incident Management System, including activation of ESF 12 Energy and Utilities Annex. Governor likely to convene Unified Command, including key state agencies and Emergency Support Function (ESF) 12 water companies, to implement response actions and coordinate messaging. Coordinate communications strategy. Web EOC incident will be established if not already in order to track water issues. Governor likely to declare a civil preparedness emergency under Title 28, and/or a public health emergency under Title 19a and/or a water supply emergency under Title 22a. Governor likely to activate State Emergency Operations Center. ESF 12 Task Force activated. State Agencies recommend waiver of appropriate state statutes and regulations by Governor under Conn. Gen. Stat. Section 28-9.
		Declare a Stage 5 Drought and notify municipal water coordinators.
		Apply for a Presidential Emergency Disaster Declaration if direct federal assistance is needed; apply for a USDA Secretarial Disaster Declaration; apply for federal assistance and funding as appropriate. Track damages and costs related to drought for potential Presidential Major Disaster Declaration.
		Declare a public drinking water supply emergency and follow the procedures listed in the amendment to Section 25-32b in Section 3 of PA 14-163, as appropriate.
		Utilize authorities under a declared water supply emergency pursuant to Section 22a-378 to undertake actions as needed relative to approving temporary suspension of diversion permits, issuing orders for new diversions.
		Improve distribution and transmission of potable water; divert water from current sources; and bridge existing water systems, including the determination of appropriate methods for financing emergency drinking water operation.
		Issue permits and conditions for the use of Class B waters for potable water purposes, as necessary, on a case-by-case basis pursuant to CGS Section 22a-378 and Section 25-32(b).
		Secure emergency legislation and funding from the General Assembly as needed.
		Activate and staff the State Emergency Operations Center as deemed appropriate, working within the State Response Framework under the National Incident Management System. Activate appropriate elements of the CT National Guard as necessary.
		Coordinate enforcement on water use bans with municipal and state law enforcement.
		Administer the emergency transfer, sale, or lease of water throughout the state.
		Initiate transporting and distributing potable water to provide essential water to key municipal emergency potable water stations (trucking water and laying water pipe, as necessary).

		Order interconnections between water utilities as needed and where feasible in accordance with CGS Sec. 22a-378.
		Consider preserving (shut valves) remaining available water in select storage tanks for rationing or fire emergency. It may be necessary to set a storage minimum to be held for extinguishing fires, the amount needed depending upon the nature of the emergency and structures in the service area.
	Water Suppliers	Initiate a drought hazard rationing plan in cooperation with appropriate local and state officials. The plan will consider needs of high priority customers, homes, commerce and fire protection.
		Maximize use of alternative supplies.
Public Outreach & Education	*State Agencies coordinated through the IDW*	Ban all non-essential water uses in accordance with CGS Section 25-32d.
		Consider closing the hunting/fishing season in accordance with CGS Sec. 26-25.
		Increase enforcement of open burning ban under CGS Sec. 23-49a, and/or prosecute violators with more severe penalties as allowed under statute.
		Close woodlands and brushlands to all persons except owners or tenants pursuant to CGS Sec. 23-50.
		Working through Governor's Unified Command and State EOC if activated, schedule a press conference to inform the public of the severity of the drought.
		Enforce compliance with mandatory drought restrictions.
	Municipalities / Local Officials	Process applications for exemptions or variances to mandatory drought restriction.
		End outdoor watering exceptions for new lawns; ban all lawn watering
		Coordinate provisions for emergency bathing services and emergency service of drinking and cooking water by tanker to any areas where normal water service must be cut off. Enforce mandatory rationing of water.
	Water Suppliers	Coordinate provisions for emergency bathing services and emergency service of drinking and cooking water by tanker to any areas where normal water service must be cut off. Enforce mandatory rationing of water.
Data collection, monitoring, & preparedness	*State Agencies coordinated through the IDW*	Prepare, based on available information and professional judgement, recommendations for the Governor on additional action steps to take in a worse-case scenario if conditions do not improve and water supplies become critical.

VI. DROUGHT RECOVERY

At such time when the drought criteria, or other information when warranted, signal that drought conditions are improving, the IDW will consider whether to scale back the drought declaration to a lower stage of drought. When considering the criteria for signs of improvement, special attention should be paid to groundwater levels, since they are more reliable for measuring long-term shifts in rainfall patterns and respond more gradually. Other criteria, such as streamflow, respond quickly to changing conditions and can be misleading during wet periods that may occur during a long-term drought. The IDW will rely on professional judgment guided by the drought criteria, when deciding whether to scale back a drought declaration.

With improving conditions, the IDW should continue to assess and recommend which drought mitigation actions (Section V) are appropriate. These actions should be communicated in the same fashion as when the drought was worsening.

6.1 Post Drought Actions

The primary objectives during post-drought recovery are to maintain, as far as possible, the resources affected by drought, and to assist in the post-drought return and restoration of those resources, taking into consideration resource maintenance and long-term sustainability. These include:

- Administering available funding of federal long-term drought relief;
- Providing risk management programs to assess the financial condition of individual agricultural enterprises and give alternatives for operators to utilize in drought recovery;
- Following-up with drought-impacted community water systems to restore operations and to ensure that drought-driven system improvements and modifications are in compliance with applicable standards;
- Evaluating the effectiveness of the triggers as defined in utility water supply plans or asset management plan;
- Preparing an After-Action / Improvement Plan Report summarizing the drought-related issues for the governor and commissioners, to include an assessment of activities undertaken to mitigate drought impacts, successes realized and recommended improvements. This report should, at a minimum:
  - recommend appropriate amendments to state legislation and municipal ordinances;
  - recommend appropriate amendments to the State Drought Plan;
  - recommend programs to encourage efficient use of potable waters;
  - recommend the level of resource monitoring that is needed to establish accurate baseline conditions;
  - recommend improvements to economic impact assessment tools;
  - recommend improvements to drought impacted public water system's water supply plan drought triggers and actions;
  - describe lessons learned with all applicable entities; and
  - summarize the use of class B water sources and emergency reservoirs and wells

APPENDIX A: DROUGHT MANAGEMENT RESPONSIBILITIES

Although all citizens are responsible for minimizing water use during a drought, managing drought is a responsibility shared by numerous organizations and agencies at all levels. Some of these organizations are responsible for data collection and dissemination, some are responsible for management and coordination, and some are responsible for declaring drought stages that allow access to financial or other assistance.

LOCAL GOVERNMENT
Involvement of local government varies widely. A small number of communities have adopted local water use restriction ordinances, which enable enforcement of drought restrictions at the local level. Because currently this is the only mechanism for enforcement before a Drought Emergency is declared, it is important that all communities adopt such ordinances. A model ordinance is available to assist communities in developing one tailored to their situation (Appendix B).

REGIONAL GOVERNMENT
Currently, Regional Councils of Governments (COGs) are not assigned specific responsibilities in drought preparedness and response outside of their roles in broader state emergency response preparedness. Nevertheless, drought conditions frequently exist in or are more intense in a portion of the state and, because of that, in combination with their close working relationships with local chief elected officials and with other regional entities, COGs might be considered an appropriate geographic level for drought response messaging and action.

In addition, all municipalities in the State are members of one of the five CT Division of Emergency Management and Homeland Security (DEMHS) Emergency Planning Regions: each region has a Regional Emergency Planning Team (REPT) to which each municipality belongs, and on which each Emergency Support Function is represented. These REPTs meet regularly to address all-hazard emergency planning issues. The REPTs also create regional emergency support plans that are used to provide mutual aid support in times of emergency.

STATE OF CONNECTICUT

Governor (OTG)
- Provide overall direction of state government drought response, including convening Governor's Unified Command as needed for a coordinated response.
- Close forestlands as necessary in extreme drought conditions pursuant to Sec. 23-50 Connecticut General Statutes (CGS)

Office of Policy and Management (OPM)
- Lead State Interagency Drought Workgroup
- Coordinate state agency drought management activities
- Maintain drought information on website

Department of Energy and Environmental Protection (DEEP)
Administratively, DEEP includes the Public Utility Regulatory Agency (PURA)
- Member of State Interagency Drought Workgroup
- During a declared water supply emergency, temporarily suspend a water diversion permit, impose conditions upon water diversion permit holders, and authorize a person or municipality to divert (or transfer) such quantities of water needed to ease emergency conditions (CGS Sec. 22a-378).
- Reduce regulated downstream release requirements in times of shortage (CGS Sec. 26-141b).

- Regulate open burning in or adjacent to woodlands and brushlands during a fire danger or drought emergency (CGS Sec. 23-49a).
- Close hunting/fishing season to prevent forest fires during dry conditions (CGS Sec. 26-25).
- PURA-specific functions:
  - Determine the terms of the sale of any water sold pursuant a declaration of a public drinking water supply emergency by Commissioner of Public Health pursuant to Section 25-32b when the water utilities that are party to the sale cannot determine such terms or if one of such water utilities is regulated by PURA.
  - Consider any special emergency regulations, which may be necessary and proper to ameliorate the present situation for those communities, which are served by private water utilities.

Department of Public Health (DPH)
- Member of State Interagency Drought Workgroup.
- Has jurisdiction over all matters concerning the purity and adequacy of any source of water and protects public health through regulatory oversight of public water systems and the provision of technical assistance pursuant to CGS Section 25-32(a).
- In consultation with DEEP and PURA, DPH may declare a public drinking water supply emergency upon receipt of information that a public water supply emergency exists or is imminent, pursuant to appendix D of CGS Section 25-32b.
- Implement Emergency Generator and Emergency Plan Regulations.
- Approve and use WebEOC for public water supply emergencies.
- Assess and respond to any impacts of water shortages on public health and on water utilities.
- Regulate permitting requirements for sale of excess water by any public water system to another public water system.
- Coordinate drought response actions with local health officials and utilities.
- Develop public education and outreach materials on conservation for the public.
- Assess any detrimental condition affecting supply adequacy and quality, pursuant to the Regulations of Connecticut State Agencies (RCSA) Section 19-13-B46.
- Require each community water supply system to maintain a supply in excess of system demands, and immediately implement conservation measures, as necessary pursuant to RCSA Section 19-13-B102 (o) and (p). RCSA Section 25-32d-1-(c)(5) also requires systems supplying water to 1,000 or more people to implement their required conservation and emergency contingency plans.
- Collect and analyze information on the status of public water supplies as the central resource for the compilation of data, coordination of activities, and information dissemination regarding instances of drought related water system failure.
- Regulate new sources of water supply.
- May identify and order the temporary emergency use of water sources.
- Work with water purveyors to develop emergency plans.
- Provide technical assistance to water utilities and local health departments and to private well users through local health departments. Also, relays drought information to the local health officers of the impacted communities.
- Lead agency with regard to monitoring public water supplies for drought impact.
- Oversight of community, transient non-community, and non-transient non-community water systems.

Department of Emergency Services and Public Protection (DESPP), Division of Emergency Management and Homeland Security (DEMHS)
- Member of the State Interagency Drought Workgroup.

- Maintain and implement the State Response Framework, including running the State Emergency Operations Center and coordinating response as directed by the Governor.
- Maintain the five DEMHS Regions, including Regional Emergency Planning Teams, to coordinate planning, response, and recovery at the local level.
- Prepare at the Governor's direction any requests to FEMA for federal assistance or a Presidential Emergency Declaration or Major Disaster Declaration.
- Establish a Web EOC incident as indicated.
- Provide precipitation and weather forecast data to State Interagency Drought Workgroup.
- Maintain the local branch of the National Warning System (NAWAS);
- Develop and maintain various types of emergency operations plans and hazard mitigation plans for state government;
- Provide technical planning assistance to communities as requested or as needed;
- Provide training programs for state and local municipalities in civil preparedness;
- Conduct emergency operations drills and exercises;
- Work with DEEP to administer the FEMA Unified Hazard Mitigation Grant Programs for the state.
- Coordinate with Governor's Info line (United Way 211) to respond to public inquiries; and
- Work with the Department of Public Health regarding the provision of emergency water and power equipment and water buffaloes for emergency use.

CT Department of Agriculture (DoAg)
- Member of the State Interagency Drought Workgroup
- Provide assistance to farmers suffering from drought.
- Provides data on Palmer Drought Severity Index, Crop Moisture Index and Vegetative Drought Response Index to Interagency Drought Workgroup
- Provide information on the impacts of a drought on the agricultural community.

CT National Guard
- Assist with emergency distribution of public water.

FEDERAL

Office of the President
- Declare drought emergencies when necessary, allowing areas of the State to receive financial and other assistance from the Federal Emergency Management Agency.

The National Oceanic and Atmospheric Administration (NOAA)
- Track national and regional weather conditions.
- Provide drought data to the State of Connecticut via the National Weather Service (NWS).

U.S. Geological Service (USGS)
- Track streamflow and groundwater levels.
- Provide information on streamflows and groundwater levels to the State Interagency Drought Workgroup.

U.S. Department of Agriculture (USDA)
- Provide assistance to farmers suffering from drought.

APPENDIX B: MODEL WATER USE RESTRICTION ORDINANCE

Note: The following model water use restriction ordinance was included in the original 2003 State Drought Plan and has not been updated. While still a valuable resource for municipalities seeking to adopt a water use restriction ordinance, the Connecticut Water Planning Council recognizes that it contains numerous deficiencies, including language that would be inapplicable to or incompatible with some municipalities. The Water Planning Council intends to reconsider this model ordinance during implementation of the State Drought Plan.

Introduction

This is a model provided by the State of Connecticut for use in developing ordinances to restrict the use of water supplied by a public water supplier. It is for communities wishing to establish enforceable limitations on the use of water during emergencies and temporary periods of high water demand. Proposed restrictions included in the ordinance should be consistent with the schedule of drought response measures indicated in the individual Water Supply Plans of the water suppliers and with the Connecticut Drought Preparedness and Response Plan. Persons violating the ordinance would be subject to civil fines. The State believes it is important for municipalities to consider exemption procedures for the restrictions included within their ordinance. These or other exemptions may be appropriate due to the economic or public health impact of water use restrictions on specific industry or population sectors. Municipalities should give careful consideration to the type of uses granted exemptions and should consider conditioning those exemptions to ensure that those granted exemptions are operating in a water efficient manner.

If a water company is experiencing issues affecting its ability to consistently provide an adequate supply of water, implementing the model ordinance may not address the problem. In severe cases, a declaration of public drinking water supply emergency under Connecticut General Statute (C.G.S.) 25-32b should be requested from the Department of Public Health. A water company operating under a declaration of public drinking water supply emergency would be expected to enter a formal agreement with the Department of Public Health to aggressively pursue measures to increase safe yield such as implementing new sources of supply, regional interconnections, increasing storage capacity, and/or improved treatment and conveyance methods.

Local requirements for adopting ordinances may vary according to the terms of individual municipal charters. Consultation with municipality counsel is encouraged before adopting any ordinance. It is essential that municipalities coordinate their activities with one another when water companies cross municipal borders and there is also a critical need to ensure consistency between water companies when municipalities are served by more than one water company. To promote such coordination and consistency, it is strongly recommended that a Memorandum of Understanding (MOU) be drafted by all involved parties. The State makes no representation concerning the legal effect or validity of this model.

1.0 Authority

The municipality, under its powers pursuant to state law, has adopted this ordinance to protect public health and welfare. This ordinance implements the municipality's authority to impose water use restrictions, conditioned upon a state of water use restrictions or a declaration of public drinking water supply emergency issued by the Department of Public Health pursuant to C.G.S. 25-32b.

2.0 Purpose

The purpose of this ordinance is to protect, preserve and maintain the public health, safety and welfare whenever there is in force a State of Water Use Restriction or State of Public Drinking Water Supply

Emergency by providing for enforcement of any duly imposed restrictions, requirements, provisions or conditions imposed by the municipality or by the State of Connecticut.

3.0 Definitions

Agriculture shall mean farming in all its branches as defined in C.G.S. Section 1-1(q).

Municipality means any town, consolidated town and city, consolidated town and borough, city, borough, and village.

Outdoor Watering shall mean any watering of decorative lawns, trees or shrubbery by water users.

Person means any individual, partnership, association, firm, limited liability company, corporation or other entity, except a municipality, and includes the federal government, the state or any instrumentality of the state, and any officer or governing or managing body of any partnership, association, firm or corporation or any member or manager of a limited liability company.

State of Public Drinking Water Supply Emergency shall mean a State of Public Drinking Water Supply Emergency declared by the Department of Public Health in consultation with the Department of Environmental Protection, and the Department of Public Utility Control under C.G.S. 25-32b.

State of Water Use Restriction shall mean a State of Water Use Restriction declared by the municipality pursuant to Section 4 of this ordinance.

Water Company means any individual, partnership, association, corporation, municipality or other entity, or the lessee thereof, who or which owns, maintains, operates, manages, controls or employs any pond, lake, reservoir, well, stream or distributing plant or system that supplies water to two or more consumers or to twenty-five or more persons on a regular basis provided if any individual, partnership, association, corporation, municipality or other entity or lessee owns or controls eighty per cent of the equity value of more than one such system or company, the number of consumers or persons supplied by all such systems so controlled shall be considered as owned by one company for the purposes of this definition.

Water Users shall mean all persons or municipalities using water from any public water source irrespective of that person's responsibility for billing purposes for use of the water.

4.0 Declaration of a State of Water Use Restriction

The municipality, in consultation with the water company, or water companies, as may be appropriate, may declare a State of Water Use Restriction. Such a declaration should be, where appropriate, conditioned on the identification of an emergency or water shortage by the water company, the local health department, a state agency or the governor which could also include or be limited to the restrictions listed in Section 5. Public notice of a State of Water Use Restriction shall be given under Section 6 of this ordinance before it may be enforced. After implementation of any state of water use restrictions, the Department of Public Health and the Department of Environmental Protection should be notified in writing within 14 days of the implementation of restrictions. These restrictions can be phased-in to tailor them according to the severity and nature of the water supply emergency.

5.0 Restricted Water Uses

A declaration of a State of Water Use Restriction shall include restrictions consistent with the response measures indicated in the individual Water Supply Plans of the water company and the Connecticut Drought Preparedness and Response Plan, as appropriate. These may include one or more of the following restrictions, conditions, or requirements limiting the use of water as necessary to protect the water supply except as provided in Section 11. The applicable restrictions, conditions or requirements shall be included in the public notice required under Section 6. Please note, the following restrictions are listed to serve as examples of the types of water use restrictions that may be implemented:

a) Automatic Sprinkler Use: The use of automatic sprinkler systems is prohibited, except lawn watering is permitted in order to establish and maintain newly laid sod or newly seeded grass associated with new construction, and the testing of a customer's newly installed or newly repaired sprinkler system by a commercial enterprise engaged in the installation or repair of lawn irrigation systems is permitted.

b) Car washing: Car or vehicle washing is prohibited, except for the washing of vehicles performed by a commercial enterprise engaged in car washing.

c) Loss of water from customer's service line: The loss of water through breaks or leaks within the customer's service line, private distribution system or plumbing for any substantial period of time within which such break or leak should reasonably have been discovered and corrected. It shall be presumed that a period of seventy-two (72) hours after the customer discovers such a break or
leak or receives notice from the water company of a break or leak is a reasonable time within which to correct such break or leak or, as a minimum, to stop the flow of water from such break or leak.

d) Off-Peak Outdoor Watering: Outdoor watering is permitted only during daily
periods of low demand, to be specified in the declaration of a State of Water Use Restriction and public notice thereof. For example, limit outdoor watering to between 8:00 p.m. and 6:00 a.m. on ____ days (specify days). (*In general, restricting outdoor water use to between sunset and early morning is best for turf needs and coincides with off peak hours. Municipalities may choose to restrict water use to one or two days per week during specified hours.*)

e) Other outdoor uses. The use of private wells or other outdoor uses not addressed in this ordinance that are, in the determination of the Director of Health, wasteful, are prohibited.

f) Outdoor Watering Ban: Outdoor watering is prohibited, except the watering of agricultural products, sod at commercial sod farms, and the watering of nursery stock at nurseries or retail outlets is permitted.

g) Outdoor Watering Method Restriction: Outdoor watering is restricted to bucket, can or hand held hose watering with automatic shutoff nozzle.

h) Swimming Pools, Wading Pools, Hot Tubs, Spas, and Jacuzzis: Filling and topping off of swimming pools, wading pools, hot tubs, spas, and jacuzzis are prohibited, unless newly constructed or installed swimming pools, wading pools, hot tubs, spas, and jacuzzis that may be filled once upon completion of construction or installation

i) Use of water for firefighting, health, sanitation, & medical purposes. The use of water for firefighting, health, sanitation, or medical purposes shall not be restricted. However, domestic water use conservation practices should be implemented wherever possible.

j) Washing impervious surfaces. The washing or cleaning of streets, driveways, sidewalks or other impervious areas is prohibited.

6.0 Notification
6.1 State of Water Use Restriction
Notification of any provision, including any restriction, requirement or condition imposed by the municipality as part of a State of Water Use Restriction shall be published by the municipality in a newspaper of general circulation within the municipality, or by such other means reasonably calculated to reach and inform all users of water of the State of Water Use Restriction. Notification of the State of Water Use Restriction shall also be provided to the Connecticut Department of Public Health, Department of Public Utility Control, and Department of Environmental Protection at the same time that notification is given.

6.2 State of Public Drinking Water Supply Emergency
When a State of Public Drinking Water Supply Emergency is declared by the Department of Public Health, the water company shall follow those procedures outlined in its approved Emergency Contingency Plan. In the event water use restrictions are necessary, the water company shall contact and consult with the affected municipality(s), in accordance with their Memorandum of Understanding (MOU). The municipality(s) would then declare a State of Water Use Restriction in accordance with Section 4.0 of this document. Appropriate notice to the public shall be provided in accordance with Section 6.0 of this document.

7.0 Termination of a State of Water Use Restriction; Notice
A State of Water Use Restriction may be terminated by a municipality upon a determination, in consultation with the water company, that the water supply shortage no longer exists. Public notification of the termination of a State of Water Use Restriction shall be given in the same manner as is required for notice of the municipality's declaration of its State of Water Use Restriction pursuant to Section 6.

8.0 State of Public Drinking Water Supply Emergency; Compliance with DPH or DEP Orders
Upon notification to the public that a declaration of a State of Public Drinking Water Supply Emergency has been declared by the Department of Public Health in consultation with the Department of Environmental Protection and the Department of Public Utility Control, no person shall violate any provision, restriction, requirement,
condition of any order approved or issued by the DPH for the purpose of bringing about an end to the State of Public Drinking Water Supply Emergency.

9.0 Enforcement and Penalties
The municipality, [through its Water Commissioner, water superintendent, building inspector, local police or water company police] may enforce this ordinance. Any person violating this ordinance shall be liable to the municipality in the amount of up to $100.00 for the first violation and up to $200.00 for the second violation. Third and subsequent violations shall require a mandatory court appearance in addition to a fine assessment of up to $500.00. Fines shall be recovered by indictment, or on complaint before the District Court, or by non-criminal disposition. In extreme cases, the municipality may order the water company to curtail water service. When enforcing water curtailment, consideration should be given to customers that have multiple tenants or at risk individuals.

10.0 Severability
The invalidity of any portion or provision of this ordinance shall not invalidate any other portion or provision thereof.

11.0 Exemptions; Application for a waiver
Any such water users that consider the restrictions, as imposed, to adversely affect their livelihood, health or sanitation, may make written application for a waiver. Any such application should be directed to the attention of the municipality. The municipality, in conjunction with the water company, will verify that the applicant is a user within the system and then forward the application, within three days, to the Director of Public Health who then makes the determination whether a waiver should be granted. This decision shall be made within three days of receipt of the application by the Director of Public Health.

APPENDIX C: NON-ESSENTIAL USES

Note: The following examples of non-essential water use restrictions formerly appeared as Section V in the 2003 Connecticut Drought Preparedness and Response Plan. Section V was not revised in the Drought Plan's 2018 update and appears here as an Appendix. It is recognized that it may contain outdated information. It may be of use to municipalities in drafting a water use restriction ordinance.

During declarations of drought, if water use demands remain at levels that cannot be sustained under current and forecasted conditions despite the coordinated water management measures implemented in previous drought stages, targeted water use restrictions may be implemented. The types of non-essential uses and their degrees of curtailment will be determined at the time each drought stage is entered based upon the specific circumstances determined at that time.

The following non-essential use restrictions are intended to guide elected officials and government agencies when it becomes necessary to enact emergency water conservation measures during a drought. This approach should enable the proper authorities to determine at what point in each of the stages certain non-essential water uses should be curtailed or banned. A flexible approach is desirable so there would be no rigid "do's" and "don'ts" that would entail unnecessary hardship during the various early stages of a drought. The State of CT Model Water Use Restriction Ordinance (Appendix B) provides a basis by which municipalities can implement this approach.

Unless otherwise determined, emergency restrictions and authorizations should apply equally to all water users in the affected regions, regardless of whether the water used is drawn from ground or surface water (such as a pond, lake, river or stream), a public water supplier, or a private well. However, the State Drought Preparedness and Response Plan does not prevent any local government from instituting water use restrictions that are more stringent, provided the local restrictions do not conflict with state or federal law. Hardship exemptions from the restrictions on water use imposed during an emergency may be obtained in accordance with procedures established by the local municipality.

Municipal and state law enforcement agencies shall be responsible for enforcement of non-essential water use restrictions.

Non-Essential Water Use Restrictions

When drought conditions become severe, all residents, visitors, businesses and government agencies must fully comply in a cooperative effort to avoid a more serious water shortage by compliance with the following restrictive measures:

1. The serving of water in restaurants, clubs or eating places is prohibited, unless specifically requested by the patron.
2. The washing of any vehicles other than fire engines, and HAZMAT vehicles is prohibited, except in the following cases:
    A. Washing of vehicles performed by a commercial enterprise engaged in car washing is permitted, provided the following requirements are met:
        1. Vehicles shall not be pre-rinsed except with recycled water;
        2. Rinse cycles shall be forty (40) seconds or less per vehicle. This may be accomplished by increasing conveyor speeds;
        3. Additional measures shall be implemented to minimize water use, such as reducing the size of water nozzles where possible and plugging all unnecessary out-flows;
        4. All fixtures and equipment shall be inspected for leaks on a daily basis. Necessary repairs shall be made immediately; and
        5. Water conservation awareness shall be encouraged by the car wash operators by the placement of posters and literature where customers and employees will have access to them.
    B. Washing of vehicles at car dealerships is permitted, provided the following requirements are met:
        1. Except as set forth in 5. below, vehicles may only be washed just prior to delivery to customers or prior to placement in display showrooms;
        2. The amount of water used shall be the minimum necessary, and rinse time shall be no longer than 2 to 3 minutes;
        3. All hoses must not leak and shall be equipped with a hand-held nozzle that automatically shuts off when released;
        4. Wash and/or rinse water shall be recycled to the extent practicable; and
        5. New vehicles at a dealership may be washed in accordance with the conditions at 2. through 4. above once per month if necessary to preserve the vehicle's finish.
    C. Washing of boats at boat dealerships and marinas is permitted, provided the following requirements are met:
        1. Except as set forth in 5. and 6. below, boats may only be washed just prior to delivery to customers or prior to placement in display showrooms;
        2. The amount of water used shall be the minimum necessary, and rinse time shall be no longer than 2 to 3 minutes per area washed;
        3. All hoses must not leak and shall be equipped with a hand-held nozzle that automatically shuts off when released;
        4. Wash and/or rinse water shall be recycled to the extent practicable;
        5. Boat bottoms may be cleaned using a powerwasher in accordance with 2., 3., and 4. above.
        6. New boats at a dealership may be washed in accordance with the conditions at 2. through 4. above once per month if necessary to preserve the boat's finish;
        7. Boats at a marina may be washed to remove salt spray and for sanitary reasons;
        8. Marine engines may be flushed with fresh water; and
        9. Trailered boats must be washed at a commercial car wash.

3. The use of water for washing paved surfaces, such as streets, roads, sidewalks, driveways, garages, parking areas and patios is prohibited, except in the following cases:

A. Water use for roadway milling, and for the preparation of asphalt street or driveway re-coating and sealing, is permitted, provided the amount of water used is the minimum necessary;

B. Washing of paved surfaces at eating and drinking establishments is permitted for sanitation purposes, provided the amount of water used is the minimum necessary;

C. Use of water for municipal street sweeping is permitted, provided that only non-potable water is used, the amount of water used is the minimum necessary; and an appropriate sign is prominently displayed on the street sweeping vehicle, clearly indicating that the water used is non-potable water; and

D. Where the municipal or county health department deems that such washing is necessary to avert a threat to public health, and provided that the amount of water used is the minimum necessary.

4. The use of water for the flushing of sewers is prohibited, except in the following cases:

A. Where non-potable water is utilized, provided that the amount of water used is the minimum necessary, and provided that an appropriate sign is prominently displayed, clearly indicating that the water used is non-potable water; and

B. Where the municipal or county health department deems that flushing is necessary to avert a threat to public health.

5. The use of fire hydrants is prohibited, except in the following cases:

A. As necessary for fire fighting or fire protection purposes;

B. As necessary for testing or fire drills only if the testing or drill is deemed necessary in the interest of public safety by the municipal governing body and the applicable water purveyor, and is specifically approved by the municipal governing body and the applicable water purveyor;

C. Where a commercial enterprise has traditionally used water from the hydrant with prior written permission from the applicable water purveyor, provided that such use is necessary for the maintenance of the business. If a hydrant is used in this manner, water usage shall be metered; and

D. As necessary to maintain distribution system water quality.

6. The use of water for power washing of buildings, vehicles, pavement, or other surfaces is prohibited, except if the power washing is performed by a commercial enterprise engaged in power washing or where the power washing is required by law, as in the case of a dairy operator. A commercial enterprise performing power washing shall comply with all applicable limits relating to specific power washing activities. For example, a commercial power washing business engaged in washing cars at a car dealership shall comply with the limits for car washing at car dealerships set forth at 2B above.

7. The outdoor use of any water for ornamental or aesthetic purposes, including fountains, artificial waterfalls and reflecting pools, is prohibited, except if necessary to preserve or support wildlife, or for sanitary or structural purposes where draining is impractical.

8. The watering of lawns is prohibited except in the following cases:

A. Lawn watering is permitted in order to establish and maintain newly laid sod or newly seeded grass associated with new construction (this exemption shall not apply to seeding over existing lawn areas), within the following limits:

1. The amount of water used shall be the minimum necessary to establish and maintain the grass;
2. The watering is permitted for the first 45 days only, starting on the date of planting or of laying the sod. Documentation of the date of seed planting or sod laying shall be produced upon the request of the appropriate authorities;
3. The watering may occur only between 6:00 A.M. and 9:00 A.M. and between 5:00 P.M. and 8:00 P.M.; and
4. The watering shall not exceed 45 minutes per area watered on any one day, except that watering may be extended to one hour per area watered on the day that sod is laid. However, sod should not be laid when conditions are dry or are expected to become dry.

B. Commercial application of fertilizes, pesticide or herbicides that require water usage should cease. If such application preceded the drought restriction the following limits should be imposed.

1. The amount of water used shall be the minimum necessary to ensure the appropriate absorption of the fertilizer, pesticide or herbicide;
2. The watering is permitted for 2 days only, starting on the date that the chemical is applied. Documentation of the date of application shall be produced upon the request of the appropriate authorities;
3. The watering may occur only between 6:00 A.M. and 9:00 A.M. and between 5:00 P.M. and 8:00 P.M.;
4. The watering shall not exceed 45 minutes per area watered on any one day; and
5. This exemption shall only be valid for a single chemical application once every three months.

C. A commercial landscaper may water newly seeded or sodded grassed areas during normal seasonal working hours outside of the hours listed above, by a means designed and operated to assure effective conservation, provided that:

1. Watering is performed in accordance with the following practices. Water needs vary considerably among the turf grasses. Consider this when establishing a lawn, for it may significantly reduce irrigation needs during the summer.

   - Lightly water newly seeded or sprigged lawns at frequent intervals. Keep the seed or sprigs moist but not saturated during this initial growth period. This may require watering four or five times on hot, windy days.
   - The first 10 days to 2 weeks are especially critical. If young plants dry out, they may die. After a couple of weeks root system development should be well under way and the watering frequency can be slowly reduced. At about 1 month after seedling or sprigging the lawn it should be treated as an established lawn.
   - Water newly sodded lawns much like established lawns except more frequently. After the sod is applied, soak it with enough water so that the soil under the sod is wet to a depth of 2 to 3 inches. Each time the sod begins to dry out, re-soak it. Roots develop fairly rapidly and within 2 weeks or so the sod can be treated like an established lawn.
   - Ideally, a lawn should be watered just before it begins to wilt. Most grasses take on a dull purplish cast and leaf blades begin to fold or roll. Grass under drought stress also shows evidence of tracks after someone walks across the

lawn. These are the first signs of wilt. With careful observation and experience, one can determine the correct number of days between waterings.

- Early morning is considered the best time to water. The wind is usually calm and the temperature is low so less water is lost to evaporation. The worst time to water is late evening because the lawn stays wet all night, making it more susceptible to disease.
- When watering a lawn, wet the soil to a depth of 4 to 6 inches. Soil type affects the amount of water needed to wet soil to the desired depth.
- It takes about 1/2 inch of water to achieve the desired wetting depth if the soil is high in sand, and about 3/4 inch of water if the soil is a loam. For soils high in clay, an inch of water is usually necessary to wet the soil to the desired depth.
- If waterings are too light or too frequent the lawn may become weak and shallow-rooted, which in turn makes it more susceptible to stress injury.
- Use the following steps to determine the amount of water the sprinkler or sprinkler system puts out and check its distribution pattern at the same time.
- Determine the rate at which the sprinkler applies water to the lawn.
    - Set out three to five empty cans in a straight line going away from the sprinkler. Set the last can near the edge of the sprinkler's coverage.
    - Run the sprinkler for a set time such as 1/2 hour.
    - Measure the amount of water in each can.
    - Each can will contain a different amount of water. Usually, the can closest to the sprinkle will have the most water. The sprinkler pattern must overlap to get an even wetness of the soil. Use this information to find out how long it takes the sprinkler to apply 1 inch of water. For example, if the can contains about 1/4 inch of water after the sprinkler runs 1/2 hour, it would take 4 x 1/2 or 2 hours to apply 1 inch.
- Run the sprinkler or sprinkler system long enough to apply at least 1 inch of water or until runoff occurs. If runoff occurs first:
    - Stop sprinkler and note running time.
    - Allow water to soak in for 1/2 hour.
    - Start sprinkler.
    - If runoff occurs, repeat above steps until at least 1 inch of water has been applied and allowed to soak into the soil.
- Do not water again until the lawn has completely dried out. (This usually takes 5 or 6 days.)
    - Apply enough water to wet the soil to a depth of 4 to 6 inches.
    - Avoid frequent light applications of water.
    - Water in early daylight hours.
    - Select a turfgrass with a low water requirement.
    - Avoid using soluble nitrogen fertilizers. (They promote high growth rates, which in turn, increase water requirements of the plant.)
- Many soils will not take an inch of water before runoff occurs. If this is a problem with a lawn, try using a wetting agent, also called a surfactant, which reduces the surface tension of water making it "wetter." This "wetter" water runs into the soil at a faster rate and goes deeper than water in a non-treated soil.
- There are a number of wetting agents available; apply them according to directions on their labels. If this does not solve to runoff problem, it may be necessary to apply 1/2 inch one day and 2 inches the next day.

2. During the initial 45 day grow-in period a sign shall be displayed on the front lawn of the property. The sign shall be at least four feet wide by four feet high, with lettering large enough to be clearly visible from the nearest road. The sign shall read:

AUTHORIZED LIMITED WATERING OF NEW LAWN
Company Name
Address
Telephone Number

3. Documentation of the planting date shall be produced upon the request of the appropriate authorities.

D. Lawn watering is permitted if it is necessary for the revegetation of land in order to prevent soil erosion following earth-moving activities, provided that:

1. The amount of water used shall be the minimum necessary to accomplish the revegetation;
2. The activity is a construction-related project that complies with the *Connecticut Guidelines for Soil Erosion and Sediment Control, 2002*, of DEEP;
3. The watering is limited to the disturbed area; and
4. Documentation of the planting date shall be produced upon the request of the appropriate authorities.

E. Testing of a customer's newly installed or newly repaired sprinkler system by a commercial enterprise engaged in the installation or repair of lawn irrigation systems is permitted, within the following limits:

1. The amount of water used shall be the minimum necessary to test the sprinkler system,
2. The test shall be limited to a maximum of ten (10) minutes per sprinkler zone, and
3. During the period of the test a sign shall be displayed on the front lawn of the property. The sign shall be at least four feet wide by four feet high, with lettering large enough to be clearly visible from the nearest road. The sign shall read:

AUTHORIZED LIMITED TESTING OF SPRINKLER SYSTEM
Company Name
Address
Telephone Number

F. All lawn watering authorized herein shall use the minimum amount of water necessary.

G. All lawn watering authorized herein shall be performed in such a way that no puddling or runoff of water occurs.

H. All lawn watering authorized herein shall be performed in such a way that no paved surfaces are included in the area watered.

9. The watering of vegetation other than lawns is prohibited, except that the watering of trees, shrubs, and vegetable or flower gardens is permitted, using the minimum amount of water necessary, within the following limits:

A. The water shall be applied with one of the following:

1. A watering can; or
2. A hose that does not leak, and is equipped with a hand-held nozzle that automatically shuts off when released.

B. All watering authorized herein shall use the minimum amount of water necessary.

C. All watering authorized herein shall be performed in such a way that no puddling or runoff of water occurs; and

D. All watering authorized herein shall be performed in such a way that no paved surfaces are included in the area watered.

10. Watering of athletic playing fields, including those used by professional, college/university and youth league sports teams, as well as those owned or operated by public and private schools and parks, is permitted within the following limits:

A. Watering may occur only between 8:00 P.M. and 6:00 A.M.;

B. Watering may not exceed 45 minutes per area watered on any one day;

C. No grass or dirt areas outside of the essential playing area may be watered;

D. Under no circumstances shall a water cannon be used; and

E. Water conservation measures shall be instituted to the maximum extent practicable.

11. The watering of agricultural food crops, sod at commercial sod farms, and the watering of nursery stock at nurseries or retail outlets is permitted and is exempt from restrictions at this time, provided that all watering is done in accordance with best management practices (See Appendix D), and:

A. The use and diversion from all sources is less than 50,000 gallons per day and

B. Any use and diversion of 50,000 gallons per day or more authorized by CGS Section 22a-368 et al of the Water Diversion Policy Act.

12. The use of water for outdoor recreational purposes, not covered by paragraph 10, is prohibited, except that:

A. Golf courses covered by a valid DEEP Water Diversion Permit or Registration may use water within the following limits:

1. For tees, greens, and fairways, watering by sprinkler or other conserving mechanical means is permitted between 9:00 P.M. and 6:00 A.M, provided that the amount of water used is the minimum necessary for vegetation survival;
2. Hot spot watering (syringing) with a hand held hose is permitted between 11:00 A.M. and 4:00 P.M., provided that no area is watered for more than 10 minutes per day;
3. If seeding or resodding is necessary, newly seeded or sodded fairways may be watered between 9:00 A.M. and 4:00 P.M., provided that no area may be watered for more than 45 minutes on any given day;
4. Rough or other grass areas not addressed above may not be watered by any means;
5. Under no circumstances shall a water cannon be used;

6. Watering shall be done in accordance with Connecticut DEEP Best Management Practices for Golf Course, Special Water Report No. 37, Connecticut Institute of Water Resources, UCONN, and

7. Under no circumstances shall total monthly cumulative water use exceed 50% of either the monthly allocated water for that golf course, or the average utilization rate the past 5 years, whichever is lower, based on the permit or registration issued by the Department. Metered usage from all water sources shall be submitted to the Inland Water Resource Division of DEEP on a monthly basis, within 7 days of the end of the calendar month.

B. Golf courses that use treated wastewater only for irrigation are exempt from these restrictions.

C. Watering of clay tennis courts is permitted, using sprinklers or hand-held watering devices, provided that watering occurs for no more than 10 minutes per day, between 8:00 P.M. and 6:00 A.M. and/or between 12:00 noon and 3:00 P.M.

D. Filling of family, public and private swimming pools, including but not limited to outdoor hot tubs, spas, jacuzzis, is prohibited, except in the following cases:

1. Newly constructed or installed swimming pools may be filled once upon completion of construction, provided that, if the water is from the local municipal water purveyor, its use is approved by that purveyor;

2. A pool that was drained prior to the declaration of the emergency may be refilled once;

3. A pool that was not drained prior to the declaration of the emergency may not be drained and refilled unless the draining is necessary in order to make structural or other essential repairs, or draining is the only way that the pool can be adequately cleaned for healthy operations;

4. If a pool requires repairs in order to preserve the structural integrity of the pool and/or its supporting infrastructure, the pool may be drained for repair and subsequently refilled one time only. A person seeking to use water for this purpose shall notify the local police, fire, and public works departments prior to draining the swimming pool to provide notice to authorities and provide for an opportunity for reuse in accordance with 5. below;

5. Every reasonable effort shall be made to collect and re-use water drained from a pool, including use by the local fire department or public works department;

6. Topping of pools (that is, adding water to a partially filled pool) is permitted, provided that the amount of water used is the minimum necessary to maintain the integrity of the pool's circulation and filtration system(s); and pools should only be drained if absolutely necessary.

APPENDIX D: LINKS TO BEST MANAGEMENT PRACTICES

EPA Water Management Plans
https://www.epa.gov/greeningepa/water-management-plans-and-best-practices
Environmental Protection Agency
February, 2016

Golf Course Best Management Practices
http://www.ct.gov/deep/lib/deep/water_inland/diversions/golfcoursewaterusebmp.pdf
CT DEEP
July, 2006

Industrial Site Specific Conservation Best Management Practices
http://www.twdb.texas.gov/conservation/BMPs/Ind/doc/2.2.pdf
Texas Water Development Board
February, 2013

Irrigation Water Management (Code 449)
http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs144p2_051336.pdf
United States Department of Agriculture – Natural Resources Conservation Service
October, 2012

Landscape Irrigation Best Management Practices
http://www.irrigation.org/uploadedFiles/Standards/BMPDesign-Install-Manage.3-18-14(2).pdf
Irrigation Association & American Society of Irrigation Consultants
May, 2014

Nursery Production Best Management Practices
http://www.twdb.texas.gov/conservation/BMPs/Ag/doc/7.1.pdf
Texas Water Development Board
November, 2014

Turf and Landscape Irrigation Best Management Practices
http://www.irrigation.org/uploadedFiles/Resources/BMP_Revised_12-2010.pdf
Water Management Committee, Irrigation Association
December, 2010

Residential Best Management Practices
http://www.cuwcc.org/Portals/0/Document%20Library/Resources/Publications/BMP%20Guidebooks/Residential%20BMP%20Guidebook.pdf?timestamp=1416507756791
California Urban Water Conservation Council
December, 2008

APPENDIX E: GLOSSARY OF STATUTES AND REGULATIONS

The following legally-granted authorities enable state, regional, and local officials to effectively plan for, respond to, and mitigate the impacts of drought in Connecticut.

Statute or Regulation Citation	Section Title of Statute or Regulation	Summary of the Responsibility or Authority Granted
CGS Section 19a-131	Declaration of Public Health Emergency by Governor.	A public health emergency is defined as one in which a communicable disease or contamination is occurring or imminent.
CGS Section 19a-2a	Powers and Duties.	Establishes the powers and duties of the Commissioner of Public Health.
CGS Section 22a-358	Sale of water by public water system.	Provides the Commissioner of Public Health the authority to approve and mechanism to review for approval the sale of excess water from one public water system to another with stipulations including the restriction of water usage by the purchasing community water system in accordance with the emergency contingency provisions of the applicant water supply plan.
CGS Section 22a-378	Water supply emergency. Violation of water supply emergency order.	Provides the Commissioner of DEEP with the power to temporarily suspend water diversion permits and enforce such suspensions.
CGS Section 23-49a	Declaration of burning ban; special burning permit; penalty; exemptions.	Restricts open burning when forest fire danger is high or when a drought emergency is declared by the State Forest Fire Warden.
CGS Section 23-50	Closing of forests by the Governor.	Governor may close public woodlands and bushlands by reason of extreme drought or other hazardous conditions.
CGS Section 25-32	Department of Public Health jurisdiction over and duties concerning water supplies, water companies and operators of water treatment plants and water distribution systems.	DPH authority to oversee drinking water.
CGS Section 25-32a	"Consumer" and "water company" defined.	Defines the terms consumer and water company.
CGS Section 25-32b	Public drinking water supply emergency.	Provides the Commissioner of Public Health the authority to declare a public drinking water emergency and stipulate some actions that can be authorized.
CGS Section 25-32d(a) thru (e)	Water supply plans.	Sets which water companies are required to submit a water supply plan, to whom the plan is submitted, and the content of the plan including contingency procedures for the shortages of water.

CGS Section 25-32g	Orders to correct immediate threats to public water supplies.	Provides the Commissioner of Public Health the authority to issue an order to discontinue, abate, alleviate or correct any actions that threatens the quality or adequacy of any source of water supply.
CGS Section 25-32l	Inclusion of educational material on water conservation may be required in the water supply plans.	Provides the Commissioner of Public Health the authority to require in a water supply plan, the inclusion of a description of a water companies program to provide education material or information on water conservation to residential customers.
CGS Section 25-32o	Water Planning Council: Composition, duties, advisory group, report.	Establishment of the Water Planning Council including its composition and duties.
CGS Section 28-11	Taking of property during emergency.	During civil preparedness or public health emergency, Governor may in the event of shortage or disaster, take possession of certain private real or personal property.
CGS Section 28-16	Stockpile of Supplies.	DESPP Commissioner can stockpile supplies in anticipation of, among other things, "any disaster."
CGS Section 28-7f	Governor's Powers in Event of Serious Disaster or Emergency.	Can authorize temporary use of civil preparedness forces.
CGS Section 28-9	Governor's Powers for Civil Preparedness Emergency.	Ability to declare emergency; can modify or waive agency laws, regulations or rules; can order forces into action; can take such other steps as are reasonably necessary in the light of the emergency to protect the health, safety, and welfare of the people of the state.
CGS Section 3-1	Governor's General Powers and Duties.	General supreme executive power of the Governor.
CGS Section 42-231	Governor's Emergency Powers for Supply Emergencies.	In the event of a shortage, or threatened shortage, Governor may proclaim a supply emergency exists and impose price restrictions or rationing of the items in short supply.
RCSA Section 19-13-B102	Standards for Water Quality of Public Drinking Water.	Various drinking water requirements that may be utilized during a drought.
RCSA Section 19-13-B102(m)	Emergency powers.	Provides the Commissioner of Public Health the authority to declare a public drinking water emergency and stipulate some actions that can be authorized.
RCSA Section 19-13-B102(n)	Reservoir, ground water and water use monitoring.	Set requirements for public water systems to submit to the DPH reservoir and ground water readings, source information, and status at a frequency set forth by the Department.

RCSA Section 19-13-B102(o)	Supply capacity requirements.	Sets requirement for community water systems to maintain in excess of the demand of the system.
RCSA Section 19-13-B102(p)	Deliver capacity requirements.	Sets requirements for community water systems to maintain sufficient capacity for source of supply, treatment, pumping, transmission, and storage facilities in excess of the maximum flows.
RCSA Section 19-13-B46	Notification by water officials in water supply emergencies.	Notification by water officials to the Department in water supply emergencies.
RCSA Section 19-13-B51c	Interconnections.	Interconnections require prior approval from the Commissioner of Public Health.
RCSA Section 25-32d-1 thru d-6	Source Water Protection Measures.	Specifically, Section 25-32d-3(d)(10)(A)-(D) "Content of the Plan"- the content of the drought portion of a water system's water supply emergency contingency plan .
RCSA Section 25-33h-1	Coordinated water system plans.	Provides for the establishment of and responsibilities of Water Utility Coordinating Committee (WUCC).
RCSA Section 26-141a-1 — 26-141a-8	Minimum Stream Flow Standards.	Regulations concerning flow of water from impoundments or diversions.
RCSA Section 26-141b-1 — 26-141b-8	Stream Flow Standards and Regulations.	Regulations establishing flow regulations for all river and stream systems.

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Drought in Connecticut

Residents in drought:
1,004,000
1,379,000 more in abnormally dry areas.

This is:
28%
of the state's population,
39% more in abnormally dry areas.

Report Your Drought Impacts

| Last Month | Last Week | Current |

Enter a city or zip code to add ⊗

Add to Map   **Clear All**

The U.S. Drought Monitor (USDM) is a map that shows the location and intensity of drought across the country. The data is updated each Tuesday and released on Thursday. This map shows the drought conditions on August 04, 2020.

Learn more about the US Drought Monitor

Highlight Counties that Produce:
Select None

|			
D0 - Abnormally Dry • Short-term dryness slowing planting, growth of crops • Some lingering water deficits • Pastures or crops not fully recovered	37.4% of State	76.9% D0-D4	
D1 - Moderate Drought • Some damage to crops, pastures • Some water shortages developing • Voluntary water-use restrictions requested	39.5% of State	39.5% D1-D4	
D2 - Severe Drought • Crop or pasture loss likely • Water shortages common • Water restrictions imposed	0.0% of State	0.0% D2-D4	
D3 - Extreme Drought • Major crop/pasture losses • Widespread water shortages or restrictions	0.0% of State	0.0% D3-D4	
D4 - Exceptional Drought • Exceptional and widespread crop/pasture losses • Shortages of water creating water emergencies	0.0% of State		
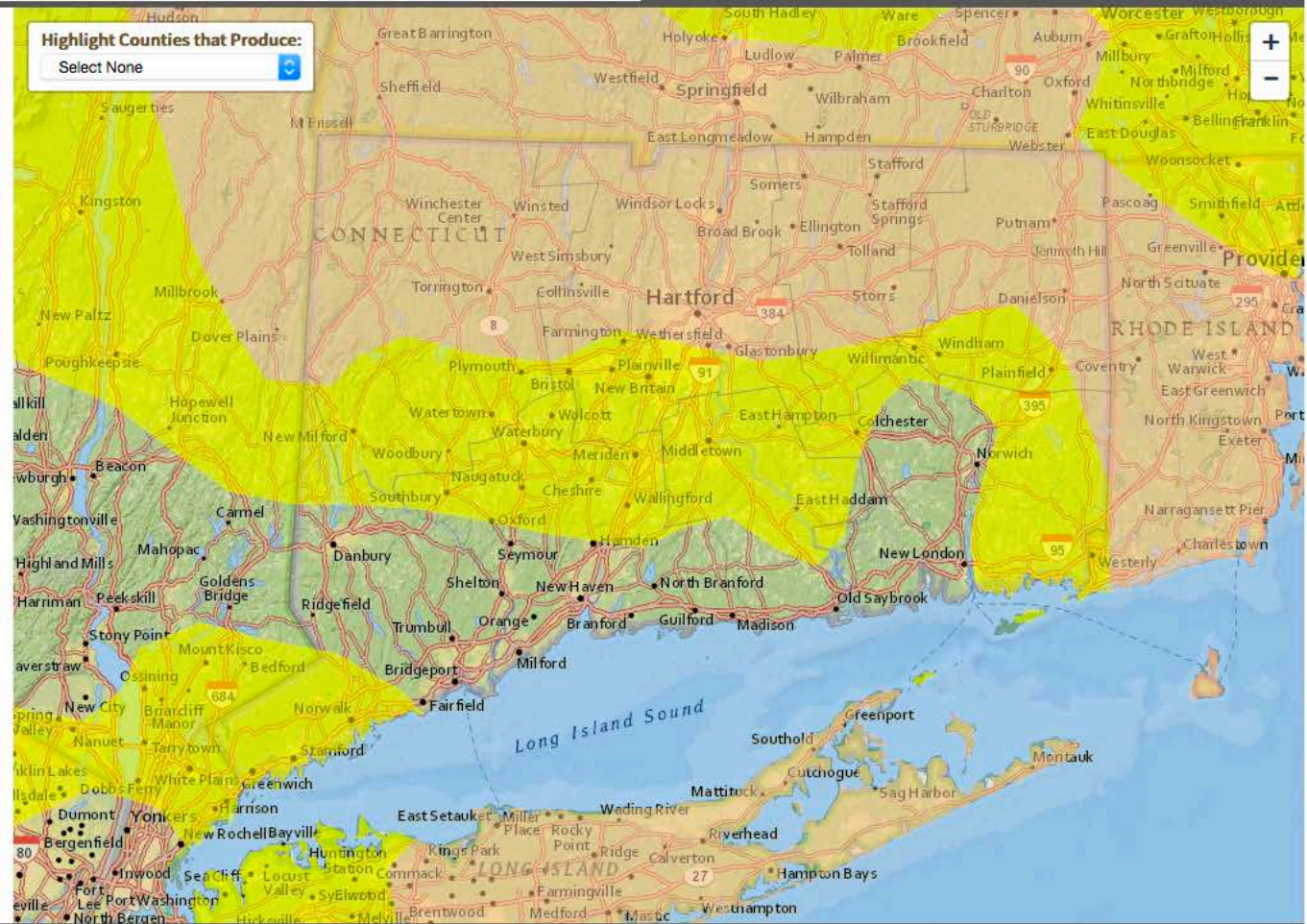
See supplemental map information

Drought in Connecticut from 2000 - 2020

The **U.S. Drought Monitor** started in 2000. Since 2000, the longest duration of drought (D1-D4) in Connecticut lasted 46 weeks beginning on June 21, 2016 and ending on May 2, 2017. The most intense period of drought occurred the week of November 15, 2016 where D3 affected 44.5% of Connecticut land.

- **Drought from 2000**
- **Drought from 1895**

Download graphic as:

| Image | JSON | XML | CSV |

Percent Area for Connecticut

Legend: D0, D1, D2, D3, D4
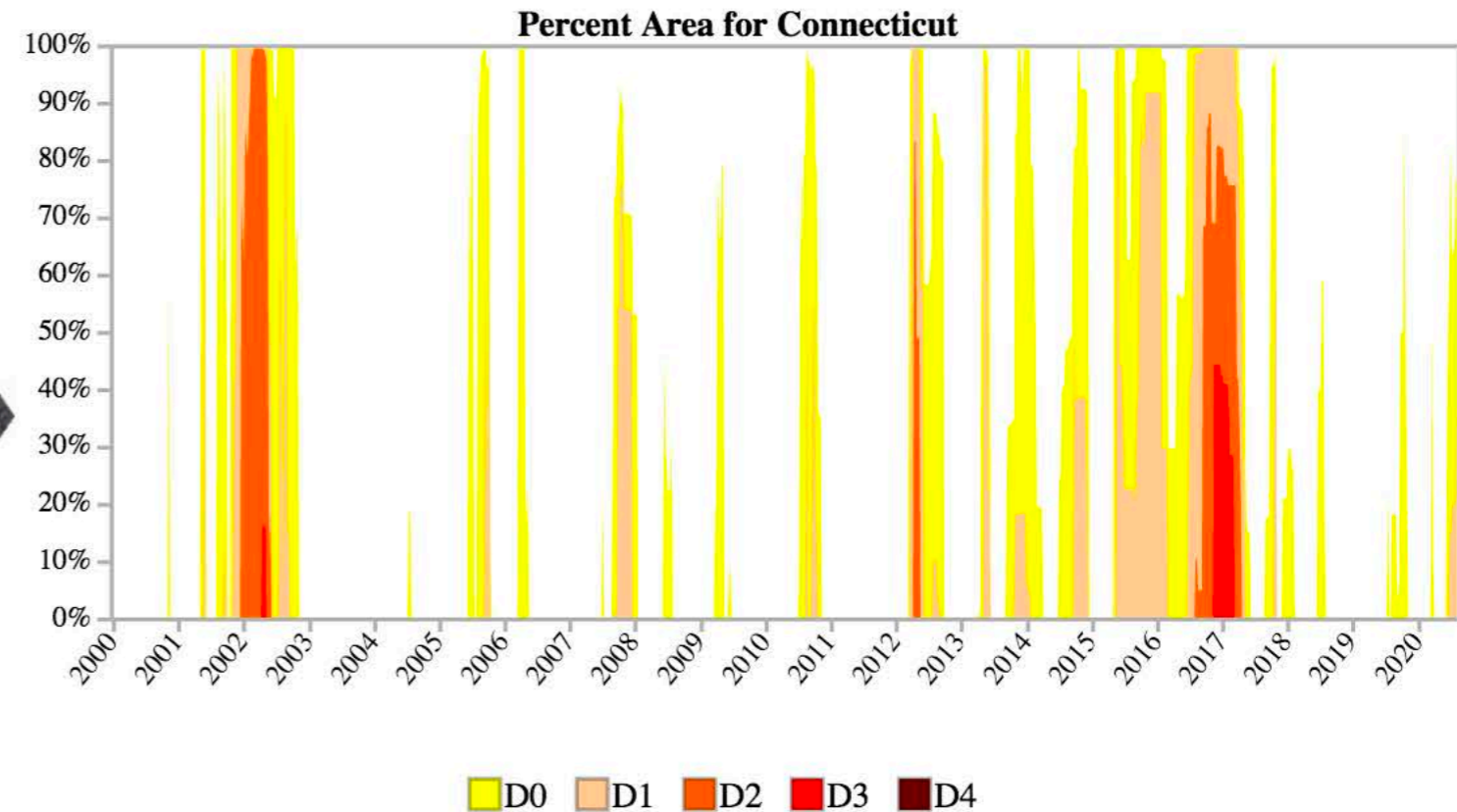
Exhibit EEEE

**TOXICOLOGICAL PROFILE FOR
BENZENE**

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

August 2007

DISCLAIMER

The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

UPDATE STATEMENT

A Toxicological Profile for Benzene, Draft for Public Comment was released in August 2005. This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary. For information regarding the update status of previously released profiles, contact ATSDR at:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine/Applied Toxicology Branch
1600 Clifton Road NE
Mailstop F-32
Atlanta, Georgia 30333

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FOREWORD

This toxicological profile is prepared in accordance with guidelines developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the hazardous substance described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a hazardous substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health are identified by ATSDR and EPA.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.
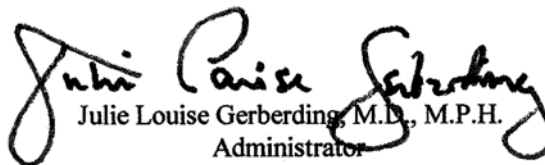
The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and is being made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

Howard Frumkin, M.D., Dr. P.H.
Director
National Center for Environmental Health/
Agency for Toxic Substances and
Disease Registry

Julie Louise Gerberding, M.D., M.P.H.
Administrator
Agency for Toxic Substances and
Disease Registry

The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on December 7, 2005 (70 FR 72840).   For prior versions of the list of substances, see *Federal Register* notices dated April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17, 1990 (55 FR 42067); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); February 28, 1994 (59 FR 9486); April 29, 1996 (61 FR 18744); November 17, 1997 (62 FR 61332); October 21, 1999 (64 FR 56792); October 25, 2001 (66 FR 54014); and November 7, 2003 (68 FR 63098). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Public Health Statement: The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

Chapter 2: Relevance to Public Health: The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

Chapter 3: Health Effects: Specific health effects of a given hazardous compound are reported by type of health effect (death, systemic, immunologic, reproductive), by route of exposure, and by length of exposure (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

Pediatrics: Four new sections have been added to each Toxicological Profile to address child health issues:
Section 1.6   **How Can (Chemical X) Affect Children?**
Section 1.7   **How Can Families Reduce the Risk of Exposure to (Chemical X)?**
Section 3.7   **Children's Susceptibility**
Section 6.6   **Exposures of Children**

Other Sections of Interest:
Section 3.8   **Biomarkers of Exposure and Effect**
Section 3.11   **Methods for Reducing Toxic Effects**

ATSDR Information Center
Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY) ***Fax:***   (770) 488-4178
E-mail: cdcinfo@cdc.gov   ***Internet***: http://www.atsdr.cdc.gov

The following additional material can be ordered through the ATSDR Information Center:

Case Studies in Environmental Medicine: Taking an Exposure History—The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental Hazards; Skin Lesions and Environmental Exposures; Cholinesterase-Inhibiting Pesticide Toxicity*; and numerous chemical-specific case studies.

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident. Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III— *Medical Management Guidelines for Acute Chemical Exposure*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs) provide answers to frequently asked questions about toxic substances.

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 200 Independence Avenue, SW, Washington, DC 20201 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19, 4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212.

Referrals

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266.

CONTRIBUTORS

CHEMICAL MANAGER(S)/AUTHOR(S):

Sharon Wilbur, M.A.
Sam Keith, M.S., C.H.P.
Obaid Faroon, Ph.D.
ATSDR, Division of Toxicology and Environmental Medicine, Atlanta, GA

David Wohlers, Ph.D.
Julie Stickney, Ph.D.
Sari Paikoff, Ph.D.
Gary Diamond, Ph.D
Antonio Quiñones-Rivera, Ph.D.
Syracuse Research Corporation, North Syracuse, NY

THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific Minimal Risk Levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

3. Data Needs Review. The Applied Toxicology Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

4. Green Border Review. Green Border review assures the consistency with ATSDR policy.

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PEER REVIEW

A peer review panel was assembled for benzene. The panel consisted of the following members:

1.   Dr. Jeffrey Fisher, Professor and Department Head, Department of Environmental Health
        Science, University of Georgia, Athens, Georgia;

2.   Dr. Tee Guidotti, Chair and Professor, Department of Occupational and Environmental Health,
        School of Public Health and Sciences, The George Washington University Medical Center,
        Washington, DC; and

3.   Dr. Rogene Henderson, Senior Scientist, Lovelace Respiratory Research Institute, Albuquerque,
        New Mexico.

These experts collectively have knowledge of benzene's physical and chemical properties, toxicokinetics,
key health end points, mechanisms of action, human and animal exposure, and quantification of risk to
humans. All reviewers were selected in conformity with the conditions for peer review specified in
Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as
amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer
reviewers' comments and determined which comments will be included in the profile. A listing of the
peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their
exclusion, exists as part of the administrative record for this compound.

The citation of the peer review panel should not be understood to imply its approval of the profile's final
content. The responsibility for the content of this profile lies with the ATSDR.

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CONTENTS

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LIST OF FIGURES

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LIST OF TABLES

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about benzene and the effects of exposure to it.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites are then placed on the National Priorities List (NPL) and are targeted for long-term federal clean-up activities. Benzene has been found in at least 1,000 of the 1,684 current or former NPL sites. Although the total number of NPL sites evaluated for this substance is not known, the possibility exists that the number of sites at which benzene is found may increase in the future as more sites are evaluated. This information is important because these sites may be sources of exposure and exposure to this substance may harm you.

When a substance is released either from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. Such a release does not always lead to exposure. You can be exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance, or by skin contact.

If you are exposed to benzene, many factors will determine whether you will be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with it. You must also consider any other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

1.1   WHAT IS BENZENE?

Benzene, also known as benzol, is a colorless liquid with a sweet odor. Benzene evaporates into air very quickly and dissolves slightly in water. Benzene is highly flammable. Most people can begin to smell benzene in air at approximately 60 parts of benzene per million parts of air (ppm) and recognize it as benzene at 100 ppm. Most people can begin to taste benzene in water at 0.5–4.5 ppm. One part per million is approximately equal to one drop in 40 gallons. Benzene is found in air, water, and soil. Benzene comes from both industrial and natural sources.

Industrial Sources and Uses. Benzene was first discovered and isolated from coal tar in the 1800s. Today, benzene is made mostly from petroleum. Because of its wide use, benzene ranks in the top 20 in production volume for chemicals produced in the United States. Various industries use benzene to make other chemicals, such as styrene (for Styrofoam® and other plastics), cumene (for various resins), and cyclohexane (for nylon and synthetic fibers). Benzene is also used in the manufacturing of some types of rubbers, lubricants, dyes, detergents, drugs, and pesticides.

Natural Sources. Natural sources of benzene, which include gas emissions from volcanoes and forest fires, also contribute to the presence of benzene in the environment. Benzene is also present in crude oil and gasoline and cigarette smoke. For more information on characteristics and uses of benzene, see Chapters 4 and 5.

1.2   WHAT HAPPENS TO BENZENE WHEN IT ENTERS THE ENVIRONMENT?

Benzene is commonly found in the environment. Industrial processes are the main sources of benzene in the environment. Benzene levels in the air can be elevated by emissions from burning coal and oil, benzene waste and storage operations, motor vehicle exhaust, and evaporation from gasoline service stations. Tobacco smoke is another source of benzene in air, particularly indoors. Industrial discharge, disposal of products containing benzene, and gasoline leaks from underground storage tanks release benzene into water and soil.

Benzene can pass into air from water and soil surfaces. Once in the air, benzene reacts with other chemicals and breaks down within a few days. Benzene in the air can also be deposited on the ground by rain or snow.

Benzene in water and soil breaks down more slowly. Benzene is slightly soluble in water and can pass through the soil into underground water. Benzene in the environment does not build up in plants or animals. For more information on what happens to benzene after it gets into the environment, see Chapters 5 and 6.

1.3   HOW MIGHT I BE EXPOSED TO BENZENE?

Everyone is exposed to a small amount of benzene every day. You are exposed to benzene in the outdoor environment, in the workplace, and in the home. Exposure of the general population to benzene mainly occurs through breathing air that contains benzene. The major sources of benzene exposure are tobacco smoke, automobile service stations, exhaust from motor vehicles, and industrial emissions. Vapors (or gases) from products that contain benzene, such as glues, paints, furniture wax, and detergents, can also be a source of exposure. Auto exhaust and industrial emissions account for about 20% of the total national exposure to benzene. About half of the exposure to benzene in the United States results from smoking tobacco or from exposure to tobacco smoke. The average smoker (32 cigarettes per day) takes in about 1.8 milligrams (mg) of benzene per day. This amount is about 10 times the average daily intake of benzene by nonsmokers.

Measured levels of benzene in outdoor air have ranged from 0.02 to 34 parts of benzene per billion parts of air (ppb) (1 ppb is 1,000 times less than 1 ppm). People living in cities or industrial areas are generally exposed to higher levels of benzene in air than those living in rural areas. Benzene levels in the home are usually higher than outdoor levels. People may be exposed to higher levels of benzene in air by living near hazardous waste sites, petroleum refining operations, petrochemical manufacturing sites, or gas stations.

For most people, the level of exposure to benzene through food, beverages, or drinking water is not as high as through air. Drinking water typically contains less than 0.1 ppb benzene. Benzene has been detected in some bottled water, liquor, and food. Leakage from underground gasoline storage tanks or from landfills and hazardous waste sites that contain benzene can result in benzene contamination of well water. People with benzene-contaminated tap water can be exposed from drinking the water or eating foods prepared with the water. In addition, exposure can result from breathing in benzene while showering, bathing, or cooking with contaminated water.

Individuals employed in industries that make or use benzene may be exposed to the highest levels of benzene. As many as 238,000 people may be occupationally exposed to benzene in the United States. These industries include benzene production (petrochemicals, petroleum refining, and coke and coal chemical manufacturing), rubber tire manufacturing, and storage or transport of benzene and petroleum products containing benzene. Other workers who may be exposed to benzene include coke oven workers in the steel industry, printers, rubber workers, shoe makers, laboratory technicians, firefighters, and gas station employees. For more information on how you might be exposed to benzene, see Chapter 6.

1.4   HOW CAN BENZENE ENTER AND LEAVE MY BODY?

Benzene can enter your body through your lungs, gastrointestinal tract, and across your skin. When you are exposed to high levels of benzene in air, about half of the benzene you breathe in passes through the lining of your lungs and enters your bloodstream. When you are exposed to benzene in food or drink, most of the benzene you take in by mouth passes through the lining of your gastrointestinal tract and enters your bloodstream. A small amount will enter your body by passing through your skin and into your bloodstream during skin contact with benzene or benzene-containing products. Once in the bloodstream, benzene travels throughout your body and can be temporarily stored in the bone marrow and fat. Benzene is converted to products, called metabolites, in the liver and bone marrow. Some of the harmful effects of benzene exposure are caused by these metabolites. Most of the metabolites of benzene leave the body in the urine within 48 hours after exposure. For more information on how benzene can enter and leave your body, see Chapter 3.

1.5   HOW CAN BENZENE AFFECT MY HEALTH?

Scientists use many tests to protect the public from harmful effects of toxic chemicals and to find ways for treating persons who have been harmed.

One way to learn whether a chemical will harm people is to determine how the body absorbs, uses, and releases the chemical. For some chemicals, animal testing may be necessary. Animal testing may also help identify health effects such as cancer or birth defects. Without laboratory

1. PUBLIC HEALTH STATEMENT

animals, scientists would lose a basic method for getting information needed to make wise decisions that protect public health. Scientists have the responsibility to treat research animals with care and compassion. Scientists must comply with strict animal care guidelines because laws today protect the welfare of research animals.

After exposure to benzene, several factors determine whether harmful health effects will occur, as well as the type and severity of such health effects. These factors include the amount of benzene to which you are exposed and the length of time of the exposure. Most information on effects of long-term exposure to benzene are from studies of workers employed in industries that make or use benzene. These workers were exposed to levels of benzene in air far greater than the levels normally encountered by the general population. Current levels of benzene in workplace air are much lower than in the past. Because of this reduction and the availability of protective equipment such as respirators, fewer workers have symptoms of benzene poisoning.

Brief exposure (5–10 minutes) to very high levels of benzene in air (10,000–20,000 ppm) can result in death. Lower levels (700–3,000 ppm) can cause drowsiness, dizziness, rapid heart rate, headaches, tremors, confusion, and unconsciousness. In most cases, people will stop feeling these effects when they are no longer exposed and begin to breathe fresh air.

Eating foods or drinking liquids containing high levels of benzene can cause vomiting, irritation of the stomach, dizziness, sleepiness, convulsions, rapid heart rate, coma, and death. The health effects that may result from eating foods or drinking liquids containing lower levels of benzene are not known. If you spill benzene on your skin, it may cause redness and sores. Benzene in your eyes may cause general irritation and damage to your cornea.

Benzene causes problems in the blood. People who breathe benzene for long periods may experience harmful effects in the tissues that form blood cells, especially the bone marrow. These effects can disrupt normal blood production and cause a decrease in important blood components. A decrease in red blood cells can lead to anemia. Reduction in other components in the blood can cause excessive bleeding. Blood production may return to normal after exposure to benzene stops. Excessive exposure to benzene can be harmful to the immune

system, increasing the chance for infection and perhaps lowering the body's defense against
cancer.

Long-term exposure to benzene can cause cancer of the blood-forming organs. This condition is
called leukemia. Exposure to benzene has been associated with development of a particular type
of leukemia called acute myeloid leukemia (AML). The Department of Health and Human
Services has determined that benzene is a known carcinogen (can cause cancer). Both the
International Agency for Cancer Research and the EPA have determined that benzene is
carcinogenic to humans.

Exposure to benzene may be harmful to the reproductive organs. Some women workers who
breathed high levels of benzene for many months had irregular menstrual periods. When
examined, these women showed a decrease in the size of their ovaries. However, exact exposure
levels were unknown, and the studies of these women did not prove that benzene caused these
effects. It is not known what effects exposure to benzene might have on the developing fetus in
pregnant women or on fertility in men. Studies with pregnant animals show that breathing
benzene has harmful effects on the developing fetus. These effects include low birth weight,
delayed bone formation, and bone marrow damage.

We do not know what human health effects might occur after long-term exposure to food and
water contaminated with benzene. In animals, exposure to food or water contaminated with
benzene can damage the blood and the immune system and can cause cancer. See Chapters 2
and 3 for more information on the health effects resulting from benzene exposure.

1.6   HOW CAN BENZENE AFFECT CHILDREN?

This section discusses potential health effects in humans from exposures during the period from
conception to maturity at 18 years of age.

Children can be affected by benzene exposure in the same ways as adults. Benzene can pass from the mother's blood to a fetus. It is not known if children are more susceptible to benzene poisoning than adults.

1.7   HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO BENZENE?

If your doctor finds that you have been exposed to substantial amounts of benzene, ask whether your children might also have been exposed. Your doctor might need to ask your state health department to investigate.

Gasoline and cigarette smoke are two main sources of human exposure to benzene. Benzene exposure can be reduced by limiting contact with these sources. People are exposed to benzene from both active and passive second-hand smoke. Average smokers take in about 10 times more benzene than nonsmokers each day. Families are encouraged not to smoke in their house, in enclosed environments, or near their children.

Benzene is a major component of gasoline and used in many manufacturing processes. Increased levels of benzene can be found at fueling stations, and in air emissions from manufacturing plants and hazardous waste sites. Living near gasoline fueling stations or hazardous waste sites may increase exposure to benzene. People are advised not to have their families play near fueling stations, manufacturing plants, or hazardous waste sites.

1.8   IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO BENZENE?

Several tests can show whether you have been exposed to benzene. Some of these tests may be available at your doctor's office. All of these tests are limited in what they can tell you. The test for measuring benzene in your breath must be done shortly after exposure. This test is not very helpful for detecting very low levels of benzene in your body. Benzene can be measured in your blood. However, because benzene rapidly disappears in the blood, measurements may be useful only for recent exposures.

In the body, benzene is converted to products called metabolites. Certain metabolites of benzene, such as phenol, muconic acid, and *S*-phenylmercapturic acid can be measured in the urine. The amount of phenol in urine has been used to check for benzene exposure in workers. The test is useful only when you are exposed to benzene in air at levels of 10 ppm or greater. However, this test must also be done shortly after exposure, and it is not a reliable indicator of how much benzene you have been exposed to, because phenol is present in the urine from other sources (diet, environment). Measurements of muconic acid or *S*-phenylmercapturic acid in the urine are more sensitive and reliable indicators of benzene exposure. The measurement of benzene in blood or of metabolites in urine cannot be used for making predictions about whether you will experience any harmful health effects. Blood counts of all components of the blood and examination of bone marrow are used to determine benzene exposure and its health effects.

For people exposed to relatively high levels of benzene, complete blood analyses can be used to monitor possible changes related to exposure. However, blood analyses are not useful when exposure levels are low. For more information on tests for benzene exposure, see Chapters 3 and 7.

1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The federal government develops regulations and recommendations to protect public health. Regulations *can* be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. Recommendations provide valuable guidelines to protect public health, but *cannot* be enforced by law. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Regulations and recommendations can be expressed as "not-to-exceed" levels, that is, levels of a toxic substance in air, water, soil, or food that do not exceed a critical value that is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans.

Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that provides it. Some regulations and recommendations for benzene include the following:

EPA has set 5 ppb as the maximum permissible level of benzene in drinking water. EPA has set a goal of 0 ppb for benzene in drinking water and in water such as rivers and lakes because benzene can cause leukemia. EPA estimates that 10 ppb benzene in drinking water that is consumed regularly or exposure to 0.4 ppb in air over a lifetime could cause a risk of one additional cancer case for every 100,000 exposed persons. EPA recommends 200 ppb as the maximum permissible level of benzene in water for short-term exposures (10 days) for children.

EPA requires that the National Response Center be notified following a discharge or spill into the environment of 10 pounds or more of benzene.

OSHA regulates levels of benzene in the workplace. The maximum allowable amount of benzene in workroom air during an 8-hour workday, 40-hour workweek is 1 ppm. Because benzene can cause cancer, NIOSH recommends that all workers wear special breathing equipment when they are likely to be exposed to benzene at levels exceeding the recommended (8-hour) exposure limit of 0.1 ppm. For more information on federal regulations, see Chapter 8.

1.10   WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department, or contact ATSDR at the address and phone number below.

ATSDR can also tell you the location of occupational and environmental health clinics. These clinics specialize in recognizing, evaluating, and treating illnesses that result from exposure to hazardous substances.

Toxicological profiles are also available on-line at www.atsdr.cdc.gov and on CD-ROM. You may request a copy of the ATSDR ToxProfiles^{TM} CD-ROM by calling the toll-free information and technical assistance number at 1-800-CDCINFO (1-800-232-4636), by e-mail at cdcinfo@cdc.gov, or by writing to:

> Agency for Toxic Substances and Disease Registry
> Division of Toxicology and Environmental Medicine
> 1600 Clifton Road NE
> Mailstop F-32
> Atlanta, GA 30333
> Fax: 1-770-488-4178

Organizations for-profit may request copies of final Toxicological Profiles from the following:

> National Technical Information Service (NTIS)
> 5285 Port Royal Road
> Springfield, VA 22161
> Phone: 1-800-553-6847 or 1-703-605-6000
> Web site: http://www.ntis.gov/

2. RELEVANCE TO PUBLIC HEALTH

2.1   BACKGROUND AND ENVIRONMENTAL EXPOSURES TO BENZENE IN THE UNITED STATES

Benzene is widely distributed in the environment. The exposure scenario of most concern to the general public is low-level inhalation over long periods. This is because the general population is exposed to benzene mainly through inhalation of contaminated air, particularly in areas of heavy traffic and around gas stations, and through inhalation of tobacco smoke from both active and passive smoking. Smoking has been identified as the single most important source of benzene exposure for the estimated 40 million U.S. smokers. Smoking accounts for approximately half of the total benzene exposure of the general population. Individuals employed in industries that make or use benzene, or products containing benzene, are probably exposed to the highest concentrations of atmospheric benzene. In addition, benzene is a common combustion product, providing high inhalation exposure potential for firefighters. Of the general population, those residing around certain chemical manufacturing sites or living near waste sites containing benzene or near leaking gasoline tanks may be exposed to concentrations of benzene that are higher than background air concentrations. In private homes, benzene levels in the air have been shown to be higher in homes with attached garages, or where the inhabitants smoke inside the house.

Although low levels of benzene have been detected in certain foods, beverages, and tap water, these do not constitute major sources of exposure for most people. However, leakage from underground gasoline storage tanks and seepage from landfills and hazardous waste sites have resulted in significant benzene contamination of well water. People with contaminated tap water can be exposed from drinking the water or eating foods prepared with it. In addition, exposure can also occur via inhalation during showering, bathing, or cooking with contaminated tap water. Showering and bathing with benzene-contaminated water can also contribute significantly to dermal exposure.

The Benzene Subregistry Baseline Technical Report of the National Exposure Registry contains information on 1,143 persons who had documented exposure to benzene in their drinking water and were exposed for at least 30 days. No causal relationship has been proposed for health conditions identified in the base subregistry or continued follow-up of the population.

2.2 SUMMARY OF HEALTH EFFECTS

The carcinogenicity of benzene is well documented in exposed workers. Epidemiological studies and case reports provide clear evidence of a causal relationship between occupational exposure to benzene and benzene-containing solvents and the occurrence of acute myelogenous leukemia (AML). The epidemiological studies are generally limited by confounding chemical exposures and methodological problems, including inadequate or lack of exposure monitoring and low statistical power, but a consistent excess risk of leukemia across studies indicates that benzene is the causal factor.

In vivo and *in vitro* data from both humans and animals indicate that benzene and/or its metabolites are genotoxic. Chromosomal aberrations (hypo- and hyperdiploidy, deletions, breaks, and gaps) in peripheral lymphocytes and bone marrow cells are the predominant effects seen in humans.

Damage to both the humoral and cellular components of the immune system has been known to occur in humans following inhalation exposure. This is manifested by decreased levels of antibodies and decreased levels of leukocytes in workers. Animal data support these findings.

The most characteristic systemic effect resulting from intermediate and chronic benzene exposure is arrested development of blood cells. Early biomarkers of exposure to relatively low levels of benzene include depressed numbers of one or more of the circulating blood cell types. A common clinical finding in benzene hematotoxicity is cytopenia, which is a decrease in various cellular elements of the circulating blood manifested as anemia, leukopenia, or thrombocytopenia in humans and in animals. Benzene-associated cytopenias vary and may involve a reduction in one (unicellular cytopenias) to all three (pancytopenia) cellular elements of the blood.

Benzene also causes a life-threatening disorder called aplastic anemia in humans and animals. This disorder is characterized by reduction of all cellular elements in the peripheral blood and in bone marrow, leading to fibrosis, an irreversible replacement of bone marrow. Benzene has also been associated with acute non-lymphocytic leukemia in humans, and aplastic anemia may be an early indicator of developing acute non-lymphocytic leukemia in some cases.

Limited information is available on other systemic effects reported in humans and is associated with high–level benzene exposure. Respiratory effects have been noted after acute exposure of humans to benzene vapors. Cardiovascular effects, particularly ventricular fibrillation, have been suggested as the

cause of death in fatal exposures to benzene vapor. Gastrointestinal effects have been noted in humans after fatal inhalation exposure (congestive gastritis), and ingestion (toxic gastritis and pyloric stenosis), of benzene. Myelofibrosis (a form of aplastic anemia) was reported by a gasoline station attendant who had been exposed to benzene by inhalation, and probably also through dermal contact. Myalgia was also reported in steel plant workers exposed to benzene vapors. Reports of renal effects in humans after benzene exposure consist of kidney congestion after fatal inhalation exposure. Dermal and ocular effects including skin irritation and burns, and eye irritation have been reported after exposure to benzene vapors. Swelling and edema have been reported to occur in a human who swallowed benzene. Studies in animals show systemic effects after inhalation exposure, including cardiovascular effects. Oral administration of benzene to animals has yielded information concerning hepatic effects. A study conducted in rabbits lends support to the finding that benzene is irritating and damaging to the skin and also shows that it is irritating and damaging to the eyes following dermal or ocular application.

Neurological effects have been commonly reported in humans following high-level exposure to benzene. Fatal inhalation exposure has been associated with vascular congestion in the brain. Chronic inhalation exposure has been associated with distal neuropathy, difficulty in sleeping, and memory loss. Oral exposure results in symptoms similar to inhalation exposure. Studies in animals suggest that inhalation exposure to benzene results in depressed electrical activity in the brain, loss of involuntary reflexes and narcosis, decrease in hind-limb grip strength and tremors, and narcosis, among other symptoms. Oral exposure to benzene has not been shown to cause significant changes in behavior. No neurological effects have been reported after dermal exposure to liquid benzene in either humans or animals.

Acute inhalation and oral exposures of humans to high concentrations of benzene have caused death. These exposures are also associated with central nervous system depression. Chronic low-level exposures have been associated with peripheral nervous system effects. Abnormalities in motor conduction velocity were noted in four of six pancytopenic individuals occupationally exposed to adhesives containing benzene.

Evidence of an effect of benzene exposure on human reproduction is not sufficient to demonstrate a causal association. Some animal studies provide limited evidence that benzene affects reproductive organs following inhalation exposure. Results from studies of benzene administered orally to rats and mice indicate no adverse effect on male or female reproductive organs at 17 weeks, but at 2 years, endometrial polyps were observed in female rats, preputial gland lesions were observed in male mice, and ovarian lesions were observed in female mice. Results are conflicting or inconclusive as to whether

inhalation of benzene vapors reduces the number of live fetuses and/or the incidences of pregnancy. Other studies are negative for effects on reproductive competence.

Epidemiological studies implicating benzene as a developmental toxicant have many limitations, and thus, it is not possible to assess the effect of benzene on the human fetus. Results of inhalation studies conducted in animals are fairly consistent across species and demonstrate that, at levels >47 ppm, benzene is fetotoxic as evidenced by decreased fetal weight and/or minor skeletal variants. Benzene has also been shown to reduce pup body weight in mice. A persistent decrease in the number of erythroid precursors was found in mice exposed *in utero*. Benzene has not been shown to be teratogenic, but has been shown to be fetotoxic in animals at high concentrations that are maternally toxic.

Cancer.   The strongest evidence for the leukemogenic potential of benzene comes from series of cohort mortality studies on workers exposed to benzene in Ohio (the Pliofilm study) and China (the NCI/CAPM study). The Pliofilm study investigated workers exposed to benzene in three rubber hydrochloride ('Pliofilm') manufacturing plants. Mortality from all leukemias was increased but declined after additional years of follow-up, suggesting that the excess risk diminished with time since exposure. Exposures in the most recent 10 years were most strongly associated with leukemia risk, and there was no significant relation between leukemia death and benzene exposures received more than 20 years previously. AML accounted for most of the increased leukemia, and the risk of AML increased with increasing cumulative exposure above 200 ppm-years.

The NCI/CAPM study, a collaboration between the National Cancer Institute and the Chinese Academy of Preventive Medicine, evaluated lymphohematopoietic malignancies and other hematologic disorders in 74,828 benzene-exposed workers employed in 672 factories in 12 cities in China. Findings included increased risks for all leukemias, acute nonlymphocytic leukemia (ANLL), and combined ANLL and precursor myelodysplastic syndromes. These risks were increased at average exposure levels of 10–24 ppm and cumulative exposure levels of 40–99 ppm-years, and tended to increase with increasing average and cumulative levels of exposure.

The results of the Pliofilm and NCI/CAPM studies are consistent with epidemiologic studies and case reports showing increased incidences of leukemia in shoe factory and rotogravure plant workers exposed to high benzene levels during its use as a solvent. No significant increases in leukemia or other lymphohematopoietic malignancies were found in chemical industry workers or petroleum industry workers exposed to lower levels of benzene.

Possible associations between occupational exposure to benzene and non-Hodgkin's lymphoma (NHL) and multiple myeloma have been suggested. The risk for mortality from NHL increased with increasing level and duration of benzene exposure the NCI/CAPM study. The significance of this finding is unclear because NHL mortality was not significantly elevated in the cohort overall, concerns regarding the adequacy of the data have been raised, and increases in NHL were not found in other cohort mortality studies or in case-control studies of benzene-exposed workers.

The risk of mortality from multiple myeloma was increased in one of the early assessments of the Pliofilm cohort. The implication of this finding is unclear because the risk declined to non-significant levels in subsequent follow-up studies, and was not supported by the findings of other cohort mortality studies. Additionally, population-based and hospital-based case-control studies indicate that benzene exposure is not likely to be causally related to the risk of multiple myeloma. A meta-analysis of case-control studies found no significant association between occupational exposure to benzene and benzene-containing products and risk of multiple myeloma from sources categorized as benzene and/or organic solvents, petroleum, or petroleum products.

Animal studies provide supporting evidence for the carcinogenicity of benzene. Benzene has been shown to be a multiple site carcinogen in rats and mice following inhalation and oral exposure. Tumors that were increased in rats that were exposed to 200 or 300 ppm benzene by inhalation for 4–7 hours/day, 5 days/week for up to 104 weeks included carcinomas of the Zymbal gland and oral cavity. Mice that were exposed to 100 or 300 ppm benzene for 6 hours/day, 5 days/week for 16 weeks and observed for 18 months or life developed a variety of tumors, including thymic lymphomas, myelogenous leukemias, and Zymbal gland, ovarian, and lung tumors.

In oral bioassays conducted by the National Toxicology Program, benzene was administered to rats and mice by gavage at dose levels of 25–200 mg/kg/day on 5 days/week for 103 weeks. Tumors that were induced in the rats included Zymbal gland carcinomas and squamous cell papillomas and carcinomas of the oral cavity and skin. In the mice, benzene caused tumors that included malignant lymphomas, Zymbal gland carcinomas, lung alveolar/bronchiolar adenomas and carcinomas, Harderian gland adenomas, preputial gland squamous cell carcinomas, and mammary gland carcinomas. Similar effects occurred in rats exposed to 50–500 mg/kg/day benzene by gavage on 4–5 days/week for up to 104 weeks and observed for life; induced tumors included carcinomas of the Zymbal gland, oral cavity, forestomach,

nasal cavity, and skin. Mice that were similarly exposed to 500 mg/kg/day for 52 or 78 weeks developed
Zymbal gland carcinomas, mammary carcinomas, and lung adenomas.

Application of benzene to the skin of animals has not produced evidence of carcinogenicity, although
most of the dermal studies were inadequate for cancer evaluation. Many dermal carcinogenicity studies
of other chemicals used benzene as a vehicle and treated large numbers of control animals (mice) with
benzene alone. None of these studies indicated that benzene induced skin tumors; however, all possible
tumor sites usually were not examined.

EPA, IARC, and the Department of Health and Human Services have concluded that benzene is a human
carcinogen. The Department of Health and Human Services determined that benzene is a known
carcinogen based on human evidence showing a causal relationship between exposure to benzene and
cancer. Two studies classified benzene in Group 1 (carcinogenic to humans) based on sufficient evidence
in both humans and animals. EPA classified benzene in Category A (known human carcinogen) based on
convincing evidence in humans supported by evidence from animal studies. Under EPA's most recent
guidelines for carcinogen risk assessment, benzene is characterized as a known human carcinogen for all
routes of exposure based on convincing human evidence as well as supporting evidence from animal
studies. Based on human leukemia data, EPA derived a range of inhalation unit risk values of 2.2×10^{-6}–
7.8×10^{-6} $(\mu g/m^3)^{-1}$ for benzene. For risks ranging from 1×10^{-4} to 1×10^{-7}, the corresponding air
concentrations range from 13.0–45.0 $\mu g/m^3$ (4–14 ppb) to 0.013–0.045 $\mu g/m^3$ (0.004–0.014 ppb),
respectively.

The consensus conclusion that benzene is a human carcinogen is based on sufficient inhalation data in
humans supported by animal evidence, including the oral studies in animals. The human cancer induced
by inhalation exposure to benzene is predominantly acute nonlymphocytic (myelocytic) leukemia,
whereas benzene is a multiple site carcinogen in animals by both the inhalation and oral routes. Due to
the lack of oral carcinogenicity data in humans, as well as the lack of a well-demonstrated and
reproducible animal model for leukemia from benzene exposure, EPA extrapolated an oral slope factor
from the inhalation unit risk range. The oral slope factor ranges from 1.5×10^{-2} to 5.5×10^{-2} $(mg/kg/day)^{-1}$,
and for cancer risks from 1×10^{-4} to 1×10^{-7}, the corresponding dose levels are 6.7×10^{-3}–1.8×10^{-3} to
6.7×10^{-6}–1.8×10^{-6} mg/kg/day, respectively.

Hematological Effects.   Both human and animal studies have shown that benzene exerts toxic effects
on various parts of the hematological system. All of the major types of blood cells are susceptible

(erythrocytes, leukocytes, and platelets). In the less severe cases of toxicity, specific deficiencies occur in individual types of blood elements. A more severe effect occurs when there is hypoplasia of the bone marrow, or hypercellular marrow exhibiting ineffective hematopoiesis so that all types of blood cells are found in reduced numbers. This is known as pancytopenia. A biphasic response (i.e., a hyperplastic effect in addition to destruction of the bone marrow cells) has been observed. Severe damage to the bone marrow involving cellular aplasia is known as aplastic anemia and can occur with prolonged exposure to benzene. This condition can lead to leukemia.

Numerous earlier studies of benzene-exposed workers demonstrated that chronic exposure to benzene air concentrations of 10 ppm or more resulted in adverse hematological effects, which increased in severity with increasing benzene exposure levels. Animal studies support the findings in humans. Significantly reduced counts for all three blood factors (white blood cells [WBCs], red blood cells [RBCs], and platelets); and other evidence of adverse effects on blood-forming units (reduced bone marrow cellularity, bone marrow hyperplasia and hypoplasia, granulocytic hyperplasia, decreased numbers of colony-forming granulopoietic stem cells and erythroid progenitor cells, damaged erythrocytes and erythroblast-forming cells) have been observed in animals at benzene concentrations in the range of 10–300 ppm and above.

Several more recent epidemiological studies have demonstrated hematological effects (including significant reductions in WBC, RBC, and platelet counts) in workers chronically exposed to benzene levels below 10 ppm, and even as low as 1 ppm or less. Results of one of these studies served as the basis for a chronic-duration inhalation MRL for benzene. Other reports demonstrated the lack of clinical signs of hematotoxicity following long-term, low-level occupational exposure to benzene levels below approximately 0.5 ppm (8-hour time-weighted average [TWA]). These investigators utilized a defined range of clinically normal hematological values and compared the prevalence of abnormal results between benzene-exposed workers and unexposed controls. The normal range for certain hematological parameters is necessarily broad due to large interindividual differences in clinical status. Restricting the comparison of benzene-exposed and nonexposed populations to only those values considered clinically abnormal or adverse may reduce the sensitivity of a particular study to detect meaningful changes at the population level.

Only one study was found that described hematological effects in humans after oral exposure to benzene. No reports describing hematological effects in humans following direct dermal exposure to benzene were found. However, intermediate- and chronic-duration animal studies show that loss of blood elements occurs in animals exposed to benzene in drinking water or by gavage at doses as low as 8–25 mg/kg/day.

Based on information found in the literature, it is reasonable to expect that adverse hematological effects
might occur in humans after inhalation, oral, or dermal exposure, since absorption of benzene through any
route of exposure would increase the risk of damage to blood elements. Studies show that the
hematological system is susceptible to chronic exposure at low levels, so people living in and around
hazardous waste sites that may be exposed to contaminated air, drinking water, soil, or food may be at an
increased risk for adverse hematological effects. Deficiencies in various types of blood cells lead to other
disorders, such as hemorrhagic conditions from a lack of platelets, susceptibility to infection from the lack
of leukocytes, and increased cardiac output from the lack of erythrocytes.

Immunological and Lymphoreticular Effects.   Benzene has been shown to have adverse
immunological effects in humans following inhalation exposure for intermediate and chronic durations.
Adverse immunological effects in animals occur following both inhalation and oral exposure for acute,
intermediate, and chronic durations. The effects include damage to both humoral (antibody) and cellular
(leukocyte) responses. Human studies of intermediate and chronic duration have shown that benzene
causes decreases in the levels of circulating leukocytes in workers at low levels (30 ppm) of exposure and
decreases in levels of circulating antibodies in workers exposed to benzene at 3–7 ppm. Other studies
have shown decreases in human lymphocytes and other blood elements after exposure; these effects have
been seen at occupational exposure levels as low as 1 ppm or less. Animal data support these findings.
Both humans and rats have shown increases in leukocyte alkaline phosphatase activity. No studies
regarding effects from oral or dermal exposure in humans were located. However, exposure to benzene
through ingestion or dermal contact could cause immunological effects similar to those seen after
inhalation exposure in humans and inhalation and oral exposure in animals.

Animal studies have also shown that benzene decreases circulating leukocytes and decreases the ability of
lymphoid tissue to produce the mature lymphocytes necessary to form antibodies. This has been
demonstrated in animals exposed for acute, intermediate, or chronic periods via the inhalation route. This
decrease in lymphocyte numbers is reflected in impaired cell-mediated immune functions in mice
following intermediate inhalation exposure to 100 ppm of benzene. The impaired cellular immunity after
benzene treatment was observed both *in vivo* and *in vitro*. Mice exposed to 100 ppm for a total of
100 days were challenged with 10^4 polyoma virus-induced tumor cells (PYB6). Nine of 10 mice had
reduced tumor resistance resulting in the development of lethal tumors. In the same study, lymphocytes
were obtained from spleens of benzene-treated mice and tested for their immune capacity *in vitro*. The
results showed that two other immune functions, alloantigen response (capacity to respond to foreign

antigens) and cytotoxicity, were also impaired. Similar effects were noted in mice exposed to benzene via the oral route for intermediate time periods, and in rats and mice exposed for chronic time periods. A decrease in spleen weight was observed in mice after acute-duration exposure to benzene at 25 ppm, the same dose levels at which a decrease in circulating leukocytes was observed. Similar effects on spleen weight and circulating leukocytes were observed in mice exposed to 12 ppm benzene 2 hours/day for 30 days. The acute-duration inhalation MRL was based on a study showing decreased mitogen-induced blastogenesis of B-lymphocytes following exposure of mice to benzene vapors at a concentration of 10 ppm, 6 hours/day for 6 days. The intermediate-duration inhalation MRL was based on a study showing delayed splenic lymphocyte reaction to foreign antigens evaluated by *in vitro* mixed lymphocyte culture following exposure of mice to benzene vapors at a concentration of 10 ppm, 6 hours/day, 5 days/week for a total of 20 exposures.

Based on information found in the literature, it is reasonable to expect that adverse immunological effects might occur in humans after inhalation, oral, or dermal exposure, since absorption of benzene through any route of exposure would increase the risk of damage to the immunological system. Studies show that the immunological system is susceptible to chronic exposure at low levels, so people living in and around hazardous waste sites who may be exposed to contaminated air, drinking water, soil, or food may be at an increased risk for adverse immunological effects.

Neurological Effects.   In humans, results of occupational studies indicate that there is a cause-and-effect relationship between acute inhalation of very high concentrations of benzene and symptoms indicative of central nervous system toxicity. These symptoms, observed following both acute nonlethal and lethal exposures, include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness. These symptoms are reversible when symptomatic workers are transferred from the problem area. Comparable toxicity in humans has been reported following ingestion of benzene at doses of 125 mg/kg and above. Occupational exposure to benzene has also been reported to produce neurological abnormalities in humans. Electromyographical and motor conduction velocity examinations were conducted on six patients with aplastic anemia, all of whom worked in environments where adhesives containing benzene were used (in one case, air concentrations bracketed around 210 ppm). Abnormalities in motor conduction velocity were noted in four of the six pancytopenic individuals and were thought to result from a direct effect of benzene on the peripheral nerves and/or spinal cord.

In its acute stages, benzene toxicity appears to be due primarily to the direct effects of benzene on the central nervous system, whereas the peripheral nervous system appears to be the target following chronic

low-level exposures. In addition, because benzene may induce an increase in brain catecholamines, it may also have a secondary effect on the immune system via the hypothalamus-pituitary-adrenal axis. Increased metabolism of catecholamines can result in increased adrenal corticosteroid levels, which are immunosuppressive.

Animal studies provide additional support that benzene affects the nervous system following acute inhalation and oral exposures, albeit at extremely high acute exposure levels. Effects reported include narcosis, nervous system depression, tremors, and convulsions. Acute and intermediate inhalation exposures have also been reported to produce adverse neurological effects in animals including a reduction in hind-limb grip strength and evoked electrical activity in the brain, and behavioral disturbances. Effects of benzene on learning were investigated in male hooded rats of the Sprague-Dawley strain given 550 mg/kg of benzene in corn oil or corn oil without benzene, intraperitoneally, on days 9, 11, and 13 postpartum. The rats exposed to benzene exhibited a significantly impaired learning ability when tested on problems of the closed-field, maze-learning task. This sign of neurotoxicity was not observed in control animals. In another study, 47-day-old juvenile cotton rats were maintained on one of two isocaloric diets containing either 4 or 16% crude protein for a 26-day experimental period. Animals were treated intraperitoneally with either 0 (corn oil), 100, 500, or 1,000 mg/kg benzene in corn oil for 3 consecutive days. The first dose was administered on days 15–17 of the experimental period. Animals were terminated on day 27. During the experimental period, severe loss of coordination was observed in some rats on the low protein diet immediately after exposure to benzene, but this subsided.

Intermediate oral exposures resulted in changes in the levels of monoamine transmitters in the brain without treatment-related behavioral changes. Mice exposed to 3 ppm for 2 hours/day for 30 days exhibited increased levels of acetylcholinesterase in the brain. *In vitro* studies suggest that benzene may have a direct effect on brain cells. Primary astrocyte cultures prepared from neonatal rat cerebella were treated with 3, 6, or 9 mmol/L benzene for 1 hour. ATPase and Mg^{2+}-ATPase activity were inhibited in a dose-related manner, and were detected at 78–92% of control values for ATPase, and 60–74% of control values for Mg^{2+}-ATPase.

These data suggest that humans exposed to benzene in the occupational setting for acute, intermediate, or chronic durations via the inhalation and oral routes are at risk of developing neurological effects. However, benzene levels in ambient air, drinking water, and at hazardous waste sites are lower and not likely to be of concern.

2.3   MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for benzene. An
MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an
appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are
derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive
health effect(s) for a specific duration within a given route of exposure. MRLs are based on
noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for
acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate
methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990),
uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional
uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an
example, acute inhalation MRLs may not be protective for health effects that are delayed in development
or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic
bronchitis. As these kinds of health effects data become available and methods to assess levels of
significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

- An MRL of 0.009 ppm has been derived for acute-duration inhalation exposure (14 days or less)
  to benzene.

The acute-duration inhalation MRL of 0.009 ppm was derived from a lowest-observed-adverse-effect
level (LOAEL) value of 10.2 ppm for reduced lymphocyte proliferation following mitogen stimulation in
mice (Rozen et al. 1984). The concentration was adjusted for intermittent exposure by multiplying the
LOAEL (10.2 ppm) by 6 hours/24 hours to correct for less than a full day of exposure. The resulting
adjusted LOAEL, 2.55 ppm, was then converted to a human equivalent concentration (HEC) according to
EPA (1994b) methodology for calculating a HEC for extrarespiratory effects of a category 3 gas (such as
benzene) as follows:

$$LOAEL_{HEC} = LOAEL_{ADJ} \times ([H_{b/g}]_A/[H_{b/g}]_H)$$

where:

LOAEL$_{HEC}$ = the LOAEL dosimetrically adjusted to a human equivalent concentration

LOAEL$_{ADJ}$ = the LOAEL adjusted from intermittent to continuous exposure

$[H_{b/g}]_A/[H_{b/g}]_H$ = the ratio of the blood:gas partition coefficient of the chemical for the laboratory animal
species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a
default value of 1 is used for the ratio. According to Wiester et al. (2002), blood:gas partition coefficients
for benzene in mice and humans are 17.44 and 8.12, respectively. Therefore, the default value of 1 is
applied, in which case, the LOAEL$_{[HEC]}$ is equivalent to the LOAEL$_{[ADJ]}$.

Therefore:

LOAEL$_{HEC}$ = LOAEL$_{ADJ}$ = 2.55 ppm

The resulting LOAEL$_{(HEC)}$ of 2.55 ppm was then divided by an uncertainty factor of 300 (10 for the use of
LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, and 10 for human
variability) to yield the MRL value of 0.009 ppm (see Appendix A). An increased number of
micronucleated polychromatic erythrocytes (MN-PCEs), decreased numbers of granulopoietic stem cells
(Toft et al. 1982), lymphopenia (Cronkite et al. 1985), lymphocyte depression, and increased
susceptibility to bacterial infection (Rosenthal and Snyder 1985) are among the adverse hematological
and immunological effects observed in several other acute-duration inhalation studies. The study by
Rozen et al. (1984) shows benzene immunotoxicity (reduced mitogen-induced lymphocyte proliferation)
at a slightly lower exposure level than these other studies. C57BL/6J mice were exposed to 0, 10.2, 31,
100, and 301 ppm benzene for 6 hours/day for 6 days. Control mice were exposed to filtered, conditioned
air only. Lymphocyte counts were depressed at all exposure levels; erythrocyte counts were elevated at
10.2 ppm, equal to controls at 31 ppm, and depressed at 100 and 301 ppm. Femoral B-lymphocyte and
splenic B-lymphocyte numbers were reduced at 100 ppm. Levels of circulating lymphocytes and
mitogen-induced blastogenesis of femoral B-lymphocytes were depressed after exposure to 10.2 ppm
benzene for 6 days. Mitogen-induced blastogeneses of splenic T-lymphocytes were depressed after
exposure to 31 ppm of benzene for 6 days. In another study, mice exhibited a 50% decrease in the

population of colony-forming granulopoietic stem cells (CFU-E) after exposure to 10 ppm benzene for
6 hours/day for 5 days (Dempster and Snyder 1991). In a study by Wells and Nerland (1991), groups of
4–5 male Swiss-Webster mice were exposed to 3, 25, 55, 105, 199, 303, 527, 1,150, or 2,290 ppm
benzene for 6 hours/day for 5 days. The number of leukocytes in peripheral blood and spleen weights
were significantly decreased compared with untreated controls at all concentrations >25 ppm. Therefore,
3 ppm was the no-observed-adverse-effect level (NOAEL) and 25 ppm was the LOAEL for these effects.
These data support the choice of Rozen et al. (1984) as the study from which to derive the MRL.

- An MRL of 0.006 ppm has been derived for intermediate-duration inhalation exposure (15–
  364 days) to benzene.

The intermediate-duration inhalation MRL of 0.006 ppm was derived from a LOAEL value of 10 ppm for
significantly delayed splenic lymphocyte reaction to foreign antigens evaluated in *in vitro* mixed
lymphocyte reaction following the exposure of male C57Bl/6 mice to benzene vapors 6 hours/day,
5 days/week for 20 exposure days (Rosenthal and Snyder 1987). The concentration was adjusted for
intermittent exposure by multiplying the LOAEL (10 ppm) by 6 hours/24 hours to correct for less than a
full day of exposure and by 5 days/7days to correct for less than a full week of exposure. The resulting
adjusted LOAEL, 1.8 ppm, was then converted to a HEC according to EPA (1994b) methodology for
calculating a HEC for extrarespiratory effects of a category 3 gas (such as benzene) as follows:

$$\text{LOAEL}_{\text{HEC}} = \text{LOAEL}_{\text{ADJ}} \times ([\text{H}_{b/g}]_A/[\text{H}_{b/g}]_H)$$

where:

$\text{LOAEL}_{\text{HEC}}$ = the LOAEL dosimetrically adjusted to a human equivalent concentration

$\text{LOAEL}_{\text{ADJ}}$ = the LOAEL adjusted from intermittent to continuous exposure

$[\text{H}_{b/g}]_A/[\text{H}_{b/g}]_H$ = the ratio of the blood:gas partition coefficient of the chemical for the laboratory animal
species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a
default value of 1 is used for the ratio. According to Wiester et al. (2002), blood:gas partition coefficients
for benzene in mice and humans are 17.44 and 8.12, respectively. Therefore, the default value of 1 is
applied, in which case, the $\text{LOAEL}_{[\text{HEC}]}$ is equivalent to the $\text{LOAEL}_{[\text{ADJ}]}$.

Therefore:

$$LOAEL_{HEC} = LOAEL_{ADJ} = 1.8 \text{ ppm}$$

The resulting $LOAEL_{(HEC)}$ of 1.8 ppm was then divided by an uncertainty factor of 300 (10 for the use of LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, and 10 for human variability) to yield the MRL value of 0.006 ppm (see Appendix A).

Results of several studies support the choice of Rosenthal and Snyder (1987) as the basis for the intermediate-duration inhalation MRL for benzene (Baarson et al. 1984; Green et al. 1981a, 1981b), although the supporting studies employed a single exposure level, which precluded consideration as critical studies for MRL derivation. Exposure of C57BL mice to 10 ppm benzene for 6 hours/day, 5 days/week caused significant depressions in numbers of lymphocytes (ca. 30% lower than controls) as early as exposure day 32; this effect was also noted at the other scheduled periods of testing (exposure days 66 and 178) (Baarson et al. 1984). Splenic RBCs were significantly reduced (ca. 15% lower than controls) at exposure days 66 and 178. The failure of the erythrons of benzene-exposed mice to support normal red cell mass was illustrated by the significant reduction in peripheral red cell numbers in these animals at 66 and 178 days of benzene exposure. Green et al. (1981a, 1981b) exposed male CD-1 mice to benzene vapors at concentrations of 0 or 9.6 ppm for 6 hours/day, 5 days/week for 50 days and assessed the effects of exposure on cellularity in the spleen, bone marrow, and peripheral blood. Exposure-related effects included a 90% increase in numbers of multipotential hematopoietic stem cells (CFU-S) (Green et al. 1981a), approximately 25% increase in spleen weight and total splenic nucleated cellularity (Green et al. 1981b), and 80% increase in nucleated RBCs (Green et al. 1981b).

One intermediate-duration inhalation study reported increased rapid response to an electrical shock in mice at 0.78 ppm (Li et al. 1992). However, this study was not selected as the critical study for deriving an intermediate-duration inhalation MRL for benzene due to apparent discrepancies between reported and actual benzene exposure levels. Other animal studies identified neurological effects only at much higher exposure levels (Carpenter et al. 1944; Dempster et al. 1984; Evans et al. 1981; Frantik et al. 1994; Green et al. 1978).

- An MRL of 0.003 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to benzene.

This MRL is based on statistically significantly decreased counts of B-lymphocytes in workers of shoe manufacturing industries in Tianjin, China (Lan et al. 2004a, 2004b), using a benchmark dose (BMD) analysis. The 250 benzene-exposed workers had been employed for an average of 6.1±2.9 years. Controls consisted of 140 age- and gender-matched workers in clothing manufacturing facilities in which measurable benzene concentrations were not found (detection limit 0.04 ppm). Benzene exposure was monitored by individual organic vapor monitors (full shift) 5 or more times during 16 months prior to phlebotomy. Benzene-exposed workers were categorized into four groups (140 controls, 109 at <1 ppm, 110 at 1–<10 ppm, and 31 at ≥10 ppm) according to mean benzene exposure levels measured during 1 month prior to phlebotomy. Complete blood count (CBC) and differential were analyzed mechanically. Coefficients of variation for all cell counts were <10%.

Mean 1-month benzene exposure levels in the four groups (controls, <1 ppm, 1–<10 ppm, and ≥10 ppm) were <0.04, 0.57±0.24, 2.85±2.11, and 28.73±20.74 ppm, respectively. Hematological values were adjusted to account for potential confounding factors (i.e., age, gender, cigarette smoking, alcohol consumption, recent infection, and body mass index). All types of WBCs and platelets were significantly decreased in the lowest exposure group (<1 ppm), ranging in magnitude from approximately 8 to 15% lower than controls. Although similar statistical analyses for the mid- and high-exposure groups were not included in the study report, decreases in all types of WBCs and platelets were noted at these exposure levels as well; the decreases in the highest exposure group ranged in magnitude from 15 to 36%. Lymphocyte subset analysis revealed significantly decreased CD4+-T cells, CD4+/CD8+ ratio, and B cells. Hemoglobin concentrations were significantly decreased only within the highest (≥10 ppm) exposure group. Tests for a linear trend using benzene air level as a continuous variable were significant for platelets and all WBC measures except monocytes and CD8+-T cells. Upon restricting the linear trend analyses to workers exposed to <10 ppm benzene, excluding controls, inverse associations remained for total WBCs, granulocytes, lymphocytes, B cells, and platelets. In order to evaluate the effect of past benzene exposures on the hematological effects observed in this study, the authors compared findings for a group of workers who had been exposed to <1 ppm benzene over the previous year (n=60) and a subset who also had <40 ppm-years lifetime cumulative benzene exposure (n=50). The authors stated that the same cell types were significantly reduced in these groups, but did not provide further information of the magnitude (i.e., percent change) of the hematological effects observed. These data suggest that the 1-month benzene exposure results could be used as an indicator of longer-term, low-level benzene hematotoxicity. To demonstrate that the observed effects were attributable to benzene, significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells were noted in a subgroup of the <1-ppm group for which exposure to other solvents was negligible.

As shown in Table A-1 of Appendix A, exposure-response relationships were noted for several blood factors. Benzene-induced decreased B cell count was selected as the critical effect for BMD modeling because it represented the highest magnitude of effect (i.e., B cell count in the highest exposure group was approximately 36% lower than that of controls). A BMD modeling approach was selected to identify the point of departure because the critical study (Lan et al. 2004a, 2004b) identified a LOAEL in the absence of a NOAEL.

As discussed in detail in Appendix A, the BMD analysis selected a point of departure ($BMCL_{0.25sd}$) of 0.1 ppm, which was adjusted from the 8-hour TWA to a continuous exposure concentration ($BMCL_{0.25sdADJ}$) using the default occupational minute volume (EPA 1994b). The resulting $BMCL_{0.25sdADJ}$ of 0.03 ppm was divided by an uncertainty factor of 10 (for human variability) to yield the chronic-duration inhalation MRL value of 0.003 ppm.

Several recent epidemiological studies provide supporting information to the Lan et al. (2004a, 2004b) findings of hematotoxicity in workers chronically exposed to relatively low levels of benzene (Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b; Ward et al. 1996). Qu et al. (2002, 2003a, 2003b) compared hematologic values among 105 healthy workers (51 men, 54 women) in industries with a history of benzene usage (Tianjin, China) and 26 age-and gender-matched workers in industries that did not use benzene. A LOAEL of 2.26 ppm was identified for significantly reduced total WBCs, neutrophils, and RBCs; there was also an indication of benzene-induced changes in some hematological values at exposure levels lower than the current industry 8-hour TWA of 1 ppm. Rothman et al. (1996a, 1996b) identified an 8-hour TWA LOAEL of 7.6 ppm for a group of 11 benzene-exposed workers in a cross-sectional study of 44 healthy workers in Chinese (Shanghai) industries with a history of benzene usage and age- and gender-matched workers in industries that did not use benzene. In a nested case-control study of a cohort of workers in the Pliofilm production departments of a rubber products manufacturer in Ohio (Ward et al. 1996), a strong exposure-response relationship was correlated with low WBC count. A weak positive exposure-response relationship was observed for RBCs, which was significant for cumulative exposure up until the blood test date. The study authors noted that there was no evidence for a threshold for hematologic effects and suggested that exposure to benzene levels <5 ppm may result in hematologic suppression.

Oral MRLs

No acute-duration oral MRL was derived due to a lack of appropriate data on the effects of acute oral exposure to benzene.

In a study by Thienes and Haley (1972), central nervous system symptoms of giddiness, vertigo, muscular incoordination, unconsciousness, and death of humans have been reported when doses of ≥125 mg kg/body weight benzene were ingested. The only lower doses in the acute oral database are 50 mg/kg/day, at which pregnant rats experienced alopecia of hindlimbs and trunk (Exxon 1986), an end point not suitable for MRL derivation for benzene because the toxicological significance is not clear; and 88 mg/kg/day, a concentration resulting in slight non-dose-related central nervous system depression in rats (Cornish and Ryan 1965).

No intermediate-duration oral MRL was derived. Examination of the literature indicated that studies by Hsieh et al. (1988b, 1991) and Wolf et al. (1956) should be considered for derivation of an intermediate-duration oral MRL. Adult male CD-1 mice were exposed to 0, 8, 40, or 180 mg/kg/day benzene in the drinking water for 4 weeks (Hsieh et al. 1988b, 1991). Serious hematological and immunological effects (leukopenia, erythrocytopenia, lymphopenia, enhanced splenic lymphocyte proliferation) occurred at the lowest dose, precluding its use for MRL derivation. In the study by Wolf et al. (1956), a NOAEL of 1 mg/kg/day and a LOAEL of 50 mg/kg/day for leukopenia were identified in rats given benzene in oil by gavage for 6 months. However, closer examination of the study by Wolf et al. (1956) revealed that the results were not adequately supported by the data and analysis presented in the paper. Therefore, Wolf et al. (1956) was not selected for derivation of the MRL.

- An MRL of 0.0005 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to benzene.

No human data are available to evaluate hematological effects following oral exposure to benzene. No adequate oral data were located from which a chronic-duration oral MRL for benzene could be derived, although a 2-year carcinogenesis bioassay of orally-exposed rats and mice is available (NTP 1986). Male rats were given 50, 100, or 200 mg/kg/day and female rats and mice of both sexes were given 0, 25, 50, and 100 mg/kg/day benzene in corn oil for 2 years. The dose of 25 mg/kg/day was a LOAEL for hematotoxicity and immunotoxicity in rats and mice, and is higher than the serious LOAEL of 8 mg/kg/day in the intermediate-duration database. Therefore, the threshold for hematological and immunological effects of benzene was not identified.

However, results of toxicokinetic studies of inhaled benzene in humans (Nomiyama and Nomiyama 1974a; Pekari et al. 1992; Srbova et al. 1950) and inhaled and orally-administered benzene in rats and mice (Sabourin et al. 1987) indicate that absorption of benzene at relatively low levels of exposure is approximately 50% of an inhaled dose and essentially 100% of an oral dose. Based on these assumptions, inhalation data can be used to estimate equivalent oral doses that would be expected to similarly affect the critical targets of benzene toxicity. Therefore, the point of departure for the chronic-duration inhalation MRL for benzene, namely the $BMCL_{0.25sdADJ}$ of 0.03 ppm for decreased B cell counts in benzene-exposed workers (Lan et al. 2004a, 2004b), serves as the point of departure for deriving the chronic-duration oral MRL as well.

The point of departure (in ppm) was converted to mg/m^3 using the molecular weight of 78.11 for benzene and assuming 25 °C and 760 mm Hg:

$$BMCL_{0.25sdADJ} \text{ of } 0.03 \text{ ppm x } 78.11/24.45 = 0.096 \text{ mg/m}^3$$

The $BMCL_{0.25sdADJ}$ of 0.096 mg/m^3 for inhaled benzene was converted to an equivalent $BMDL_{0.25sdADJ}$ for ingested benzene using EPA (1988b) human reference values for inhalation rate (20 m^3/day) and body weight (70 kg) and a factor of 0.5 to adjust for differences in absorption of benzene following inhalation versus oral exposure (50 versus 100%, respectively) as follows:

$$BMDL_{0.25sdADJ} = BMCL_{0.25sdADJ} \text{ of } 0.096 \text{ mg/m}^3 \text{ x } 20 \text{ m}^3\text{/day x } 0.5 \div 70 \text{ kg} = 0.014 \text{ mg/kg/day}$$

A total uncertainty factor of 30 (10 for human variability and 3 for uncertainty in route-to-route extrapolation) was applied to the $BMDL_{0.25sdADJ}$ of 0.014 mg/kg/day; the resulting chronic-duration oral MRL is 0.0005 mg/kg/day.

3. HEALTH EFFECTS

3.1   INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of benzene. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2   DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not

the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of benzene are indicated in Tables 3-1 and 3-2 and Figures 3-1 and 3-2. Because cancer effects could occur at lower exposure levels, Figures 3-1 and 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1   Inhalation Exposure

Although occupational or environmental exposure to benzene or benzene-containing materials may include inhalation, oral, and dermal exposure routes, the inhalation and dermal routes are usually of primary concern in such scenarios. Data regarding occupational or environmental exposure in which inhalation is considered to have been the primary exposure route are summarized in this section (Section 3.2.1). Information regarding adverse health effects following oral or dermal exposure to benzene or benzene-containing materials is summarized in Sections 3.2.2 and 3.2.3, respectively.

3.2.1.1   Death

Case reports of fatalities due to acute benzene exposures have appeared in the literature since the early 1900s (Cronin 1924; Greenburg 1926; Hamilton 1922). Deaths occurred suddenly or within several hours after exposure (Avis and Hutton 1993; Cronin 1924; Greenburg 1926; Hamilton 1922; Winek et al. 1967). The benzene concentrations encountered by the victims were not often known. However, it has been estimated that 5–10 minutes of exposure to 20,000 ppm benzene in air is usually fatal (Flury 1928). Lethality in humans has been attributed to asphyxiation, respiratory arrest, central nervous system

depression, or suspected cardiac collapse (Avis and Hutton 1993; Hamilton 1922; Winek and Collom 1971; Winek et al. 1967). Cyanosis, hemolysis, and congestion or hemorrhage of organs were reported in the cases for which there were autopsy reports (Avis and Hutton 1993; Greenburg 1926; Hamilton 1922; Winek et al. 1967). No studies were located regarding noncancer-related mortality in humans following long-term inhalation exposure to benzene. Cancer-related mortality data for chronic-duration human occupational exposure to benzene are presented in Section 3.2.1.7.

In animals, acute inhalation exposure to high concentrations of benzene has caused death. An inhalation LC_{50} value for rats was calculated as 13,700 ppm for a 4-hour exposure (Drew and Fouts 1974). Additionally, 4 of 6 rats died following a 4-hour exposure to 16,000 ppm benzene (Smyth et al. 1962). However, in a study by Green et al. (1981b), male CD-1 mice exposed by inhalation to doses of benzene up to 4,862 ppm, 6 hours/day for 5 days showed no lethality. Lower doses (up to 400 ppm) for longer periods of time (2 weeks) did not cause death in mice (Cronkite et al. 1985). Lethality in monkeys and cats exposed to unspecified concentrations has been ascribed to ventricular fibrillation due to increased release of adrenaline (Nahum and Hoff 1934). Exposure of rabbits to 45,000 ppm of benzene for approximately 30 minutes caused narcosis that was followed by the death of all exposed animals (Carpenter et al. 1944). Furthermore, early deaths of rats and mice have occurred from intermediate and chronic exposure to air concentrations of 200 or 300 ppm of benzene in cancer studies (Cronkite et al. 1989; Farris et al. 1993; Maltoni et al. 1982a, 1983). Intermediate exposures (6 hours/day, 5 days/week for 50 days) of male CD-1 mice to benzene at doses of 9.6 ppm caused no increase in mortality, although mice exposed to 302 ppm benzene under the same regimen for a total of 26 weeks showed mortality approaching 50% (Green et al. 1981b). Mortality was observed in 97% of the CBA/Ca mice exposed to 300 ppm benzene for 16 weeks, as compared to 20% mortality in sham-exposed mice (Cronkite 1986). In Sprague-Dawley rats that received 300 ppm benzene vapor for 6 hours/day, 5 days/week for 691 days, the calculated median survival time was shown to be 51 weeks as compared to 65 weeks for controls (Snyder et al. 1978a). However, Snyder et al. (1984) reported a median survival time of 546 days for male Sprague-Dawley rats exposed to 100 ppm benzene for 5 days/week, 6 hours/day for life, compared to 560 days for air-exposed controls. It is not clear whether the difference in survival was significant or due to benzene exposure since both controls and exposed rats experienced early mortality from respiratory infections. Companion studies were also conducted with AKR and C57BL mice exposed to 300 ppm benzene (Snyder et al. 1978a, 1980). The calculated median survival time for AKR and C57BL mice exposed to 300 ppm benzene was shown to be 11 and 41 weeks compared to 39 and 75 weeks, respectively, for controls. For AKR mice exposed to 100 ppm, the calculated median survival time was 39 weeks (number of deaths not shown) as compared to 41 weeks for controls (Snyder et al. 1980).

The LC$_{50}$ value and all reliable LOAEL values for each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

No studies were located regarding endocrine, metabolic, or body weight effects in humans or gastrointestinal, musculoskeletal, endocrine, metabolic, or dermal effects in animals following inhalation exposure to benzene. Available data pertaining to systemic effects are presented below.

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects.   Respiratory effects have been reported in humans after acute exposure to benzene vapors (Avis and Hutton 1993; Midzenski et al. 1992; Winek and Collom 1971; Winek et al. 1967; Yin et al. 1987b). Fifteen male workers employed in removing residual fuel from shipyard tanks were evaluated for benzene exposure (Midzenski et al. 1992). Mucous membrane irritation was noted in 80% and dyspnea was noted in 67% of the workers at occupational exposures of >60 ppm for up to 3 weeks. Nasal irritation and sore throat were reported by male and female workers exposed to 33 and 59 ppm benzene, respectively, for more than 1 year (Yin et al. 1987b). After a fatal occupational exposure to benzene vapors on a chemical cargo ship for only minutes, autopsy reports on three victims revealed hemorrhagic, edematous lungs (Avis and Hutton 1993). Acute granular tracheitis, laryngitis, bronchitis, and massive hemorrhages of the lungs were observed at autopsy of an 18-year-old male who died of benzene poisoning after intentional inhalation of benzene (Winek and Collom 1971). Similarly, acute pulmonary edema was found during the autopsy of a 16-year-old who died after sniffing glue containing benzene (Winek et al. 1967).

Snyder et al. (1978a, 1984) reported no treatment-related effects on lung tissue in male Sprague-Dawley rats exposed to 0, 100, or 300 ppm benzene 5 days/week, 6 hours/day for life. In addition, no adverse histopathological effects on lung tissue were observed in AKR/J or C57BL/65 mice exposed to 300 ppm benzene for life (Snyder et al. 1978a, 1980).

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
ACUTE EXPOSURE								
Death								
1	Human	1 d 5-10 min				20000 (death)	Flury 1928	
2	Rat (Sprague-Dawley)	4 hr				13700 (LC50)	Drew and Fouts 1974	
3	Rat (NS)	4 hr				16000 (4/6 died)	Smyth et al. 1962	
4	Rabbit (NS)	3.7-36.2 min				45000 (death in 36.2 min)	Carpenter et al. 1944	
Systemic								
5	Human	1-21 d 2.5-8 hr/d	Resp		60 M (mucous membrane irritation, dyspnea)		Midzenski et al. 1992	
			Hemato			60 M (leukopenia, anemia, thrombocytopenia, MCV elevation)		
			Dermal		60 M (skin irritation)			
6	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d	Bd Wt	300 F	2200 F (decreased maternal body weight)		Green et al. 1978	
7	Rat (Sprague-Dawley)	Gd 6-15 7 hr/d	Bd Wt	10 F	50 F (decreased maternal body weight and weight gain)		Kuna and Kapp 1981	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation **(continued)**

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
8	Rat (Wistar)	7 d 8 hr/d	Hemato	50 F	100 F (leukopenia)		Li et al. 1986	
9	Rat (Wistar)	15 min	Cardio			3526 M (ventricular arrhythmia)	Magos et al. 1990	
10	Rat (CFY)	Gd 7-14 24 hr/d	Hepatic	125 F			Tatrai et al. 1980a	
			Bd Wt			125 F (decreased maternal weight gain of <22.08% of controls)		
11	Rat (CFY)	Gd 7-14 24 hr/d	Hepatic	47 F	141 F (increased relative liver weight)		Tatrai et al. 1980b	
			Bd Wt		47 F (decreased maternal weight gain)			
12	Rat (Sprague-Dawley)	2 wk 5 d/wk 6 hr/d	Hemato	30	300 (decrease in leukocytes, males; decrease in lymphocytes)		Ward et al. 1985	
13	Mouse (BALB/c)	7 d 6 hr/d	Hemato	47 M	211 M (depressed WBC count)		Aoyama 1986	
			Bd Wt	47 M	211 M (16% decrease in body weight)			

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
14	Mouse (BALB/c)	14 d 6 hr/d	Hemato		48 M (depressed WBC count)		Aoyama 1986	
			Bd Wt	48 M	208 M (18% decrease in body weight)			
15	Mouse (DBA/2)	2 wk 6 hr/d 5 d/wk	Hemato			300 M (hematocrit decreased by 26%, leukocytes decreased by 80%, bone marrow cellularity decreased by 93%)	Chertkov et al. 1992	
			Bd Wt		300 M (15% decrease)			
16	Mouse (Hale- Stoner)	11 d 5 d/wk 6 hr/d	Hemato			400 M (decreased erythrocytes and leukocytes)	Cronkite et al. 1982	
17	Mouse (C57B1/6 BNL)	2 wk 5 d/wk 6 hr/d	Hemato	25		100 (decreased hematocrit, hemolytic anemia)	Cronkite et al. 1985	
18	Mouse (Hale- Stoner)	2 d 5 d/wk 6 hr/d	Hemato		400 M (decreased CFU-E cells)		Cronkite et al. 1989	
19	Mouse (C57BL/6BNL)	8 d 6 hr/d	Hemato		3000 F (decreased marrow cellularity)		Cronkite et al. 1989	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
20	Mouse (DBA/2J)	5 d 6 hr/d	Hemato		10 M (50% decrease in CFU-E numbers)		Dempster and Snyder 1991	
21	Mouse (C57B1/6)	2-8 d 24 hr/d	Hemato			100 M (leukopenia; decrease in marrow cellularity)	Gill et al. 1980	
22	Mouse (CD-1)	5 d 6 hr/d	Hemato	9.9 M		103 M (decreased marrow cellularity; granulocytopenia, lymphocytopenia; decreased polymorphonucleucytes)	Green et al. 1981b	
			Bd Wt	4862 M				
23	Mouse (Swiss Webster, C57B1/6J)	2 wk 4 d/wk 6 h/d	Hemato			300 M (reduced bone marrow cellularity and CFU-E development)	Neun et al. 1992	
24	Mouse (Hybrid)	5 d 5 d/wk 6 hr/d	Hemato	300 F	900 F (CFU-E depression)		Plappert et al. 1994a	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
25	Mouse (C57Bl/6J)	6 d 6 hr/d	Hemato		10.2 M (depressed lymphocyte counts; elevated RBCs)		Rozen et al. 1984	
26	Mouse (NMRI)	1-10 d 24 hr/d	Hemato			21 M (reduced bone marrow cellularity; increased polychromatic erythrocytes; decreased granulopoietic stem cells)	Toft et al. 1982	
27	Mouse (NMRI)	1 wk	Hemato	14 M			Toft et al. 1982	
28	Mouse (NMRI)	2 wk 5 d/wk 8 hr/d	Hemato	10.5 M	21 M (increased micronucleated polychromatic erythrocytes; decreased granulopoietic stem cells)		Toft et al. 1982	
29	Mouse (CD-1)	2 wk 5 d/wk 6 hr/d	Hemato	30		300 (anemia, decreased hemoglobin,erythrocytes and hematocrit; hypoplasia of bone marrow; leukopenia)	Ward et al. 1985	
			Bd Wt	300				

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation **(continued)**

Key to Figure	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	Less Serious (ppm)	Serious (ppm)	Reference Chemical Form	Comments
30	Mouse (Swiss-Webster)	5 d 6 hr/d	Hemato	3 M	25 M (decrease in WBC count)		Wells and Nerland 1991	
31	Rabbit	Gd 7-20 24 hr/d	Bd Wt	156.5 F	313 F (reduced maternal weight gain)		Ungvary and Tatrai 1985	
Immuno/ Lymphoret								
32	Rat (Wistar)	7 d 8 hr/d		50 F	100 F (leukopenia; increased leukocyte alkaline phosphatase)		Li et al. 1986	
33	Rat (Sprague-Dawley)	2 wk 5 d/wk 6 hr/d		200 M	400 M (29% reduction in total splenic cells, 28% lower thymus weight)		Robinson et al. 1997	
34	Mouse (BALB/c)	7 d 6 hr/d			47 M (depressed T- and B-lymphocytes; decreased spleen weight and WBC count)		Aoyama 1986	
35	Mouse (BALB/c)	14 d 6 hr/d			48 M (depressed T- and B-lymphocytes; decreased spleen and thymus weights and WBC count)		Aoyama 1986	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
36	Mouse (DBA/2)	2 wk 6 hr/d 5 d/wk				300 M (leukocytes decreased by 80%, bone marrow cellularity decreased by 93%)	Chertkov et al. 1992	
37	Mouse (CBA/Ca)	2 wk 5 d/wk 6 hr/d		10	25 (lymphopenia)		Cronkite 1986	
38	Mouse (Hale- Stoner)	11 d 5 d/wk 6 hr/d				400 M (decreased bone marrow cellularity)	Cronkite et al. 1982	
39	Mouse (C57B1/6 BNL)	2 wk 5 d/wk 6 hr/d		10	25 (lymphopenia)		Cronkite et al. 1985	
40	Mouse (CBA/Ca BNL)	2 d 5 d/wk 6 hr/d			3000 M (decreased lymphocytes, CFU-S content in marrow)		Cronkite et al. 1989	
41	Mouse (C57B1/6)	2-8 d 24 hr/d				100 M (leukopenia; decrease in marrow cellularity)	Gill et al. 1980	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
42	Mouse (CD-1)	5 d 6 hr/d		9.9 M		103 M (decreased femoral marrow and splenic cellularities; reduced splenic granulocytes)	Green et al. 1981a	
43	Mouse (Swiss Webster, C57B1/6J)	2 wk 4 d/wk 6 h/d				300 M (reduced bone marrow cellularity)	Neun et al. 1992	
44	Mouse (Hybrid)	5 d 5 d/wk 6 hr/d		100 F	300 F (increased helper lymphocytes)		Plappert et al. 1994a	
45	Mouse (C57BL/6)	1-12 d 6 hr/d		10 M	30 M (Listeria infection, T and B lymphocyte depression)		Rosenthal and Snyder 1985	
46	Mouse (C57Bl/6J)	6 d 6 hr/d			10.2 [b] M (decreased circulating lymphocytes and mitogen-induced blastogenesis of femoral T and B-lymphocytes)		Rozen et al. 1984	
47	Mouse (NMRI)	2 wk 5 d/wk 8 hr/d		10.5 M	21 M (decreased granulopoietic stem cells)		Toft et al. 1982	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
48	Mouse (NMRI)	1-10 d 24 hr/d			21 M (decreased granulopoietic stem cells)		Toft et al. 1982	
49	Mouse (CD-1)	2 wk 5 d/wk 6 hr/d		30		300 (leukopenia; lymphopenia; bone marrow hypoplasia; histopathological lesions in spleen, thymus, selected lymph nodes)	Ward et al. 1985	
50	Mouse (Swiss-Webster)	5 d 6 hr/d		3 M	25 M (decrease in spleen weight and WBC count)		Wells and Nerland 1991	
Neurological								
51	Human	30 min			300 (drowsiness, dizziness, headaches)		Flury 1928	
52	Human	1-21 d 2.5-8 hr/d			60 M (dizzyness, nausea, headache, peculiar or strong odor, chemical taste, fatigue)		Midzenski et al. 1992	
53	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		300 F	2200 F (lethargy)		Green et al. 1978	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL			Reference Chemical Form	Comments
					Less Serious (ppm)		Serious (ppm)		
54	Mouse (C57BL)	1-14 d 5 d/wk 6 hr/d			100 M (increased milk licking: behavioral index)		3000 M (tremors; decreased grip strength)	Dempster et al. 1984	
55	Mouse (CD1, C57BL/6J)	5 d 6 hr/d			300 (hyperactivity)		900 (narcosis)	Evans et al. 1981	
56	Rabbit (NS)	3.7-36.2 min					45000 (narcosis, tremors, excitement, chewing, loss of pupillary and blink reflex; pupillary contraction and involuntary blinking)	Carpenter et al. 1944	
Reproductive									
57	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		100 F				Coate et al. 1984	
58	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		2200 F				Green et al. 1978	
59	Rat (CFY)	Gd 7-14 24 hr/d		125 F				Tatrai et al. 1980a	
60	Mouse (CF-1)	Gd 6-15 7 hr/d		500 F				Murray et al. 1979	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
61	Rabbit (New Zealand)	Gd 6-18 7 hr/d		500 F			Murray et al. 1979	
62	Rabbit	Gd 7-20 24 hr/d		156.5 F		313 F (increased abortions and resorptions)	Ungvary and Tatrai 1985	
Developmental								
63	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		40 F	100 F (decreased fetal weight)		Coate et al. 1984	
64	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d			100 F (increased incidence of missing sternebrae)		Green et al. 1978	
65	Rat (Sprague-Dawley)	Gd 6-15 7 hr/d		10 F	50 F (decreased fetal weight)		Kuna and Kapp 1981	
66	Rat (CFY)	Gd 7-14 24 hr/d			125 F (decreased mean fetal weight; increased fetal weight retardation; skeletal retardation; 17% decrease in mean placental weight)		Tatrai et al. 1980a	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
67	Rat (CFY)	Gd 7-14 24 hr/d			47 F (decreased fetal weight; signs of skeletal retardation)	141 F (significant increase in fetal mortality)	Tatrai et al. 1980b	
68	Mouse (Swiss-Webster)	Gd 6-15 6 hr/d		10 F	20 F (decreased circulating erythroid precursors, elevation of granulocytic precourser cells in neonates and 6-week old offspring)		Keller and Snyder 1988	
69	Mouse (CF-1)	Gd 6-15 7 hr/d			500 F (decreased fetal body weight; delayed skeletal ossifications)		Murray et al. 1979	
70	Mouse (CFLP)	Gd 6-15 12 hr/d			156.5 F (decreased fetal body weight and skeletal retardation)		Ungvary and Tatrai 1985	
71	Rabbit (New Zealand)	Gd 6-18 7 hr/d			500 F (increased minor skeletal variants)		Murray et al. 1979	
72	Rabbit (New Zealand)	Gd 7-20 24 hr/d		156.5 F	313 F (decreased fetal weight; increased minor anomalies)		Ungvary and Tatrai 1985	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
INTERMEDIATE EXPOSURE								
Death								
73	Rat (Sprague-Dawley)	15 wk 4-5 d/wk 4-7 hr/d				200 (death)	Maltoni et al. 1983, 1985	
74	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M (97% mortality)	Cronkite 1986	
75	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				300 (deaths in males during exposure; deaths in females shortly after exposure)	Cronkite et al. 1989	
76	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M (11/125 died during first 9 months after initiation of exposure)	Farris et al. 1993	
77	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d				302 M (50% mortality)	Green et al. 1981b	
Systemic								
78	Human	4 mo- 1 yr (occup)	Hemato			150 (pancytopenia)	Aksoy and Erdem 1978	
79	Human	4 mo -1 yr (occup)	Hemato			210 (pancytopenia, hypocellular to hypercellular bone marrow)	Aksoy et al. 1972	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
80	Human	1 yr (occup)	Hemato		40 (decrease in WBC counts in first 4 months)		Cody et al. 1993	
81	Human	3.5 mo- 19 yr (occup)	Hemato			29 (aplastic anemia)	Yin et al. 1987c	
82	Rat (Sprague-Dawley)	3 wk 5 d/wk 6 hr/d	Hemato			500 (decreased WBC and lymphocytes; increased RBC and hemoglobin)	Dow 1992	
83	Rat (Sprague-Dawley)	10 wk Gd 0-20 Ld 5-20 5 d/wk 6 hr/d	Bd Wt	300 F			Kuna et al. 1992	
84	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d	Hemato	30		300 (decrease in leukocytes, slight decrease in marrow cellularity)	Ward et al. 1985	
			Bd Wt	300				
85	Rat (Wistar)	204 d 5 d/wk 7 hr/d	Hemato		88 M (leukopenia)		Wolf et al. 1956	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   **(continued)**

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
86	Mouse (C57BL)	24 wk 5 d/wk 6 hr/d	Hemato		10 M (depressed peripheral red blood cells, CFU-E)		Baarson et al. 1984	The use of one dose precludes a dose-response assessment.
87	Mouse (C57BL)	24 wk 5 d/wk 6 hr/d	Hemato		10 M (depressed splenic red cells)		Baarson et al. 1984	The use of one dose precludes a dose-response assessment.
88	Mouse (Hale- Stoner)	9.5 wk 5 d/wk 6 hr/d	Hemato			400 M (decreased erythrocytes and leukocytes, decreased bone marrow cellularity)	Cronkite et al. 1982	
89	Mouse (C57B1/ 6BNL)	4-16 wk 5 d/wk 6 hr/d	Hemato		300 (stem cell depression in bone marrow, reversible after 2-4 weeks)		Cronkite et al. 1985	
90	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d	Hemato	25 M	316 M (decreased stem cells in bone marrow)		Cronkite et al. 1989	
91	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d	Hemato		300 M (granulocytic hyperplasia in bone marrow)		Farris et al. 1993	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
92	Mouse (B6C3F1)	up to 8 wk 5 d/wk 6 hr/d	Hemato	10 M	100 M (decreased numbers of differentiating and maturing hematopoietic bone marrow cells)		Farris et al. 1997a	
93	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d	Hemato			302 M (decreased WBC, RBC; altered RBC morphology)	Green et al. 1981b	
			Bd Wt	302 M				
94	Mouse (CD-1)	50 d 5 d/wk 6 hr/d	Hemato	9.6 M			Green et al. 1981b	
			Bd Wt	9.6 M				
95	Mouse (Kunming)	30 d 6 d/wk 2 hr/d	Hepatic	12.52 M			Li et al. 1992	Exposures conducted under static conditions; measured only on first 3 of 30 days.
			Renal	12.52 M				
			Bd Wt	12.52 M				
96	Mouse (DBA/2, B6C3F1, C57B1/6)	13 wk 3 or 5 d/wk 6 hr/d	Hemato			300 M (depressed rate of erythropoiesis, increased frequency of MN-PCE and MN-NCE)	Luke et al. 1988b	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
97	Mouse (Hybrid)	8 wks 5 d/wk 6 hr/d	Hemato	100 F	300 F (slight anemia; BFU-E and CFU-E depression in bone marrow)		Plappert et al. 1994a	
98	Mouse (Hybrid)	8 wk 5 d/wk 6 hr/d	Hemato			300 F (decreased Hgb, Hct, erythrocyte counts)	Plappert et al. 1994b	
99	Mouse (BDF1)	8 wk 5 d/wk 6 hr/d	Hemato		100 F (BFU-E and CFU-E depression)		Seidel et al. 1989b	
100	Mouse (NMRI)	8 wk	Hemato	14 M			Toft et al. 1982	
101	Mouse (Hybrid)	6 or 7 wk 5 d/wk 6 h/d	Hemato			300 F (decreased CFU-C, BFU-E and CFU-E)	Vacha et al. 1990	
102	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d	Hemato	30		300 (pancytopenia, bone marrow hypoplasia)	Ward et al. 1985	
			Bd Wt	300				
103	Gn Pig (NS)	32 or 269 d 5 d/wk 7 hr/d	Hemato		88 (leukopenia)		Wolf et al. 1956	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL			Reference Chemical Form	Comments
					Less Serious (ppm)		Serious (ppm)		
104	Rabbit (NS)	243 d 5 d/wk 7 hr/d	Hemato		80	(leukopenia)		Wolf et al. 1956	
105	Pig (Duroc-Jersey)	3 wk 5 d/wk 6 hr/d	Hemato	20	100	(decreased peripheral WBC, and increased erythroid cells)		Dow 1992	
Immuno/ Lymphoret									
106	Human	4 mo- 1 yr (occup)					210 (pancytopenia, hypoplastic to hyperplastic bone marrow, enlarged spleen)	Aksoy et al. 1972	
107	Human	1 yr (occup)			40	(decreased lymphocytes)		Cody et al. 1993	
108	Rat (Sprague-Dawley)	3 wk 5 d/wk 6 hr/d					500 (decreased myeloid and lymphoid cells)	Dow 1992	
109	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d		200 M	400 M	(29% reduction in total splenic cells, 28% lower thymus weight)		Robinson et al. 1997	
110	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d		30	300	(leukopenia and lymphopenia)		Ward et al. 1985	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
111	Rat (Wistar)	204 d 5 d/wk 7 hr/d			88 (leukopenia, increased spleen weight)		Wolf et al. 1956	
112	Rat (NS)	20 wk 6 d/wk 4 hr/d			4570 (increased leukocyte alkaline phosphatase, decreased white blood cell count)		Yin et al. 1982	
113	Mouse (C57B1)	24 wk 5 d/wk 6 hr/d			10 M (decreased number of splenic lymphocytes)		Baarson et al. 1984	The use of one dose precludes a dose-response assessment.
114	Mouse (C57B1/ 6BNL)	4-16 wk 5 d/wk 6 hr/d				300 (reduced bone marrow cellularity; stem cell depression, reversible after 2-4 weeks)	Cronkite et al. 1985	
115	Mouse (CBA/Ca BNL)	20 d 5 d/wk 6 hr/d				316 M (decreased lymphocytes, CFU-S content in marrow)	Cronkite et al. 1989	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
116	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d			300 M (granulocytic hyperplasia)		Farris et al. 1993	
117	Mouse (B6C3F1)	up to 8 wk 5 d/wk 6 hr/d		10 M	100 M (reduced numbers of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, peripheral blood leukocytes and RBCs)		Farris et al. 1997a	
118	Mouse (B6C3F1)	8 wk 5 d/wk 6 hr/d		10 M	100 M (reduced lymphocyte and total nucleated cell counts)		Farris et al. 1997b	
119	Mouse (C57B1/6)	6 wk 5 d/wk 6 hr/d				1000 (leukopenia, granulocytopenia, lymphocytopenia)	Gill et al. 1980	
120	Mouse (CD-1)	50 d 6 hr/d 5 d/wk			9.6 M (increased splenic CFU-S)		Green et al. 1981a	The use of one exposure level precludes usefulness for dose-response assessment.

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
121	Mouse (CD-1)	26 wk 6 hr/d 5 d/wk				302 M (reduced marrow and spleen cellularity; decreased spleen weight)	Green et al. 1981a	
122	Mouse (CD-1)	50 d 5 d/wk 6 hr/d			9.6 M (increased spleen weight, total splenic nucleated cellularity and NRBC)		Green et al. 1981b	The use of one exposure level precludes usefulness for dose-response assessment.
123	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d				302 M (lymphocytopenia, anemia, decreased spleen weight, decreased spleen and marrow cellularities)	Green et al. 1981b	
124	Mouse (Kunming)	30 d 6 d/wk 2 hr/d		3.13 M	12.52 M (26% decrease in relative spleen weight; decrease in myelocytes, premyelocytes, myeloblasts, and metamyelocytes in the bone marrow)		Li et al. 1992	Exposures conducted under static conditions; measured only on first 3 of 30 days.

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
125	Mouse (Hybrid)	8 wks 5 d/wk 6 hr/d		100 F	300 F (increased T4/T8 ratio)		Plappert et al. 1994a	
126	Mouse (C57B1/6)	20 d 5 d/wk 6 hr/d			10 [c] M (delayed splenic lymphocyte reaction to foreign antigens evaluated in in vitro mixed lymphocyte reaction)		Rosenthal and Snyder 1987	
127	Mouse (C57B1/6)	100 d 5 d/wk 6 hr/d				100 M (death in 9/10 mice due to depressed cell-mediated immunity)	Rosenthal and Snyder 1987	
128	Mouse (Hale- Stoner)	4-5 wk 5 d/wk 6 hr/d		50 F	200 F (suppressed antibody response to fluid tetanus toxoid)		Stoner et al. 1981	
129	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d		30		300 (leukocyte & lymphocyte depression; bone marrow hypoplasia; histopathologic lesions in spleen and selected lymph nodes)	Ward et al. 1985	
130	Gn Pig (NS)	32 or 269 d 5 d/wk 7 hr/d			88 (leukopenia, increased spleen weight)		Wolf et al. 1956	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
131	Rabbit (NS)	243 d 5 d/wk 7 hr/d			80 (leukopenia)		Wolf et al. 1956	
132	Pig (Duroc-Jersey)	3 wk 5 d/wk 6 hr/d		20 F	100 F (T-cell depression; decreased peripheral WBC; decreased total lymphocytes)		Dow 1992	
Neurological								
133	Rat (Wistar)	3 wk 3-4 x 4 hr				929 M (calculated 30% depression of evoked electrical activity)	Frantik et al. 1994	
134	Mouse (H)	3 wk 3-4 x 2 hr				856 F (calculated 30% depression of evoked electrical activity)	Frantik et al. 1994	
135	Mouse (Kunming)	30 d 6 d/wk 2 hr/d			0.78 M (increased rapid response)		Li et al. 1992	Exposures conducted under static conditions; measured only on first 3 of 30 days.
Reproductive								
136	Rat (Sprague-Dawley)	10 wk Gd 0-20 Ld 5-20 5 d/wk 6 hr/d		300 F			Kuna et al. 1992	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
137	Rat (Wistar)	93 d 5 d/wk 7-8 hr/d			6600 M (testicular weight increase)		Wolf et al. 1956	
138	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d		30		300 (bilateral cyst in ovaries; atrophy/degeneration of testes; decrease in spermatozoa; increase in abnormal sperm)	Ward et al. 1985	
139	Gn Pig (NS)	32 or 269 d 5 d/wk 7-8 hr/d			88 M (testicular weight increase)		Wolf et al. 1956	
140	Rabbit (NS)	243 d 5 d/wk 7-8 hr/d			80 M (degeneration of germinal epithelium in testes)		Wolf et al. 1956	
Cancer								
141	Human	3.5 mo- 19 yr (occup)				29 (CEL: humanlymphocytic leukemia)	Yin et al. 1987c	
142	Rat (Sprague-Dawley)	15 wk 4-5 d/wk 4-7 hr/d				200 (CEL: hepatomas)	Maltoni et al. 1982a, 1983, 1985	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
143	Rat (Sprague-Dawley)	15 wk 5 d/wk 4-7 hr/d				200 (CEL: hepatomas; onset of Zymbal gland carcinoma)	Maltoni et al. 1982b, 1983, 1985	
144	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				100 M (CEL: leukemia)	Cronkite 1986	
145	Mouse (C57BL/ 6BNL)	4-16 wk 5 d/wk 6 hr/d				300 (CEL: thymic and non-thymic lymphoma)	Cronkite et al. 1984, 1985	
146	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				300 (Harderian and Zymbal gland, squamous cell and mammary carcinoma, papillary adenocarcinoma of the lung)	Cronkite et al. 1989	
147	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				100 M (CEL: hepatomata, lymphomatous and myelogenous neoplasms)	Cronkite et al. 1989	
148	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M (CEL: lymphoma in 12%)	Farris et al. 1993	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
149	Mouse (C57BL, CD-1)	10 wk 5 d/wk 6 hr/d				1200 M (CEL: 46% lung adenoma on CD-1 mice)	Snyder et al. 1988	

CHRONIC EXPOSURE

Death

150	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (61% died, versus 46% in controls)	Maltoni et al. 1982a	
151	Rat (Sprague-Dawley)	691 d 5 d/wk 6 hr/d				300 M (median lifespan 51 weeks versus 65 weeks in controls)	Snyder et al. 1978a	
152	Mouse (AKR/J, C57Bl)	lifetime 5 d/wk 6 hr/d				300 M (median lifespan 11-41 weeks versus 39-75 weeks in controls)	Snyder et al. 1978a, 1980	
153	Mouse (CD-1)	222 d 5 d/wk 6 hr/d				300 M (decreased survival)	Snyder et al. 1982	

Systemic

154	Human	4 mo- 15 yr (occup)	Hemato			150 (pancytopenia)	Aksoy and Erdem 1978	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
155	Human	14 yr (Occup)	Hemato	0.55			Collins et al. 1997	The NOAEL is for abnormal hematological values, not significantly altered values that would still fall within the range of normal values.
156	Human	1-3 yr (occup)	Hemato			3 (anemia, lymphocytosis, thrombocytopenia, leukopenia, leukocytosis)	Doskin 1971	
157	Human	NS (occup)	Hemato			24 (pancytopenia, hypoplastic bone marrow)	Erf and Rhoads 1939	
158	Human	3-29 yr (occup)	Hemato		25 M (increased mean corpuscular volume)		Fishbeck et al. 1978	
159	Human	0.5-5 yr (occup)	Hemato			11 (anemia, macrocytosis, thrombocytopenia)	Goldwater 1941, Greenburg et al. 1939	
160	Human	1-25 yr (occup)	Hemato	20		75 (anemia and leukopenia)	Kipen et al. 1989	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
161	Human	6.1 yr (avg) (Occup)	Hemato		0.57[d] (reduced WBC and platelet counts, approximately 7-18% lower than control values)		Lan et al. 2004a	
162	Human	4.5-9.7 yr (mean duration)	Hemato		2.26 (reduced neutrophils and RBC counts, approximately 12% lower than controls)		Qu et al. 2002, 2003	
163	Human	6.3 yr (avg) (Occup)	Hemato		7.6 (reduced absolute lymphocyte count, approximately 16% lower than controls)		Rothman et al. 1996a, 1996b	
164	Human	1-21 yr (occup)	Hemato	0.53 M			Tsai et al. 1983	
165	Human	>1 yr (occup)	Hemato		0.69 (leukopenia)		Xia et al. 1995	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
166	Human	>1 yr (occup)	Resp		33[e] M (sore throat; nasal irritation)		Yin et al. 1987b	
					59 F (sore throat; nasal irritation)			
			Hemato	33[e] M				
				59 F				
			Renal	33[e] M				
				59 F				
			Ocular		33[e] M (eye irritation)			
					59 F (eye irritation)			
167	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d	Resp	300 M			Snyder et al. 1978a, 1984	
			Hemato			100 M (anemia, leukopenia)		
			Hepatic	300 M				
			Renal	300 M				
			Bd Wt	100 M	300 M (unspecified decreased body weight gain relative to controls)			

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation   (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
168	Mouse (AKR/J, C57BL/6J)	lifetime 5 d/wk 6 hr/d	Resp	300 M			Snyder et al. 1978a, 1980	
			Hemato			100 M (anemia, increased neutrophil levels; pancytopenia; bone marrow hypoplasia)		
			Hepatic	300 M				
			Renal	300 M				
			Bd Wt			300 M (59% decrease in weight gain)		
169	Mouse (CD-1)	222 d 5 d/wk 6 hr/d	Hemato		300 M (decreased RBCs and lymphocytes)		Snyder et al. 1982	
			Bd Wt		300 M (reduced body weight gain)			
Immuno/ Lymphoret								
170	Human	3-5 yr (occup)				11 (macrocytosis, thrombocytopenia)	Goldwater 1941, Greenburg et al. 1939	
171	Human	1-25 yr (occup)		20	75 (leukopenia)		Kipen et al. 1989	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
172	Human	>1 yr (occup)			0.69 (leukopenia)		Xia et al. 1995	
173	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d			100 M (decreased lymphocyte counts, splenic hyperplasia)		Snyder et al. 1978a, 1984	
174	Mouse (AKR/J, C57Bl)	lifetime 5 d/wk 6 hr/d			100 M (lymphocytopenia, bone marrow hypoplasia)		Snyder et al. 1978a, 1980	
175	Mouse (C57BL, CD-1)	lifetime 6 hr/d 5 d/wk			300 M (lymphopenia)		Snyder et al. 1988	
Cancer								
176	Human	4-15 yr (occup)				150 (CEL: leukemia)	Aksoy and Erdem 1978	
177	Human	28 mo- 40 yr				1 (CEL: leukemia, lymphoma)	Aksoy et al. 1987	
178	Human	1-10 yr (occup) (occup)				10 M (CEL: leukemia)	Infante 1978; Infante 1977	
179	Human	18 mo (occup)				0.3 M (CEL: leukemia)	Ott et al. 1978	

					LOAEL			
Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	Less Serious (ppm)	Serious (ppm)	Reference Chemical Form	Comments
180	Human	1-14 yr (occup)				16 M (CEL: leukemia)	Rinsky et al. 1981; Infante et al. 1977a	
181	Human	1-30 yr (occup)				200 (CEL: leukemia)	Vigliani and Forni 1976	
182	Human	>1 yr (occup)				2 (CEL: chronic erythroid leukemia)	Yin et al. 1989	
183	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (CEL: hepatomas)	Maltoni et al. 1982a	
184	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (CEL: hepatomas)	Maltoni et al. 1983, 1985	
185	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d				100 M (CEL: Zymbal gland carcinoma, myelogenous leukemia, liver tumors)	Snyder et al. 1984	
186	Mouse (AKR/J, C57BL6J)	lifetime 5 d/wk 6 hr/d				300 M (CEL: hematopoietic neoplasms [8/40], including 6 thymic lymphomas)	Snyder et al. 1978a, 1980	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
187	Mouse (C57BL, CD-1)	lifetime every 3rd wk 7 d/wk				300 M (CEL: 35% increase of Zymbal gland carcinomas in C57BL mice)	Snyder et al. 1988	

a The number corresponds to entries in Figure 3-1.

b Used to derive an acute-duration inhalation minimal risk level (MRL) of 0.009 ppm for benzene. Concentration was adjusted for intermittent exposure by multiplying by 6 hours/24 hours and converted to a human equivalent concentration, which was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric adjustment, and 10 for human variability) (see Appendix A).

c Used to derive an intermediate-duration inhalation minimal risk level (MRL) of 0.006 ppm for benzene. Concentration was adjusted for intermittent exposure by multiplying by 6 hours/24 hours and 5 days/7 days and converted to a human equivalent concentration, which was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric adjustment, and 10 for human variability) (see Appendix A).

d Study results used to derive a chronic-duration inhalation minimal risk level (MRL) of 0.003 ppm for benzene, as described in detail in Appendix A. Benchmark dose (BMD) analysis was performed on B-lymphocyte counts to select a point of departure, which was adjusted for intermittent exposure and divided by an uncertainty factor of 10 for human variability. Study results also used to derive a chronic-duration oral minimal risk level (MRL) of 0.0005 mg/kg/day based on route-to-route extrapolation, as described in detail in Chapter 2 and Appendix A. Benchmark dose (BMD) analysis was performed on B-lymphocyte counts in benzene-exposed workers to select a point of departure, which was adjusted for intermittent exposure. An equivalent oral dose was estimated based on route-to-route extrapolation to determine a point of departure for deriving a chronic-duration oral MRL for benzene, which was divided by an uncertainty factor of 30 (10 for human variability and 3 for uncertainty in route-to-route extrapolation).

e Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-1. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

AChE = acetylcholinesterase; Bd Wt = body weight; BFU-E = burst-forming units - erythroid; Cardio = cardiovascular; CEL = cancer effect level; CFU-E = colony-forming units - erythroid progenitor cells; CFU-G = colony-forming units - granulopoietic stem cells; CFU-GM = colony-forming units - macrophages; CFU-S = colony-forming units - spleen; CNS = central nervous system; d = day(s); F = female; Gd = gestational day; Hct = hematocrit; Hemato = hematological; Hgb = hemoglobin; hr = hour(s); LC50 = lethal concentration, 50% kill; Ld = lactational day(s); LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); MN-NCE = micronucleated normochromatic erythrocytes; MN-PCE = micronucleated polychromatic erythrocytes; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NRBC = nucleated red blood cells; NS = not specified; occup = occupational exposure; RBC = red blood cell; WBC = white blood cell; wk = week(s); yr = year(s)

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation
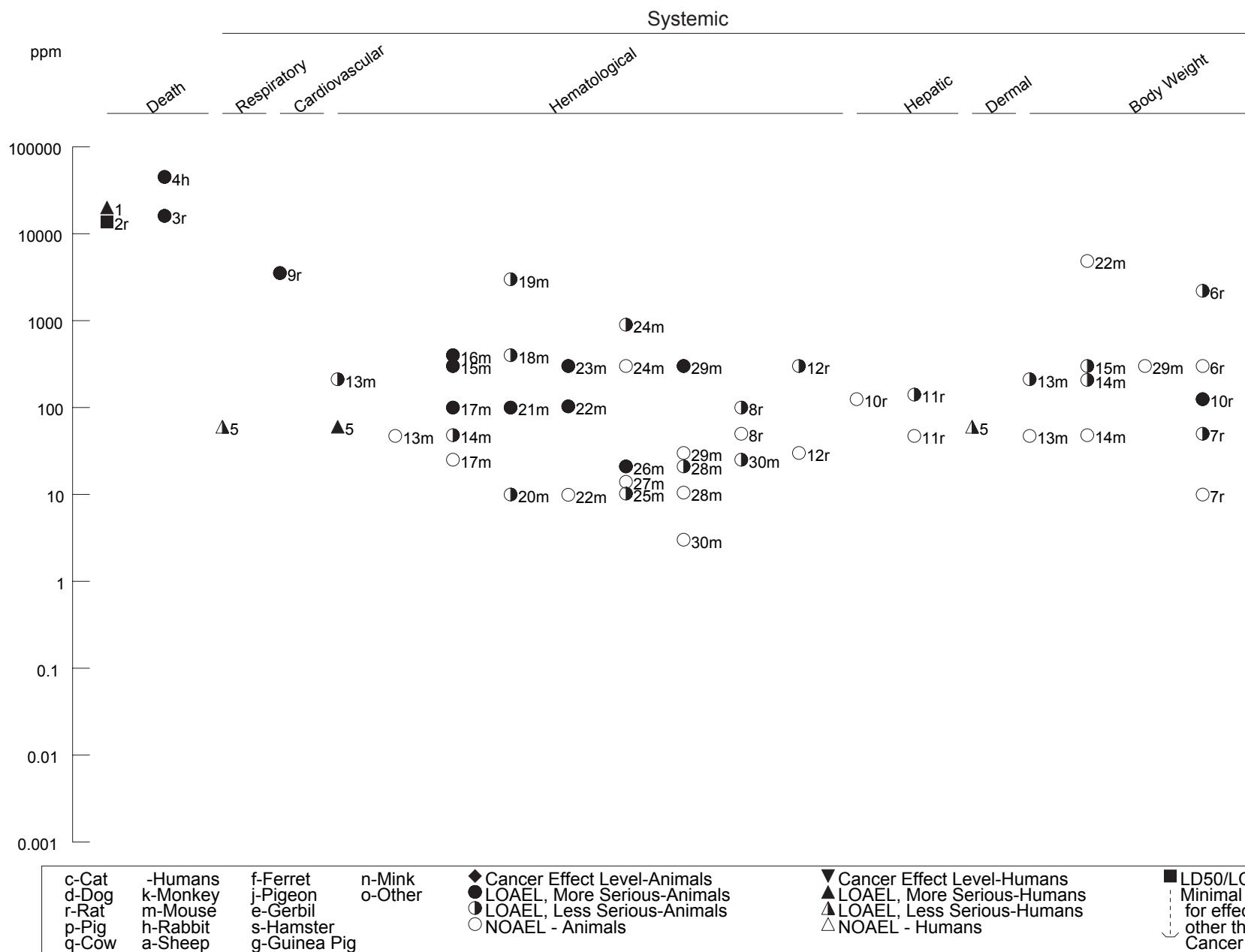
Acute (≤14 days)

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*
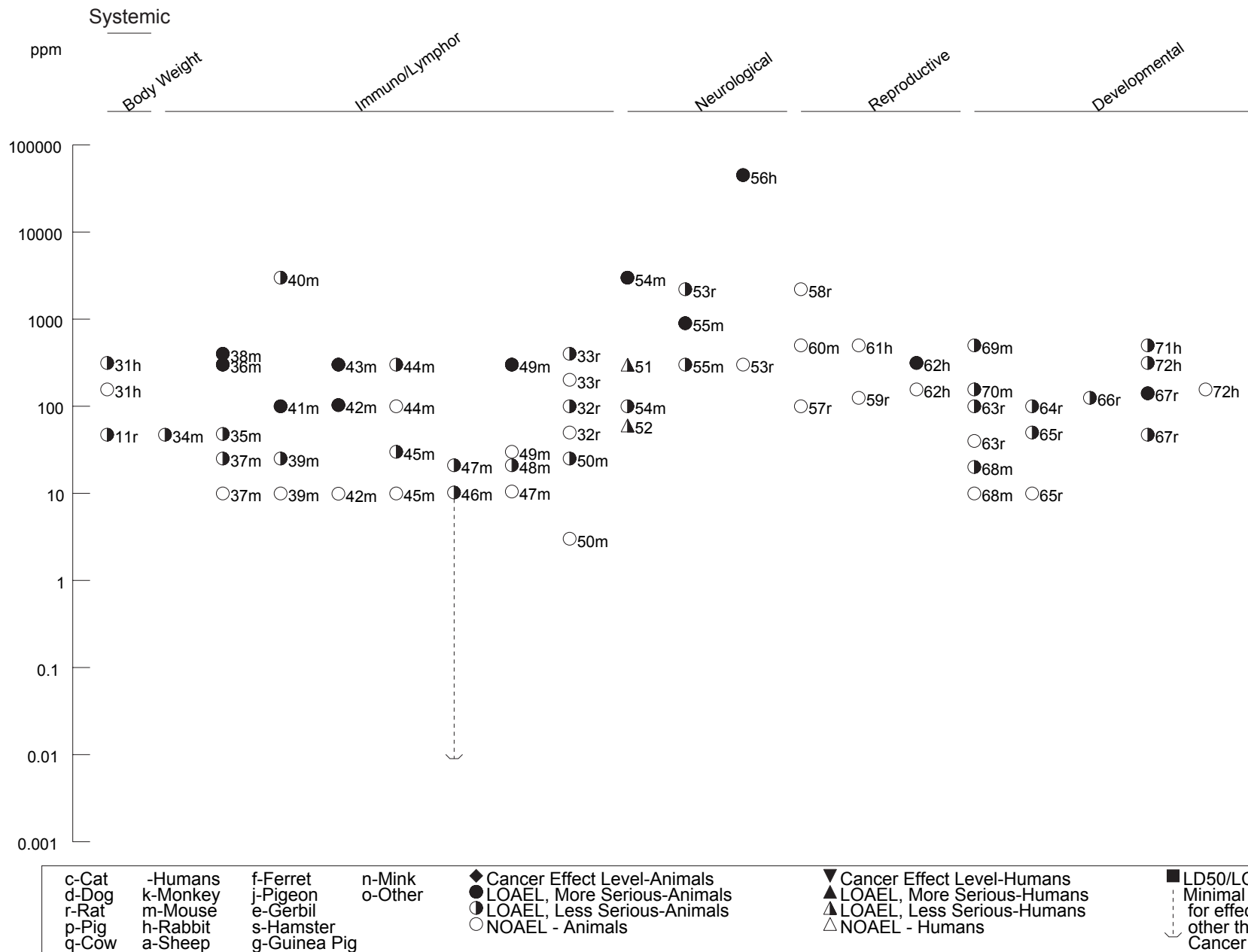
Acute (≤14 days)

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*
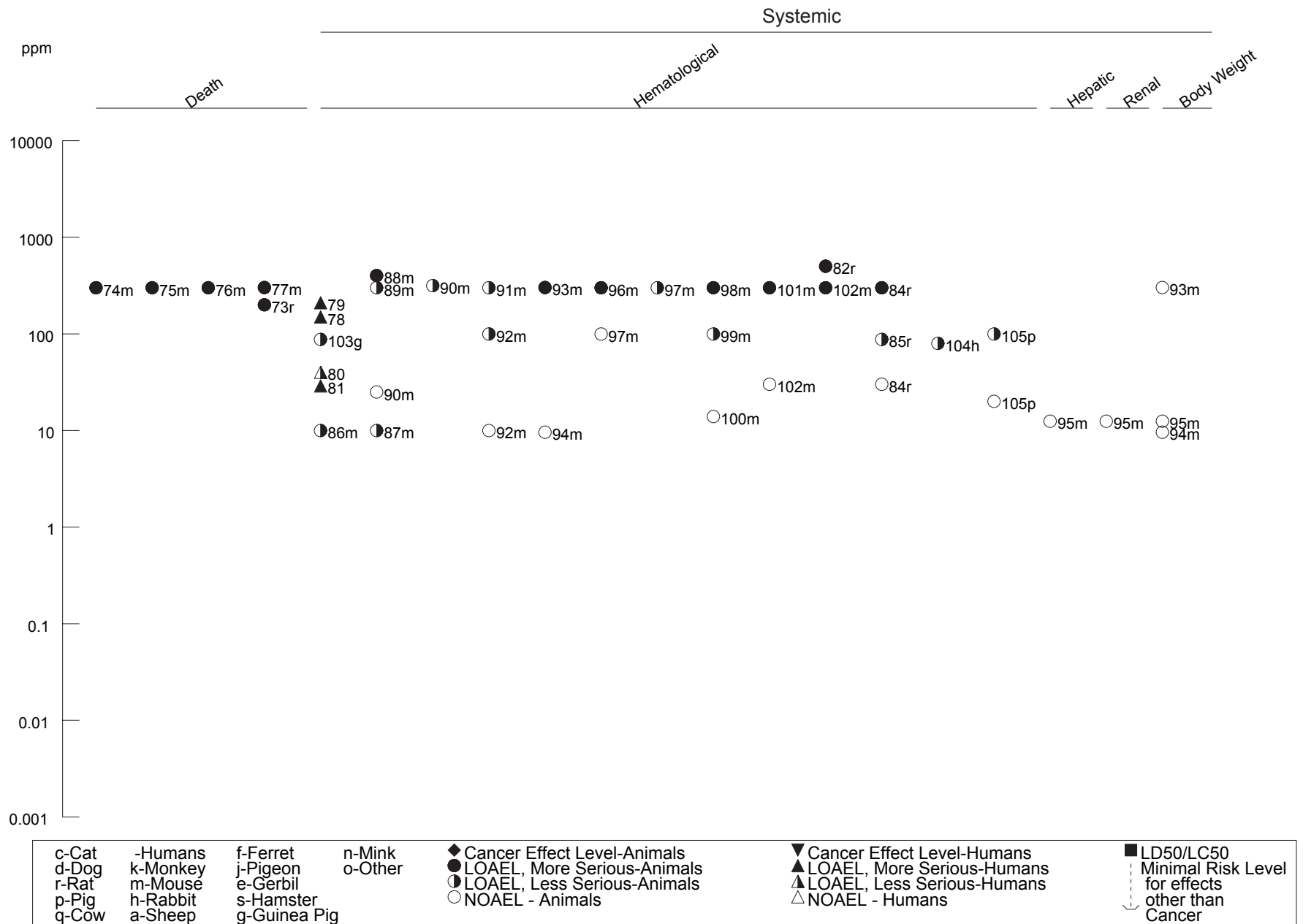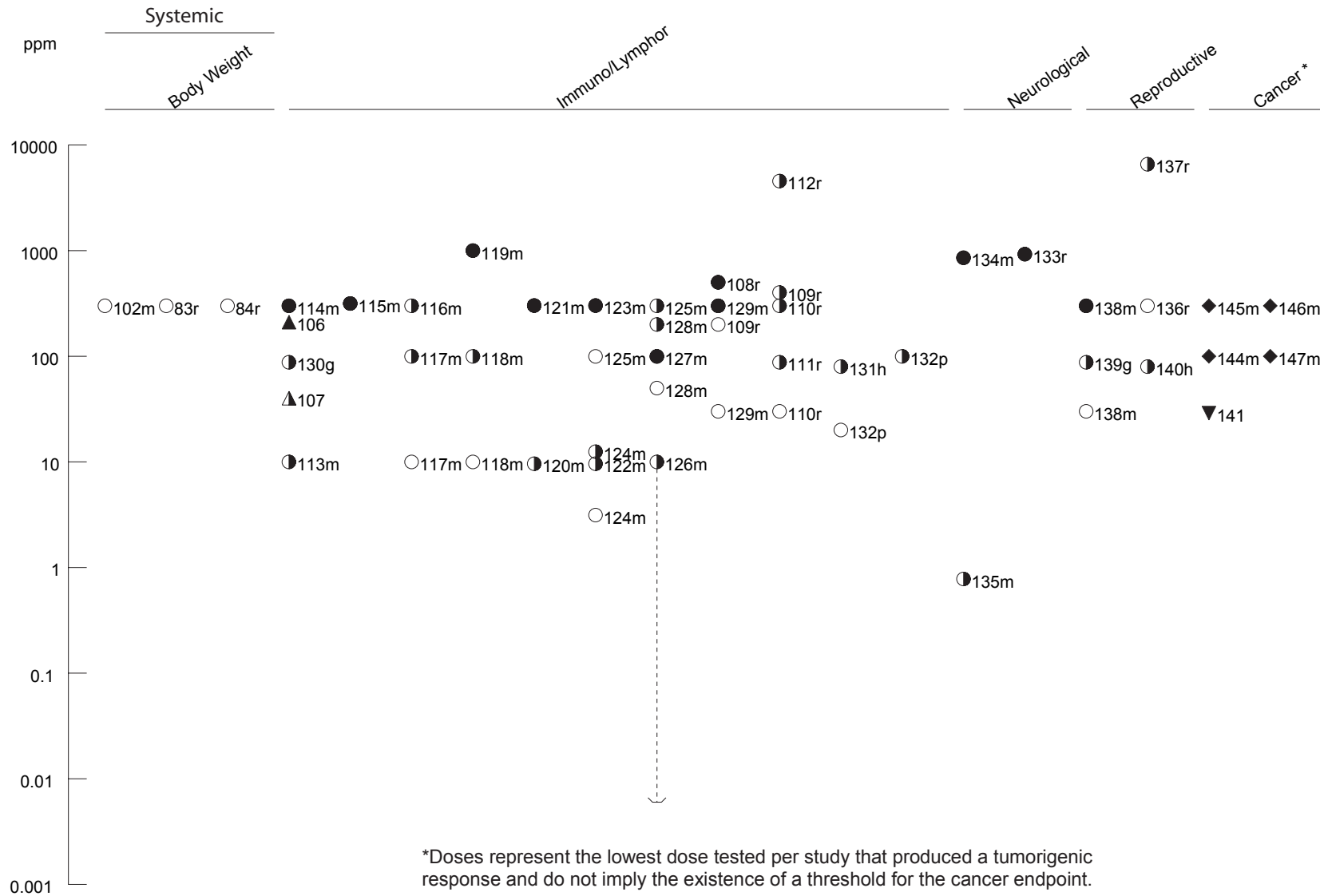
Intermediate (15-364 days)

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*
## Intermediate (15-364 days)

*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink
d-Dog	k-Monkey	j-Pigeon	o-Other
r-Rat	m-Mouse	e-Gerbil	
p-Pig	h-Rabbit	s-Hamster	
q-Cow	a-Sheep	g-Guinea Pig	

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
▲ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level
for effects
other than
Cancer

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Intermediate (15-364 days)

ppm

Cancer *

10000

1000 ◆149m

◆148m
◆142r ◆143r

100

10

1

0.1

0.01

0.001

*Doses represent the lowest dose tested per study that produced a tumorigenic
response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	¦ Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◖ LOAEL, Less Serious-Animals	▲ LOAEL, Less Serious-Humans	for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	↓ other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*
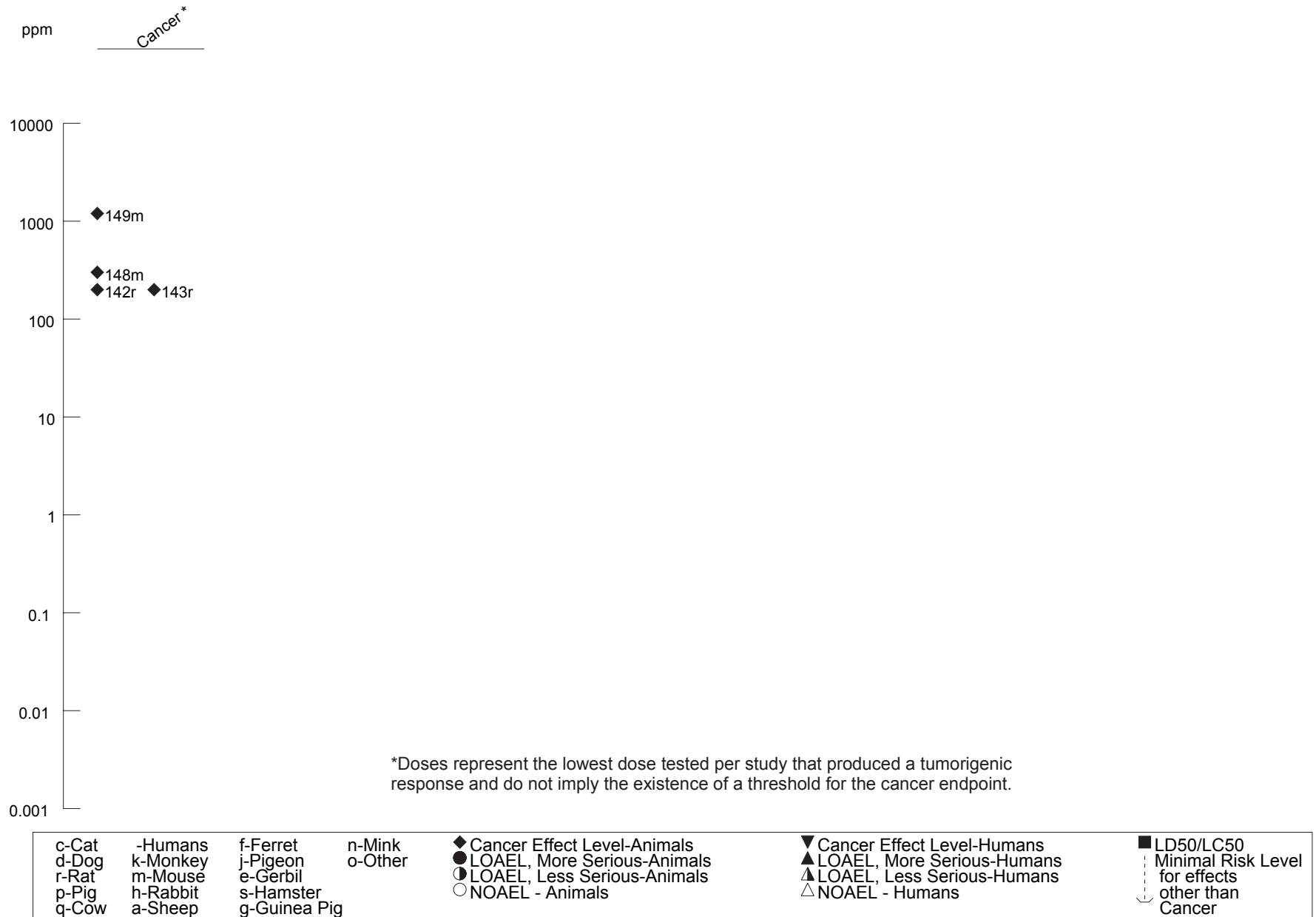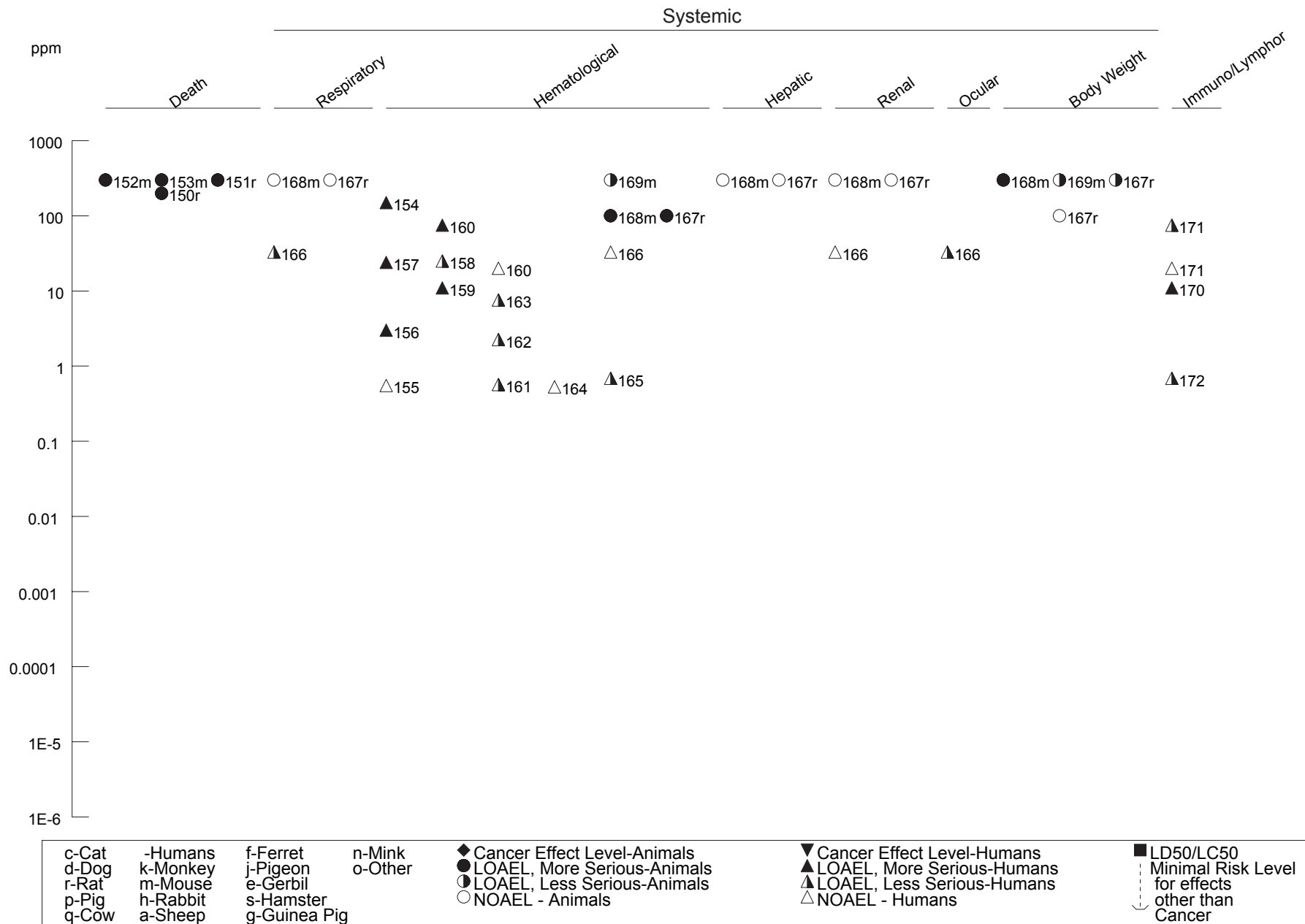
Chronic (≥365 days)

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Chronic (≥365 days)
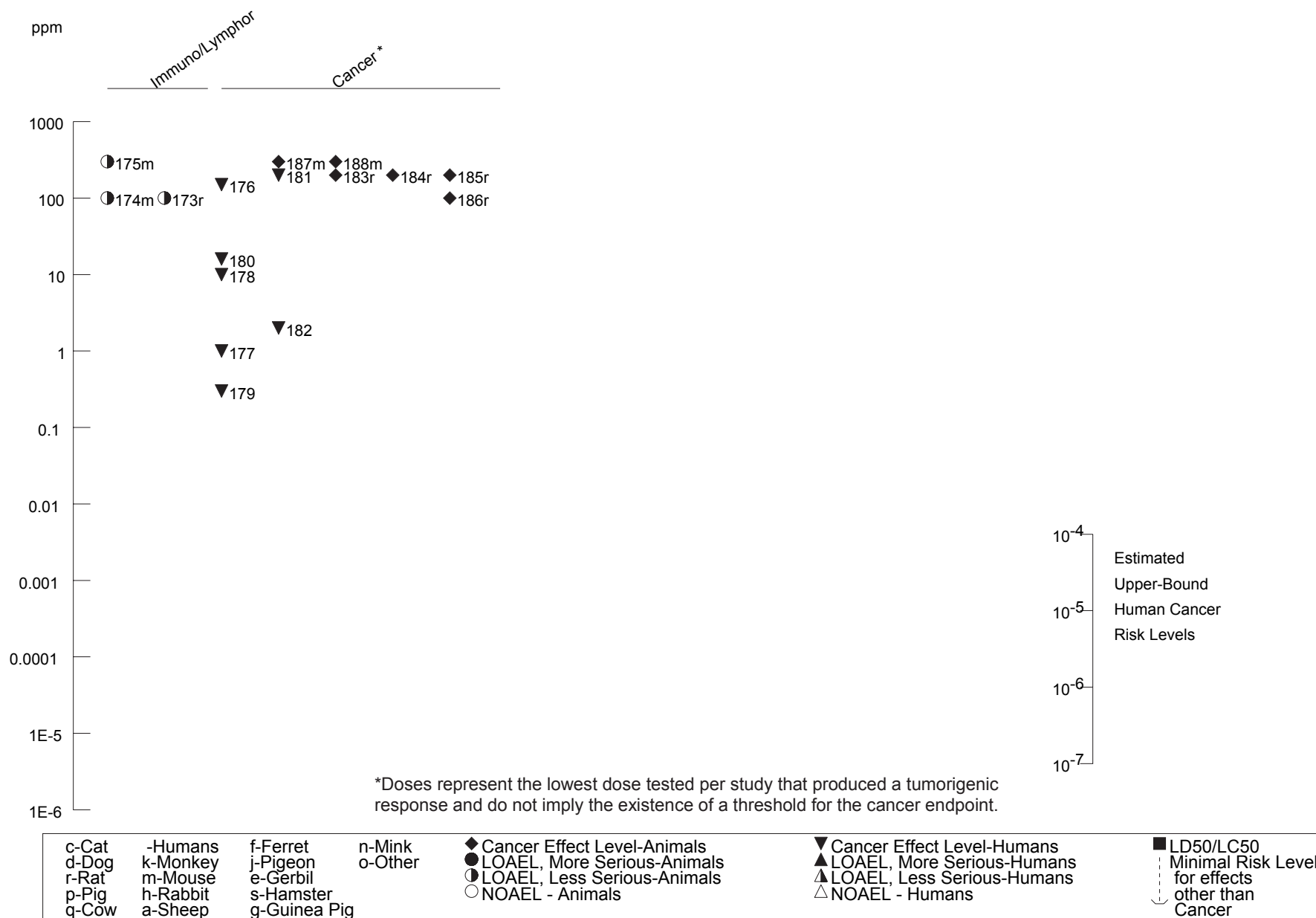
Cardiovascular Effects.   No studies were located regarding cardiovascular effects in humans after inhalation exposure to benzene, although ventricular fibrillation has been proposed as the cause of death in some human poisonings (Avis and Hutton 1993; Winek and Collom 1971).

One animal study was found that investigated the effects of acute inhalation exposure to high concentrations of benzene vapor on the heart muscle of cats and monkeys (Nahum and Hoff 1934). Information from the electrocardiograms indicated that exposure to benzene vapor caused extra systoles and ventricular tachycardia of the prefibrillation type. Animals that had their adrenals and stellate ganglias removed did not exhibit extra systoles or ventricular tachycardia. These findings suggest that the arrhythmias were caused by catecholamine release and sympathetic discharge. This study is limited in that exact levels of exposure are not available. An additional study investigated the influence of benzene inhalation on ventricular arrhythmia in the rat (Magos et al. 1990). Rats exposed to 3,526–8,224 ppm of benzene in a closed chamber for 15 minutes exhibited an increased number of ectopic ventricular beats.

Gastrointestinal Effects.   Very few data are available describing gastrointestinal effects in humans after inhalation exposure to benzene. In a case study involving the death of an 18-year-old boy who intentionally inhaled benzene, the autopsy revealed congestive gastritis (Winek and Collom 1971). No other details or data were given.

Hematological Effects.   Data regarding effects on the human hematological system following acute inhalation exposure to benzene are scant, but indicate leukopenia, anemia, and thrombocytopenia after more than 2 days of occupational exposure to more than 60 ppm benzene (Midzenski et al. 1992). Epidemiological studies on persons exposed to various levels of benzene in the workplace for intermediate and chronic periods of time also indicate hematological effects. Deficiencies in most of these studies include uncertainty in estimates of historical exposure levels, concomitant exposure to other chemicals, and lack of appropriate control groups. However, sufficient data are available to show that the hematopoietic system is a critical target for benzene toxicity. Studies that were conducted well and that show effects linked to specific exposure levels are presented in Table 3-1 and Figure 3-1. Effects on leukocytes, lymphocytes, and bone marrow are also discussed in Section 3.2.1.3.

Inhalation exposure to benzene levels in excess of regulated workplace limits (8-hour TWA of 1 ppm) for several months to several years can result in deficits in the relative numbers of circulating blood cells, which may be severe enough to be considered clinical pancytopenia. Continued exposure to benzene can

also result in aplastic anemia or leukemia (Aksoy et al. 1974; EPA 1995; Hayes et al. 1997; IARC 1982, 1987; IRIS 2007; Rinsky et al. 1987, 2002; Yin et al. 1987c, 1996a, 1996b).

Pancytopenia is the reduction in the number of all three major types of blood cells: erythrocytes (red blood cells), thrombocytes (platelets), and leukocytes (white blood cells). In adults, all three major types of blood cells are produced in the red bone marrow of the vertebrae, sternum, ribs, and pelvis. The red bone marrow contains immature cells, known as multipotent myeloid stem cells, that later differentiate into the various mature blood cells. Pancytopenia results from a reduction in the ability of the red bone marrow to produce adequate numbers of these mature blood cells.

Aplastic anemia is a more severe effect of benzene and occurs when the bone marrow ceases to function and the stem cells never reach maturity. Depression in bone marrow function occurs in two stages— hyperplasia (increased synthesis of blood cell elements), followed by hypoplasia (decreased synthesis). As the disease progresses, bone marrow function decreases and the bone marrow becomes necrotic and filled with fatty tissue. This myeloblastic dysplasia without acute leukemia has been seen in persons exposed to benzene (Erf and Rhoads 1939). Aplastic anemia can progress to a type of leukemia known as acute myelogenous leukemia (Aksoy 1980), which is discussed in Section 3.2.1.7.

Early biomarkers of exposure to relatively low levels of benzene include depressed numbers of one or more of the circulating blood cell types. For example, statistically significantly decreased total red blood cells (RBCs), white blood cells (WBCs), absolute lymphocyte count, platelets, and hematocrit were reported for a group of 44 healthy subjects exposed to benzene in the workplace (median 8-hour time-weighted average [TWA] of 31 ppm; minimal exposure to other solvents) for an average of 6.3 years in China (Rothman et al. 1996a, 1996b). Age- and gender-matched workers with no history of occupational exposure to benzene served as controls. Among the 22 workers whose mean 5-day benzene exposure levels did not exceed 31 ppm (median 8-hour TWA of 13.6 ppm), significantly depressed absolute lymphocyte count, RBCs, and platelets were noted. Only absolute lymphocyte count was significantly decreased in a subgroup of 11 workers with no 8-hour TWA exceeding 31 ppm (median 8-hour TWA of 7.6 ppm).

Qu et al. (2002, 2003a, 2003b) compared hematology values in a group of 130 chronically exposed workers in China with those obtained from 51 age- and gender-matched subjects without occupational exposure to benzene. Statistically significant trends for depressed RBCs, WBCs, and neutrophils were observed in the benzene-exposed workers (average measured 4-week benzene exposure levels ranged

from 0.08 to 54.5 ppm just prior to blood testing). A subgroup of 73 of these workers, whose average 4-week benzene exposure level was 2.26 ppm, exhibited significantly depressed RBCs and neutrophils. This study provided exposure-response data, but apparent discrepancies in reported low-concentration results render the study of limited value for MRL derivation.

One recent cross-sectional study (Lan et al. 2004a, 2004b), performed on 250 workers exposed to benzene in shoe manufacturing industries in Tianjin, China, and 140 age- and gender-matched workers in clothing manufacturing facilities that did not use benzene, was of sufficient quality to serve as the basis for deriving a chronic-duration inhalation MRL for benzene (see footnote to Table 3-1 and Appendix A). The benzene-exposed workers had been employed for an average of 6.1±2.9 years. Controls consisted of 140 age-and gender-matched workers in clothing manufacturing facilities in which measurable benzene concentrations were not found (detection limit 0.04 ppm). Benzene exposure was monitored by individual organic vapor monitors (full shift) 5 or more times during 16 months prior to phlebotomy. Benzene-exposed workers were categorized into four groups (controls, <1, 1–<10, and ≥10 ppm) according to mean benzene exposure levels measured during 1 month prior to phlebotomy. Complete blood count (CBC) and differential were analyzed mechanically. Coefficients of variation for all cell counts were <10%.

Mean 1-month benzene exposure levels in the four groups (controls, <1, 1–<10, and ≥10 ppm) were <0.04, 0.57±0.24, 2.85±2.11, and 28.73±20.74 ppm, respectively. Hematological values were adjusted to account for potential confounding factors (i.e., age, gender, cigarette smoking, alcohol consumption, recent infection, and body mass index). All types of WBCs and platelets were significantly decreased in the lowest exposure group (<1 ppm), ranging in magnitude from approximately 8 to 15% lower than controls. Although similar statistical analyses for the mid- and high-exposure groups were not included in the study report, decreases in all types of WBCs and platelets were noted at these exposure levels as well; the decreases in the highest exposure group ranged in magnitude from 15 to 36%. Lymphocyte subset analysis revealed significantly decreased CD4+-T cells, CD4+/CD8+ ratio, and B cells. Hemoglobin concentrations were significantly decreased only within the highest (≥10 ppm) exposure group. Tests for a linear trend using benzene air level as a continuous variable were significant for platelets and all WBC measures except monocytes and CD8+-T cells. Upon restricting the linear trend analyses to workers exposed to <10 ppm benzene, excluding controls, inverse associations remained for total WBCs, granulocytes, lymphocytes, B cells, and platelets. In order to evaluate the effect of past benzene exposures on the hematological effects observed in this study, the authors compared findings for a group of workers who had been exposed to <1 ppm benzene over the previous year (n=60) and a subset

who also had <40 ppm-years lifetime cumulative benzene exposure (n=50). The authors stated that the same cell types were significantly reduced in these groups, but did not provide further information of the magnitude (i.e., percent change) of the hematological effects observed. These data suggest that the 1-month benzene exposure results could be used as an indicator of longer-term low-level benzene hematotoxicity. To demonstrate that the observed effects were attributable to benzene, significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells were noted in a subgroup (n=30; mean 1-month exposure level of 0.29±0.15 ppm) of the <1 ppm group for which exposure to other solvents was negligible. Lan et al. (2004a, 2004b) also presented information on the effect of benzene on colony forming progenitor cells (data were only presented for the mid- and high-exposure groups). Benzene exposure was associated with a concentration-dependent decrease in colony formation and progenitor cells were suggested to be more sensitive than circulating cells.

As described above, several epidemiology studies compared hematological variables for benzene-exposed workers to gender- and age-matched controls and noted hematological effects at relatively low concentrations (well below 1 ppm in some exposure groups) (Lan et al. 2004a, 2004b; Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b). An alternative approach was used by Collins et al. (1991, 1997) and Tsai et al. (1983, 2004). This approach utilized a defined range of clinically normal hematological values and compared the prevalence of abnormal results between benzene-exposed workers and unexposed controls. Collins et al. (1991) found no significant correlations between benzene exposure and the prevalence of abnormal hematological values among 200 workers exposed to benzene at estimated concentrations ranging from 0.01 to 1.4 ppm, relative to the prevalence of abnormal hematological values obtained from 268 unexposed workers in the same plant. In a more recent evaluation, Collins et al. (1997) found no significant correlation between exposure to benzene at an 8-hour TWA of 0.55 ppm and prevalence of clinically-defined lymphopenia (or other measures of hematotoxicity including mean corpuscular volume [MCV], and counts of WBCs, RBCs, hemoglobin, and platelets) among a group of 387 workers exposed for ≥5 years. Tsai et al. (1983) measured benzene levels in a Texas refinery at one time point (mean benzene concentration of 0.53 ppm) and found that hemoglobin, hematocrit, RBCs, WBCs, and thrombocytes of workers exposed for up to 21 years were within the range of normal values. Tsai et al. (2004) found no clinically adverse hematotoxic effects among a large group of 1,200 petrochemical employees with mean 8-hour TWA benzene exposure levels of 0.6 ppm from 1977 to 1988 and 0.14 ppm from 1988 to 2002. The normal range for certain hematological parameters is necessarily broad due to large interindividual differences in clinical status. Restricting the comparison of benzene-exposed and nonexposed populations to only those values

considered clinically abnormal or adverse may reduce the sensitivity of the study to detect meaningful changes at the population level.

Many reports of hematological effects in benzene-exposed workers involve estimated exposure levels well in excess of 1 ppm. A series of studies conducted on Turkish workers exposed to benzene-containing adhesives in various occupations showed increased severity of effects with increased levels or duration of exposure. The initial hematological study examined 217 male workers who were exposed for between 4 months and 17 years to benzene-containing solvents (Aksoy et al. 1971). The concentration of benzene in the work area ranged from 15 to 30 ppm outside work hours and reached a maximum of 210 ppm when benzene-containing adhesives were being used. Fifty-one of the workers showed clinical hematological abnormalities such as leukopenia, thrombocytopenia, eosinophilia, and pancytopenia. An additional cohort was identified that included 32 shoe manufacturers who had worked with benzene for 4 months to 15 years at concentrations of 15–30 ppm outside work hours and 210–640 ppm during the use of benzene, and who showed pancytopenia (Aksoy et al. 1972). Examination of these people revealed disruptions in bone marrow function, including cases of hypoplastic, acellular, hyperplastic, or normoblastic bone marrow. The continuing assessment further identified that ineffective erythropoiesis or increased hemolysis may have been responsible for the reticulocytosis, hyperbilirubinemia, erythroblastemia, increase in quantitative osmotic fragility, and elevated serum lactate dehydrogenase levels observed in some patients.

Leukopenia, lymphocytosis, a biphasic leukocyte response, and bone marrow hypercellularity were reported for workers in a Russian industry who were exposed to a complex of hydrocarbons that included benzene, cyclohexane, and 1,3-butadiene (Doskin 1971). Benzene levels were estimated at 3.2–12.8 ppm, which are 2–8 times the Russian standard. Leukopenia was observed by Xia et al. (1995) in Chinese workers exposed to 0.69–140 ppm (mean=6 ppm) benzene for more than 1 year. Enlarged RBCs, transient anemia, reduced hemoglobin concentrations, and clinically increased MCV were reported in workers in a chemical factory who were exposed to over 25 ppm of benzene in the workplace for an average of 9 years (Fishbeck et al. 1978).

Dosemeci et al. (1996) assessed the relative risk of abnormal hematological values with increasing benzene levels in a study of workers employed in 672 rubber and rubber glue application facilities in China between 1949 and 1987. Compared to workers with estimated exposures <5 ppm, relative risks of abnormal hematological values (indicating benzene poisoning) by estimated exposure intensity at 1.5 years prior to clinically-diagnosed benzene poisoning were 2.2 (95% confidence interval [CI] 1.7–

2.9), 4.7 (95% CI 3.4–6.5), and 7.2 (95% CI 5.3–9.8) for exposures in the ranges of 5–19, 20–39, and >40 ppm, respectively.

Examination of individual hematological records, demographic data, and chronological work histories of 459 workers employed at a rubber products manufacturing plant in Ohio between 1940 and 1975 revealed significant decreases in WBC and RBC counts and hemoglobin for the period of 1940–1948 when the exposure was 75 ppm (Kipen et al. 1989). The trend was not apparent during later years (1949–1975) when the exposure was decreased to 15–20 ppm. A more focused study of many of the same workers during their first year of employment revealed significantly lower WBC and RBC counts in employees exposed to benzene levels greater than the median (estimated at 40–54 ppm), compared to those with lower estimated exposure levels (Cody et al. 1993). Using a nested case-control approach at the same rubber products manufacturing plant to assess a possible exposure-response relationship between benzene and the risk of developing a low WBC or RBC count in workers for whom hematologic screening data were available, Ward et al. (1996) reported a strong correlation between low WBC counts and benzene exposure and a weak correlation between RBC count and benzene exposure.

More severe effects, including preleukemia or acute leukemia, were observed in 26 out of 28,500 benzene workers exposed to 210–650 ppm for 1–15 years (Aksoy et al. 1974). Clinical features of the preleukemia included one or more of the following: anemia, leukopenia, pancytopenia, bone marrow hyperplasia, pseudo-Pelger-Huet anomaly, and splenomegaly. A study was conducted 2–17 years following the last exposure of 44 pancytopenic patients exposed to benzene (150–650 ppm) in adhesives for 4 months to 15 years (Aksoy and Erdem 1978). Of these patients, complete remission was seen in 23, death due to complications of pancytopenia in 14, death due to myeloid metaplasia in 1, and leukemia in 6. When benzene concentrations in factories decreased in later years, less severe effects were seen. At 40 tire manufacturing plants, 231 workers were exposed for 28 months to 40 years (mean 8.8 years) to benzene-containing solvents and thinners (Aksoy et al. 1987). The decrease in benzene content of materials used in these workshops and the corresponding reduction in air concentration (most samples <1 ppm) paralleled the decrease in the number of hematological abnormalities reported for benzene-exposed workers.

Another study revealed effects, ranging from mild to severe, of benzene exposure in factory workers in China (Yin et al. 1987c). Of the 528,729 workers, 95% were exposed to mixtures of benzene, toluene, and xylene, while 5% (26,319 workers) were exposed to benzene alone at 0.02–264 ppm in air in 95% of the work stations. Over half of the work stations had levels of benzene in the air of less than 13 ppm;

about 1% had levels of 13–264 ppm. Benzene toxicity, as indicated by leukopenia, aplastic anemia, and leukemia, was seen in 0.94% of the workers exposed to benzene and 0.44% of the workers exposed to the mixtures. Similar toxicity was found in employees of 28 of the 141 shoe factories studied (124 cases in 2,740 employees) (Yin et al. 1987c). A positive correlation was observed for prevalence of adverse benzene effects and benzene concentration in data from these 28 shoe factories. The authors determined that the affected people were exposed to benzene concentrations >29 ppm. In one workshop, there were 4 cases of aplastic anemia in 211 workers. These workers were exposed to benzene at a mean concentration of 324 ppm during an 8-month period of employment. The prevalence of aplastic anemia in the shoe-making industry was about 5.8 times that in the general population. The main limitation of this study is the lack of information on the duration of exposure.

People who were exposed to high levels of benzene vapors in the printing industry also showed severe hematological effects. One study evaluated 332 workers who were exposed to 11–1,060 ppm of benzene for 6 months to 5 years. Detailed blood studies performed on 102 of these workers revealed benzene poisoning in 22 workers characterized by pancytopenia or other clinical signs (Goldwater 1941; Greenburg et al. 1939). Another study reported 6 cases of pancytopenia and bone marrow dysplasia in printers exposed to 24–1,060 ppm of benzene (Erf and Rhoads 1939).

Animal studies have been designed to characterize exposure-response relationships of benzene hematotoxicity; the results provide support to the human data. Animal responses to benzene exposure are variable and may depend on factors such as species, strain, duration of exposure, and whether exposure is intermittent or continuous. Wide variations have also been observed in normal hematological parameters, complicating statistical evaluation. However, the studies show that benzene exerts toxic effects at all phases of the hematological system, from stem cell depression in the bone marrow, to pancytopenia, to histopathological changes in the bone marrow. Effects on leukocytes, lymphocytes, and bone marrow are also discussed in Section 3.2.1.3.

Repeated acute-, intermediate-, and chronic-duration inhalation exposure of laboratory animals (mainly mice, but also rats, rabbits, and guinea pigs) to benzene vapor concentrations ranging from 10 to >300 ppm demonstrate significant decreases in blood values that include RBCs, total WBCs, lymphocytes, granulocytes, hematocrit, and hemoglobin (Aoyama 1986; Baarson et al. 1984; Chertkov et al. 1992; Cronkite et al. 1982; Farris et al. 1997a, 1997b; Gill et al. 1980; Green et al. 1981a, 1981b; Li et al. 1986; Rozen and Snyder 1985; Rozen et al. 1984; Snyder et al. 1982; Ward et al. 1985; Wells and Nerland 1991; Wolf et al. 1956). Pancytopenia was noted in the study of Ward et al. (1985).

Short-duration exposure of mice to benzene has been shown to cause both concentration- and duration-related reductions in bone marrow cellularity (number of nucleated cells) and the number of colony-forming granulopoietic stem cells (CFU-C), and an increased frequency of micronucleated polychromatic erythrocytes (MN-PCE) (Toft et al. 1982). Mice that were exposed continuously at benzene concentrations ≥21 ppm in air for 4–10 days showed significant changes in all three parameters. Intermittent exposure (8 hours/day, 5 days/week for 2 weeks) to 21 ppm significantly reduced the number of CFU-C and elevated the frequency of MN-PCE, but did not affect bone marrow cellularity. Intermittent exposure at concentrations ≥50 ppm caused significant changes in all three parameters and decreased the ability of the spleen to form mature cells (as measured by numbers of colony-forming units of stem cells). When mice were intermittently exposed for 2 weeks, decreased cellularity and CFU-C per tibia were observed at 95 ppm after 6–8 hours/day exposure, whereas at 201 ppm benzene, decreased cellularity (but no effect on CFU-C) was noted after 2 hours/day exposure. A decrease in cellularity and CFU-C was observed after 4–8 hours/day exposure to 201 ppm benzene.

Results of numerous additional studies of laboratory animals (mainly mice, which are particularly sensitive to benzene hematotoxicity, but also rats and pigs) support findings of benzene-induced effects on bone marrow cellularity (hyper- and/or hypocellularity) and colony-forming stem cells, as well as granulocytic hyperplasia, following repeated acute-, intermediate-, or chronic-duration inhalation exposure to benzene vapors at concentrations ranging from 10 to 500 ppm (Baarson and Snyder 1991; Baarson et al. 1984; Chertkov et al. 1992; Corti and Snyder 1996; Cronkite et al. 1982, 1985, 1989; Dempster and Snyder 1991; Dow 1992; Farris et al. 1993, 1997b; Neun et al. 1992, 1994; Plappert et al. 1994a, 1994b; Snyder et al. 1978a, 1980, 1982; Vacha et al. 1990). For example, Dempster and Snyder (1991) observed a 50% reduction in CFU-E (erythroid progenitor cells) in bone marrow of DBA/2 mice exposed to 10 ppm benzene for 6 hours/day for 5 days. Farris et al. (1997b) reported benzene-induced decreased numbers of total bone marrow forming cells, progenitor cells, and differentiating hematopoietic cells in mice exposed to benzene vapor concentrations ≥200 ppm, 6 hours/day, 5 days/week for up to 8 weeks. Replication of primitive progenitor cells in the bone marrow was increased during the exposure period, presumably as compensation for cytotoxicity. Granulocytic hyperplasia was detected in the bone marrow of mice exposed to 300 ppm benzene in air for 6 hours/day, 5 days/week for 16 weeks, and held 18 months after the last exposure (Farris et al. 1993). Prolonged exposure to lower levels had a greater hematotoxic effect than exposure to higher levels for a shorter period of time. Recovery from hematotoxicity has been demonstrated following the cessation of exposure (Cronkite et al. 1982, 1985, 1989) and may be most closely associated with rate of exposure, since longer-term exposure of mice to

316 ppm of benzene caused irreparable hematologic injury, while the hematotoxic effects caused by shorter-term exposure to 10 times higher benzene concentrations were reversible (Cronkite et al. 1989).

One study revealed damaged erythrocytes in the peripheral blood of mice (Luke et al. 1988b). Cytotoxic damage in the bone marrow was dependent on strain and exposure duration. Peripheral blood smears were analyzed weekly from three strains of mice (DBA/2, B6C3F$_1$, and C57BL/6) exposed to 300 ppm benzene for 13 weeks (6 hours/day) for either 5 days/week (Regimen 1) or 3 days/week (Regimen 2). In all three strains, an initial severe depression in rate of erythropoiesis was observed. The return to normal was dependent on strain (Luke et al. 1988b) and regimen (Cronkite et al. 1989; Luke et al. 1988b). An increase in frequency of micronucleated normochromatic erythrocytes (MN-NCE) was observed to be dependent on strain (C57BL/6=B6C3F$_1$>DBA/2) and regimen (Regimen 1 > Regimen 2), whereas the increase in frequency of MN-PCE was dependent on strain (DBA/2>C57BL/6=B6C3F$_1$) but, for the most part, was not dependent on exposure regimen.

Damaged erythroblast-forming cells were also noted in bone marrow (Seidel et al. 1989). A substantial decrease in erythroid colony-forming units and smaller decreases in erythroid burst-forming units occurred in BDF$_1$ mice intermittently exposed to 100 ppm of benzene for 8 weeks (6 hours/day, 5 days/week). Although the effects in the 100 ppm group were not apparent at 8 weeks (the end of experiment), they did occur, and it took over 3 weeks for the erythroid burst-forming units and erythroid colony-forming units to return to their initial values. This reduction in the number of erythroid precursors was reflected by a slight reduction in the number of erythrocytes.

Benzene-induced hematotoxicity was also demonstrated in the spleen of rats and mice following intermediate- or chronic-duration repeated inhalation exposure (Snyder et al. 1978a, 1984; Ward et al. 1985). Snyder et al. (1978a, 1984) reported benzene-induced increased extramedullary hematopoiesis in the spleen. Ward et al. (1985) noted that the finding of hemosiderin in the spleen of benzene-exposed rats could be due to erythrocyte hemolysis.

Musculoskeletal Effects.   A case of myelofibrosis was diagnosed in a 46-year-old man in October 1992 (Tondel et al. 1995). The patient worked from 1962 to 1979 as a gasoline station attendant. The patient was referred to the Department of Hematology, University Hospital in Linkoping, Sweden, where a bone marrow biopsy was performed. The patient described symptoms of increasing muscle pain for 1 year, fatigue for 3 weeks, night sweats, and weight loss. A bone marrow biopsy showed myelofibrosis. The TWA concentration for gasoline station attendants was estimated to be <0.2 ppm. The occupational

standard for benzene in Sweden was 0.5 ppm (TWA) and the Swedish short-term exposure limit was
3 ppm. Ruiz et al. (1994) reported musculoskeletal effects in employees from a steel plant of Cubatao,
S. Paulo, Brazil, who presented with neutropenia due to benzene exposure. Patients either were employed
at the steel plant (mean time of 7 years and 4 months), or were employees of a building construction
company working at repairs in the steel plant (mean time of 5 years and 5 months). Sixty percent of the
workers had nonspecific clinical complaints such as myalgia.

Hepatic Effects.   No specific reports of adverse hepatic effects of inhalation exposure to benzene in
humans were found, although Aksoy et al. (1972) reported enlarged livers in workers chronically exposed
to benzene at airborne concentrations ranging from 150 to 650 ppm.

CFY rats (20/group) were exposed to pure air, benzene (125 ppm) or benzene (400 ppm) and toluene
(265 ppm) for 24 hours/day from gestational day (Gd) 7 through 14 (Tatrai et al. 1980a). The rats were
then sacrificed on day 21 of pregnancy. Exposure to 125 ppm benzene caused a slight increase in relative
liver weight of 4.67% compared to 4.25% in controls, which was not considered adverse. In a companion
study, CFY rats were exposed to continuous benzene inhalation 24 hours/day from day 7 to day 14 of
gestation at 0, 47, 141, 470, or 939 ppm atmospheric concentrations (Tatrai et al. 1980b). At 141 ppm
benzene, there was a significant increase in relative maternal liver weight.

No treatment-related non-neoplastic histopathological effects on hepatic tissue were found in male
Sprague-Dawley rats exposed to 0, 100, or 300 ppm benzene 5 days/week, 6 hours/day for life (Snyder et
al. 1978a, 1984) or in AKR/J or C57B1/6J mice similarly exposed to 300 ppm for life (Snyder et al.
1978a, 1980).

Renal Effects.   Very little data are available describing renal effects in humans after inhalation
exposure to benzene. In a case study involving the death of an 18-year-old boy who intentionally inhaled
benzene, the autopsy revealed acute kidney congestion (Winek and Collom 1971). No other details or
data were given.

No treatment-related histopathological effects on kidney tissue were found in male Sprague-Dawley rats
were exposed to 0, 100, or 300 ppm benzene 5 days/week, 6 hours/day for life (Snyder et al. 1978a, 1984)
or in AKR/J or C57B1/6J mice similarly exposed to 300 ppm (Snyder et al. 1978a, 1980).

Dermal Effects.   Dermal effects in humans have been reported after acute exposure to benzene vapors (Avis and Hutton 1993). After a fatal occupational exposure to benzene vapors on a chemical cargo ship for only minutes, autopsy reports on three victims revealed hemorrhagic respiratory tissues, and second degree burns on the face, trunk, and limbs (Avis and Hutton 1993). Skin irritation has been noted at occupational exposures of >60 ppm for up to 3 weeks (Midzenski et al. 1992). These effects are due to direct contact of the skin with the vapor, and other dermal effects resulting from direct contact of the skin are discussed in Section 3.2.3.2.

Ocular Effects.   Three hundred solvent workers who had inhalation exposures for >1 year to benzene at 33 and 59 ppm for men and women, respectively, complained of eye irritation (Yin et al. 1987b).

Male Charles River CD rats exposed to 0, 1, 10, 30, or 300 ppm benzene 6 hours/day, 5 days/week for 10 weeks exhibited lacrimation at concentrations >10 ppm during the first 3 weeks of treatment (Shell 1980).

These effects are due to direct contact of the eyes with the vapor, and other ocular effects resulting from direct contact of the eyes are discussed in Section 3.2.3.2.

Body Weight Effects.   Relatively few studies in animals report changes in body weight after inhalation exposure to benzene. No change in body weight was noted in Sprague-Dawley rats or CD-1 mice exposed to 300 ppm benzene for 13 weeks (Ward et al. 1985). No significant decrease in body weight was observed in CD-1 mice exposed to doses up to 4,862 ppm for 6 hours/day, for 5 days, or at lower doses of 9.6 ppm for 50 days (Green et al. 1981b). Decreases in body weight (15%) were seen in DBA/2 mice after exposure to 300 ppm benzene in air for 6 hours/day, 5 days/week for 2 weeks (Chertkov et al. 1992). Decreased body weight (16–18%) has also been noted in mice exposed to doses of approximately 200 ppm of benzene for 6 hours/day for 7 or 14 days (Aoyama 1986). C57BL mice exhibited a 59% decrease in body weight gain after exposure to 300 ppm benzene, 5 hours/day, 6 days/week over their lifetime (Snyder et al. 1980). Decreased maternal body weight and weight gain have been observed in Sprague-Dawley rats exposed to 50 ppm benzene during gestation days (Gds) 6-15 (Kuna and Kapp 1981), but not in rats exposed to doses up to 300 ppm during premating, mating, gestation, and lactation (Kuna et al. 1992). CFY rats (20/group) were exposed to pure air, benzene (125 ppm), or benzene (400 ppm) plus toluene (265 ppm) for 24 hours/day from Gd 7 through 14 (Tatrai et al. 1980a). The rats were then sacrificed on day 21 of pregnancy. Exposure to 125 ppm benzene caused decreased maternal weight gain (46.74% of starting weight as opposed to 68.82% of starting

weight in controls). Decreased maternal weight gain was also observed in a companion experiment in which the rats were exposed to doses as low as 47 ppm using the same study design (Tatrai et al. 1980b). Decreased maternal body weight was also observed in rats exposed to 2,200 ppm benzene during gestation (Green et al. 1978). Rabbits exposed to 313 ppm benzene on Gd 7–20 exhibited decreased maternal weight gain (Ungvary and Tatrai 1985). Kunming mice exposed to 12.52 ppm benzene for 2 hours/day, 6 days/week for 30 days exhibited no adverse effect on body weight (Li et al. 1992). Sprague-Dawley rats received 100 or 300 ppm benzene vapor for 6 hours/day, 5 days/week for life (Snyder et al. 1978a, 1984). Decreased weight gain, which continued throughout the study, was observed at 30 weeks at 300 ppm, but not at 100 ppm. AKR mice exposed to 100 or 300 ppm and C57BL mice exposed to 300 ppm benzene vapor for 6 hours/day, 5 days/week for life had decreased weight gain at 300 ppm (Snyder et al. 1978a, 1980).

3.2.1.3   Immunological and Lymphoreticular Effects

Immunological effects have been reported in humans with occupational exposure to benzene. There are two types of acquired immunity, humoral and cellular, and benzene damages both. First, benzene has been shown to alter humoral immunity (i.e., to produce changes in levels of antibodies in the blood). Painters who were exposed to benzene (3–7 ppm), toluene, and xylene in the workplace for 1–21 years showed increased serum immunoglobulin values for IgM and decreased values for IgG and IgA (Lange et al. 1973b). The decreased levels of immunoglobulins may represent suppression of immunoglobulin-producing cells by benzene. Other adverse reactions, characterized by a reaction between leukocytes and agglutinins, occurred in 10 of 35 of these workers (Lange et al. 1973a). These results suggest the occurrence of allergic blood dyscrasia in some persons exposed to benzene. However, since the workers were exposed to multiple solvents, the specific role of benzene is uncertain.

The second type of immunity, cellular immunity, is affected by changes in circulating leukocytes and a subcategory of leukocytes, called lymphocytes. Leukopenia was found in a series of studies of workers exposed to benzene at levels ranging from 15 to 210 ppm in various manufacturing processes in Turkey (Aksoy et al. 1971, 1987). Another study also noted signs of preleukemia that included loss of leukocytes and other blood elements, bone marrow histopathology, and enlarged spleens (Aksoy et al. 1972, 1974). Other studies in chronically exposed workers also showed losses of lymphocytes and other blood elements (Cody et al. 1993; Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Ruiz et al. 1994; Yin et al. 1987c). In these studies, benzene levels in workplace air ranged from 1 to 1,060 ppm. Hematological effects reported in these studies are described in Section 3.2.1.2. In one study, routine

leukocyte counts conducted every 3 months on employees of a small-scale industry in China revealed leukopenia in workers exposed to as little as 0.69–140 ppm (mean=6 ppm) for an average period of 5–6 years (Xia et al. 1995). Leukocyte alkaline phosphatase (LAP) activity was increased in benzene workers exposed to about 31 ppm for a chronic time period (Songnian et al. 1982). Increased LAP activity is an indicator of myelofibrosis and is associated with both decreased white blood cell counts and with changes in bone marrow activity. The change in LAP activity could be used in the diagnosis of benzene poisoning since it was more sensitive than the change in the leukocyte count, although it is not a biomarker that is specific for benzene exposure. A study conducted by Li et al. (1994) during 1972–1987 examined 74,828 benzene-exposed workers employed in 672 factories and 35,805 unexposed workers from 109 factories located in 12 cities in China. Estimates of gender-specific rate ratios and a comparison of the rate ratios for females to the rate ratios for males were calculated for the incidence of hematopoietic and lymphoproliferative (HLP) disorders, comparing all exposed workers in each of the occupational groups to unexposed workers. Small increases in relative risks for all HLP disorders for both genders were observed among chemical and rubber manufacturing workers, painters, and paint manufacturers. In another study, an increase in leukocyte count and alkaline phosphatase score was observed in a pipe-fitter who was chronically exposed to 0.9 ppm benzene in addition to other solvents (Froom et al. 1994).

Animal studies support the observations made in humans and show that benzene affects humoral and cellular immunity. A decrease in spleen weight was observed in mice after exposure to benzene at a concentration of 25 ppm, 6 hours/day for 5 days, the same exposure concentration at which a decrease in circulating leukocytes was observed (Wells and Nerland 1991). Benzene decreases the formation of the B-lymphocytes that produce the serum immunoglobulins or antibodies. Exposure to benzene at 10 ppm and above for 6 days decreased the ability of bone marrow cells to produce mature B-lymphocytes in C57BL/6 mice (Rozen et al. 1984). The spleen was also inhibited from forming mature T-lymphocytes at exposure levels of 31 ppm and above. Mitogen-induced blastogenesis of B- and T-lymphocytes was depressed at 10 ppm and above. Peripheral lymphocyte counts were depressed at all levels, whereas erythrocyte counts were depressed only at 100 and 300 ppm. This study is the basis for the acute-duration inhalation MRL of 0.009 ppm (see footnote to Table 3-1 and Appendix A).

A continuation of this line of studies for 6 days to 23 weeks at 300 ppm showed continued decreases in numbers of mature B- and T-lymphocytes produced in the bone marrow, spleen, and thymus (Rozen and Snyder 1985). Abnormalities of humoral and cell-mediated immune responses following benzene exposure are presumably caused by a defect in the lymphoid stem cell precursors of both T- and B-lymphocytes. Bone marrow cellularity increased 3-fold, and the number of thymic T-cells increased

15-fold in benzene-exposed mice between the 6th and the 30th exposure. No corresponding increase in splenic cells was noted. The marked increase in the numbers of cells in bone marrow and thymus was interpreted by the authors to indicate a compensatory proliferation in these cell lines in response to benzene exposure, which may play a role in the carcinogenic response of C57BL mice to inhaled benzene. The lack of response in the spleen suggests a lack of lymphoid restorative capacity in that organ.

Other studies have also shown similar effects on immune functions following inhalation exposure to benzene. These include decreased numbers of circulating leukocytes and decreases in bone marrow cellularity in mice exposed to 100 ppm and higher, 24 hours/day for up to 8 days (Gill et al. 1980); decreased leukocytes and increased leukocyte alkaline phosphatase in rats exposed to 100 ppm for 7 days (Li et al. 1986); decreased leukocytes and bone marrow cellularity in DBA/2 mice exposed to 300 ppm benzene for 2 weeks (Chertkov et al. 1992); decreased leukocytes in mice exposed to 300 ppm for 10 days (Ward et al. 1985); bone marrow hyperplasia, lymphocytopenia, and anemia in mice repeatedly exposed to 100 ppm or higher for a lifetime (Snyder et al. 1978a, 1980); and leukopenia and lymphopenia in rats repeatedly exposed to 100 ppm for life (Snyder et al. 1978a, 1984). Aoyama (1986) noted decreased spleen and thymus weights and decreased levels of B- and T-lymphocytes in blood and spleen in mice exposed 6 hours/day to approximately 48 ppm of benzene for 14 days. Exposures of mice to benzene vapor concentrations ≥100 ppm, 6 hours/day, 5 days/week resulted in significantly reduced numbers of lymphocytes (ranging from 25 to 43% lower than controls) in the spleen, thymus, and femur as early as 1 week following the initial exposure, and persisting throughout an 8-week exposure period (Farris et al. 1997a). In rats, exposure to benzene vapors at a concentration of 400 ppm (but not 200 ppm) for 6 hours/day, 5 days/week for 4 weeks resulted in reduced thymus weight and decreased splenic lymphocytes (Robinson et al. 1997).

Reduced bone marrow cellularity was observed in Swiss Webster and C57BL/6J mice after exposure to 300 ppm benzene for 2 weeks (Neun et al. 1992). Decreased granulopoietic stem cells were observed at 21 ppm in NMRI mice after exposures of 10 days to 2 weeks (Toft et al. 1982). Green and colleagues examined the effect of benzene inhalation on peripheral blood and bone marrow and spleen cells (Green et al. 1981a, 1981b) of CD-1 mice following exposures to a number of regimens. In the acute studies, individual test groups were exposed by inhalation to mean benzene concentrations of 0, 1.1, 9.9, 103, 306, 603, 1,276, 2,416, or 4,862 ppm, 6 hours/day for 5 days. Intermediate studies consisted of two different exposure regimens and concentrations: 6 hours/day, 5 days/week for 10 weeks (50 days) to a mean concentration of 0 or 9.6 ppm benzene; or 6 hours/day, 5 days/week for 26 weeks to a mean concentration

of 0 or 302 ppm benzene. In the acute studies, marrow and splenic cellularities were reduced at 103 ppm. Splenic granulocytes were reduced at all exposure levels except at 9.9 ppm (Green et al. 1981b). The decrease in spleen cellularity correlated with a reduction in spleen weight at all concentrations ≥103 ppm. Mean spleen weights were significantly depressed at 1.1 ppm and at doses above 9.9 ppm, but not at 9.9 ppm. In Green et al. (1981a), marrow concentration of GM-CFU-C was equivalent to or greater than control values at all levels; however, splenic GM-CFU-C concentration was decreased at 103 ppm. Femoral and splenic CFU-S and GM-CFU-C per organ were depressed at 103 ppm. Absolute numbers of GM-CFU-C/femur or spleen were significantly reduced at all higher concentrations. There was no change in the colony/cluster ratio observed in the 5-day experiment. In the 50-day experiments, increased spleen weight, splenic nucleated cellularity, splenic nucleated erythrocytes, and CFU-S were seen at 9.6 ppm (Green et al. 1981a, 1981b). Exposure to 302 ppm for 26 weeks resulted in reduced marrow and spleen cellularity and decreased spleen weight (Green et al. 1981a, 1981b).

Sprague-Dawley SD/Tex rats were exposed to benzene vapor at 0 or 500 ppm for 5 days/week, 6 hours/day for 3 weeks (Dow 1992). In the bone marrow differential counts, rats showed a relative decrease in lymphoid cells at the 500 ppm dose level. There were also decreases in myeloid cells of animals exposed to 500 ppm benzene. In a companion study, purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours/day, 5 days/week for 3 weeks (Dow 1992). Exposure to 500 ppm resulted in significant decreases in total white blood cells, T-cells, peripheral blood lymphocytes, and proportion of myeloid cells in bone marrow counts.

Another series of experiments revealed that exposures as low as 25 ppm for 2 weeks caused decreases in the numbers of circulating lymphocytes but did not affect the bone marrow cellularity in C57BL/6 or CBA/Ca mice (Cronkite 1986; Cronkite et al. 1985). Increasing the duration (up to 16 weeks) or concentration (up to 300–400 ppm) produced the same effect of reduced peripheral blood lymphocytes as well as decreased bone marrow cellularity that persisted for up to 8 weeks after exposure (Cronkite et al. 1982, 1985). Similar observations were made in two groups of male CBA/Ca mice exposed to a total of 6,000 ppm of benzene by inhalation using two different regimens (Cronkite et al. 1989). One group was exposed to 316 ppm for a total of 19 times, while the second group was exposed to 3,000 ppm twice. Although both groups had significantly decreased lymphocyte counts, the lymphocyte numbers were much more depressed in the group of mice exposed to 316 ppm of benzene over 19 days. This suggests that, at relatively high exposure levels, repeated or prolonged exposure is more potent than the magnitude of exposure in causing lymphopenia. In both groups, the lymphocyte numbers had not returned to normal

values by 214 days post exposure. The femoral bone marrow cellularity returned to normal levels 32 days after exposure in both groups of mice.

In female BDF_1 mice (C57BL/6xDBA/$2F_1$ hybrids) exposed to 0, 300, or 900 ppm benzene 6 hours/day for 5 days, there was a relative increase in helper lymphocytes (CD4+) at days 3–5, leading to an increase of the T4/T8 (CD4+–CD8+) ratio from 2 in controls to higher values at 8 weeks (Plappert et al. 1994a). No concentration dependency was observed. Granulocytic hyperplasia of the spleen was observed in CBA/Ca mice exposed to 300 ppm benzene for 16 weeks (Farris et al. 1993).

Leukopenia, granulocytopenia, and lymphocytopenia were observed in C57BL/6 mice exposed to 1,000 ppm benzene for 6 hours/day, 5 days/week for up to 6 weeks (Gill et al. 1980). Decreased leukocyte counts were also noted in Sprague-Dawley rats and CD-1 mice exposed to 300 ppm benzene 6 hours/day, 5 days/week for up to 13 weeks (Ward et al. 1985). In mice, the most common compound-related histopathological findings were splenic periarteriolar lymphoid sheath depletion, lymphoid depletion in the mesenteric lymph node, and plasma cell infiltration of the mandibular lymph node. Exposure to the highest concentration caused a decrease in leukocyte and lymphocyte counts in Sprague-Dawley rats. Hematological changes at 300 ppm were accounted for by decreased leukocyte counts in males on day 14 and in females on day 91. Decreases in percentage of lymphocytes in males and females started on day 14 and lasted through day 91. Rats exhibited decreased femoral marrow cellularity as the only histological change. Treatment-related changes were not observed at lower concentrations. Rabbits, rats, and guinea pigs exposed to 80–88 ppm for 32–269 days also had decreased leukocyte counts (Wolf et al. 1956). Leukocyte alkaline phosphatase values were increased and leukocyte counts were decreased in rats exposed to 4,570 ppm for 20 weeks (Songnian et al. 1982). Exposure of C57BL mice to 10 ppm benzene for 6 hours/day, 5 days/week, for 24 weeks caused depressions in the numbers of splenic nucleated red cells and lymphocytes (Baarson et al. 1984).

Li et al. (1992) observed a 26% decrease in spleen weight in male Kunming mice exposed to 12.52 ppm benzene 2 hours/day, 6 days/week for 30 days. Examination of the bone marrow showed decreases in myelocytes, premyelocytes, myeloblasts, and metamyeloblasts at the same dose level.

Benzene also affects functional immune responses, as indicated by decreased resistance to infectious agents. Pre-exposure to benzene at 30 ppm for 5–12 days increased the bacterial counts in mice on day 4 of infection with *Listeria monocytogenes* (Rosenthal and Snyder 1985). Recovery of the immune system was noted on day 7. The effects did not occur at 10 ppm. In addition, a concentration-dependent

statistically significant depression was noted in T- and B-lymphocyte populations from day 1 through day 7 at 30 ppm and above. B-cells were more sensitive to benzene than were T-cells on a percentage-of-control basis. These results indicate a benzene-induced delay in immune response to *L. monocytogenes*. Concentrations of 200 or 400 ppm for 4–5 weeks (5 days/week) suppressed the primary antibody response to tetanus toxin in mice, but there was no effect at 50 ppm (Stoner et al. 1981). In another intermediate-duration exposure study, no changes were noted in the numbers of splenic B-cells, T-cells, or T-cell subsets in C57BL/6 mice exposed to 100 ppm of benzene 5 days/week for 20 days (Rosenthal and Snyder 1987). However, when splenic T-cells from mice treated with 10 ppm and 100 ppm were tested *in vitro* for their capacity to respond to foreign antigens (alloantigens) in the mixed lymphocyte reaction (MLR), the MLR response was delayed. Further analysis showing that this delayed MLR response was not due to the presence of benzene-induced suppressor cells and indicated that benzene impaired the functional abilities of alloreactive T-cells. This study is the basis for the intermediate-duration inhalation MRL of 0.006 ppm (see footnote to Table 3-1 and Appendix A). A similar *in vitro* observation was made using T-cells from mice exposed to 100 ppm of benzene 5 days/week for 3 weeks. These cells had a reduced tumor cytolytic activity, suggesting a benzene-induced impairment of cell-mediated immunity. The impaired cell-mediated immune function was also apparent *in vivo*. Mice exposed to 100 ppm for a total of 100 days were challenged with 10,000 polyoma virus-induced tumor cells (PYB6), and 9 of 10 mice had reduced tumor resistance and developed tumors that were lethal (Rosenthal and Snyder 1987).

The highest NOAEL values and all reliable LOAEL values for immunological effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.4 Neurological Effects

Following acute inhalation of benzene, humans exhibit symptoms indicative of central nervous system effects (Cronin 1924; Flury 1928; Greenburg 1926; Midzenski et al. 1992). These symptoms, reported to occur at levels ranging from 300 to 3,000 ppm, include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness. Acute exposure (5–10 minutes) to higher concentrations of benzene (approximately 20,000 ppm) can result in death, which has been associated with vascular congestion in the brain (Avis and Hutton 1993; Flury et al. 1928). Lethal exposures are also associated with nonspecific neurological symptoms similar to those reported for nonlethal exposures. These symptoms are similar to the consequences of exposure to multiple organic solvents and are reversible when symptomatic workers are removed from the problem area (Kraut et al. 1988; Yin et al. 1987b). In reports

of cases of benzene poisoning, subjects exhibited headaches, nausea, tremor, convulsions, and unconsciousness, among other neurological effects (Cronin 1924; Greenburg 1926; Midzenski et al. 1992; Tauber 1970).

Chronic exposure to benzene has been reported to produce neurological abnormalities in humans. Of eight patients (six with aplastic anemia and two with preleukemia) with previous occupational exposure to adhesives and solutions containing 9–88% benzene, four of the six patients with aplastic anemia showed neurological abnormalities (global atrophy of lower extremities and distal neuropathy of upper extremities) (Baslo and Aksoy 1982). Air concentrations in the workplace were reported to have reached levels of ≥210 ppm. These findings suggest that benzene may induce toxic effects on the nervous system involving peripheral nerves and/or spinal cord. The limitations of this study are that benzene exposure levels were not monitored and that there was a possibility of an additional exposure to toluene (6.37–9.25%).

Chronic exposure to benzene and toluene was studied in 121 workers exposed to benzene for 2–9 years (Kahn and Muzyka 1973). The air concentration of benzene between 1962 and 1965 was 6–15.6 ppm (20–50 mg/m^3), while the toluene vapors did not exceed the 5 mg/m^3 level. Subsequently (the authors do not specify when), the air levels of both benzene and toluene have not exceeded the 5 mg/m^3 level. Seventy-four of the examined workers complained of frequent headaches (usually at the end of the work day), became tired easily, had difficulties sleeping, and complained of memory loss. The limitations of this study are that workers were exposed to both benzene and toluene and that the precise dose and duration of exposure are not known.

Tondel et al. (1995) reported the case of a gasoline station attendant who had worked from 1962 to 1979. The patient described symptoms of fatigue for 3 weeks and night sweats, among other symptoms.

The neurotoxicity of benzene has not been studied extensively in animals. Female Sprague-Dawley rats exhibited lethargy after exposure to 2,200 ppm benzene, but not 300 ppm, on Gd 6–15 (Green et al. 1978). Male albino SPF rats from a Wistar-derived strain exposed to benzene for 4 hours in glass chambers (dose not specified) exhibited depression of evoked electrical activity in the brain; the authors calculated the 30% effect level (depressed activity) as 929 ppm (Frantik et al. 1994). When female H strain mice were exposed to benzene for 2 hours, the 30% effect level for depression of evoked electrical activity in the brain was 856 ppm (Frantik et al. 1994). In rabbits, symptoms that occurred 3.7 minutes following acute exposure to benzene at 45,000 ppm were relaxation and light narcosis

(Carpenter et al. 1944). As the time after exposure progressed, so did the symptoms to include excitation, chewing, and tremors (after 5 minutes), loss of pupillary reflex to strong light (after 6.5 minutes), loss of blinking reflex (after 11.4 minutes), pupillary contraction (after 12 minutes), and involuntary blinking (after 15.6 minutes). Behavioral tests of C57BL/6 mice showed significant increase in licking of sweetened milk after 1 week of exposure to 300 ppm; a 90% decrease in hind limb grip strength after one exposure to 1,000 or 3,000 ppm (data for 100 ppm were not reported); and tremors after one exposure to 3,000 ppm that subsided 30 minutes after the exposure (Dempster et al. 1984). In another study, designed to reflect occupational exposure, male CD-1 and C57BL/6 mice were exposed to 300 or 900 ppm of benzene 6 hours/day for 5 days followed by 2 weeks of no exposure after which the exposure regimen was repeated for an unspecified amount of time (Evans et al. 1981). The following seven categories of behavioral activities were monitored in exposed and control animals: stereotypic behavior, sleeping, resting, grooming, eating, locomotion, and fighting. Only minimal and insignificant differences were observed between the two strains of mice. Increased behavioral activity was observed after exposure to benzene in both strains of mice. Mice exposed to 300 ppm of benzene had a greater increase than those exposed to 900 ppm, probably because of narcosis-like effects induced at the higher exposure level (Evans et al. 1981). It is not known if benzene induces behavioral changes by directly acting upon the central nervous system. It is also not known whether these changes occur before or after hematological changes.

Li et al. (1992) exposed male Kunming mice to 0, 0.78, 3.13, or 12.52 ppm benzene for 2 hours/day, 6 days/week for 30 days, and then monitored brain and blood acetylcholinesterase, forelimb grip strength, locomotor activity, and rapid response. Significantly increased grip strength was observed at 0.78 ppm, whereas at the higher doses, grip strength decreased significantly. Rapid response showed a significant increase at the low dose; the two higher doses showed a significant depressed rapid response. Locomotor activity increased at the low dose, was similar to control values at the middle dose, and decreased at the high dose. However, these changes were not significantly different from the control values. A significant decrease in acetylcholinesterase activity was noted in the brain, but it was not large enough to be considered adverse; no change in acetylcholinesterase levels in the blood was observed. However, the exposure levels used by Li et al. (1992) were more than 10-fold lower than those eliciting signs of neurotoxicity in other animal studies, an indication that the actual exposure levels in the study of Li et al. (1992) may have been higher than indicated. Uncertainty regarding actual exposure levels renders this study of little value for purposes of risk assessment.

All reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

Data on the reproductive effects of occupational exposure to benzene suggest that benzene may impair fertility in women (Mukhametova and Vozovaya 1972; Vara and Kinnunen 1946). However, the findings are inconclusive due to uncertainties in exposure assessment and the limited data collected. In one study, 30 women with symptoms of benzene toxicity were examined (Vara and Kinnunen 1946). The levels of benzene in air were not specified, but are assumed to have been much greater than the 1 ppm permitted in today's working environment. Twelve of these women had menstrual disorders (profuse or scanty blood flow and dysmenorrhea). Ten of the 12 women provided information on fertility. Of these 10 women, 2 had spontaneous abortions, and no births occurred during their employment, even though no contraceptive measures had been taken. This led the investigators to suggest that benzene has a detrimental effect on fertility at high levels of exposure. However, the study failed to provide verification that the absence of birth was due to infertility. Gynecological examinations revealed that the scanty menstruations of five of the patients were due to ovarian atrophy. This study is limited in that an appropriate comparison population was not identified. Additionally, little follow-up was conducted on the 30 women with regard to their continued work history and possible symptoms of benzene toxicity.

Disturbances of the menstrual cycle were found in women workers exposed to aromatic hydrocarbons (benzene, toluene, xylene) (Michon 1965). The exposure levels of benzene and toluene were below 0.25 ppm. The observed group consisted of 500 women, 20–40 years old. One hundred controls were included in the study. The results showed that 21% of exposed women whose work involved sitting or standing had irregular menstrual cycles compared to 12% in the control group. Brief (up to 2 days), long (6–9 days), and prolonged (over 9 days) menstrual cycles were present in 26% of women who performed lifting during their work as compared to 13% in the control group. Irregular amounts of menstrual flow and pain were also observed in female workers exposed to aromatic hydrocarbons. The major limitations of this study are that the exposure occurred from a mixture of chemicals, levels of exposure were not well defined, duration of exposure was not stated, and activities of the controls were not provided.

Another study examined the reproductive function and incidence of gynecological effects in 360 women exposed to petroleum (a major source of benzene) and chlorinated hydrocarbons both dermally and by inhalation (Mukhametova and Vozovaya 1972). However, dermal exposure was considered to be

negligible. The concentrations of benzene in the air were not well documented. When compared to female workers with no chemical exposure, female gluers had developed functional disturbances of the menstrual cycle. Additionally, as chemical exposure time increased, there were increases in the number of premature interruptions of pregnancy, the percentage of cases in which the membranes ruptured late, and the number of cases of intrauterine asphyxia of the fetus. The study limitations (including lack of exposure history, simultaneous exposure to other substances, and lack of follow-up) make it difficult to assess the effects of benzene on reproduction.

Reproductive competence of male workers in two organic chemical factories in France was evaluated by Stucker et al. (1994). Analysis of 1,739 pregnancies that ended in spontaneous abortion or birth was presented. Paternal exposure to benzene for each pregnancy was described as exposure in the 3 months immediately before conception, and as previous job exposures. Benzene exposure was graded at two levels: <5 ppm (low) and ≥5 ppm (moderate). Of the 1,739 pregnancies described, 171 ended in a spontaneous abortion (rate=9.8%). According to exposure categories, 1,277 pregnancies were defined as non-exposed (mate not exposed) and 270 whose mates were exposed at some time before conception. For the 270 pregnant women, 145 of their mates were exposed during the 3 months immediately preceding conception. The frequency of spontaneous abortion was not significantly higher for the paternal group exposed at any time before conception than in the non-exposed group; nor was it higher for the group exposed during the 3 months immediately before conception.

In CFY rats exposed to either pure air or benzene (125 ppm) for 24 hours/day from Gd 7 through 14, no effect on implantation number was observed (Tatrai et al. 1980a). No changes in maternal body weight were observed in Sprague-Dawley rats exposed to 100 ppm benzene for 6 hours/day on Gd 6–15 (Coate et al. 1984). Pregnant rabbits exposed 12 hours/day to 156.5 or 313 ppm benzene on Gd 7–20 showed an increase in the number of abortions and resorptions at 312 ppm (Ungvary and Tatrai 1985). However, in other developmental toxicity studies, no effect on the number of resorptions was seen in rats at doses as high as 2,200 ppm (Green et al. 1978), in mice at 500 ppm (Murray et al. 1979), or in rabbits at doses of 500 ppm (Murray et al. 1979).

Reproductive effects have been noted in experimental animals exposed for intermediate durations, but the levels of exposure were higher (80–6,600 ppm) than those to which humans are exposed in the modern industrial environment (Ward et al. 1985; Wolf et al. 1956). In an intermediate-duration inhalation study, groups of male and female CD-1 mice were exposed to benzene vapor concentrations of 0, 1, 10, 30, or 300 ppm, 5 days/week, 6 hours/day to benzene vapor for 13 weeks (Ward et al. 1985). Histopathological

changes were observed in ovaries (bilateral cysts) and testes (atrophy/degeneration, decrease in spermatozoa, moderate increase in abnormal sperm forms) of mice exposed to 300 ppm benzene; the severity of gonadal lesions was greater in the males. An inhalation study was conducted exposing rats (6,600 ppm), rabbits (80 ppm), and guinea pigs (88 ppm) to benzene for 7–8 hours/day, 5 days/week for 93, 243, and 32 or 269 days, respectively (Wolf et al. 1956). Male rats showed an increase in testicular weight after 93 days at the 6,600 ppm level. The guinea pigs showed a slight increase in average testicular weight at the 88 ppm level. Rabbits showed slight histopathological testicular changes (degeneration of the germinal epithelium) when exposed to 80 ppm. Since only one or two rabbits were used in this study, it was not possible to draw any conclusions regarding benzene's ability to induce testicular damage in the rabbits. Continuous exposure of female rats to 210 ppm benzene for 10–15 days before cohabitation with males and 3 weeks after cohabitation resulted in a complete absence of litters (Gofmekler 1968). It is not known whether this was due to failure to mate, infertility, or early preimplantation losses of fertilized ova. In a fertility study, female rats exposed up to 300 ppm benzene for 10 weeks during premating, mating, gestation, and lactation showed no effect on indices of fertility, reproduction, and lactation (Kuna et al. 1992).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.6 Developmental Effects

The available human data on the developmental effects of benzene after inhalation exposure are inconclusive. The studies designed specifically to investigate developmental effects are limited, primarily because of concomitant exposure to other chemicals, inadequate sample size, and lack of quantification of exposure levels (Budnick et al. 1984; Goldman et al. 1985; Heath 1983; Olsen 1983). Benzene crosses the human placenta and is present in the cord blood in amounts equal to or greater than those in maternal blood (Dowty et al. 1976). In a study of subjects with known benzene poisoning in Italy, Forni et al. (1971a) reported the case of one pregnant worker exposed to benzene in the air during her entire pregnancy. Although she had severe pancytopenia and increased chromosomal aberrations, she delivered a healthy son with no evidence of chromosomal alterations. The following year, she delivered a healthy daughter. However, increased frequency of chromatid and isochromatid breaks and sister chromatid exchange was found in lymphocytes from 14 children of female workers exposed by inhalation to benzene (dose not specified) and other organic solvents during pregnancy (Funes-Cravioto et al. 1977).

No mention was made of whether the mothers showed signs of toxicity or whether physical abnormalities occurred among their offspring.

There are numerous inhalation studies in which animals have been exposed to benzene during pregnancy (Coate et al. 1984; Green et al. 1978; Kuna and Kapp 1981; Murray et al. 1979; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985). None of these studies demonstrated that benzene was teratogenic even at levels that induced maternal and fetal toxicity. Fetotoxicity was evidenced by decreased body weight and by increased skeletal variants such as missing sternebrae and extra ribs, which were not considered to be malformations. Alterations in hematopoiesis have also been observed in the fetuses and offspring of pregnant mice exposed to low levels of benzene (Keller and Snyder 1986, 1988). These studies are discussed below.

Mice exposed to 500 ppm benzene for 7 hours/day on days 6–15 of pregnancy exhibited fetal growth retardation (i.e., decreased fetal body weight) and increased minor skeletal variants (i.e., delayed ossification) (Murray et al. 1979). There were no fetal malformations and no significant effect on the incidence of pregnancy, average number of live fetuses, resorptions per litter, or maternal weight gain. No malformations in fetuses and no significant effects on incidence of pregnancy, average number of live fetuses, or resorptions per litter were observed when rabbits were exposed to benzene 7 hours/day at 500 ppm during gestation for 9 days, although an increase in minor skeletal variations was observed (Murray et al. 1979).

Pregnant mice exposed 12 hours/day to 156.5 or 313 ppm benzene on Gd 6–15 had pups with significant weight retardation and retardation of skeletal development, but no malformations (Ungvary and Tatrai 1985). A parallel study in rabbits showed that inhalation of benzene at 313 ppm caused fetal weight reduction, and an increase in minor fetal anomalies (Ungvary and Tatrai 1985).

As was the case with mice and rabbits, the fetotoxicity of benzene in rats is also demonstrated by retarded fetal weight and/or minor skeletal variants (Coate et al. 1984; Green et al. 1978; Kuna and Kapp 1981; Tatrai et al. 1980b). In an experiment conducted by Green et al. (1978), pregnant Sprague-Dawley rats were exposed to 100, 300, or 2,200 ppm benzene for 6 hours/day on Gd 6–15. Exposure to high levels of benzene (2,200 ppm) during gestation resulted in a significant decrease in fetal weight, whereas dams breathing air containing lower levels of benzene (100 or 300 ppm) during gestation bore young that were similar in weight and crown–rump length to control pups. Statistically significant numbers of fetuses with delayed ossification were found in groups exposed to concentrations of 300 and 2,200 ppm. The

litter incidence of missing sternebrae was significantly increased in the 100 and 2,200 ppm exposure groups. Maternal toxicity, as indicated by a decrease in maternal weight gain, was evident only at the 2,200 ppm level. The female offspring appeared to be affected to a greater extent than males with respect to delayed ossification and missing sternebrae.

Kuna and Kapp (1981) found decreased fetal weight after exposure to 50 ppm. From a group of 151 pups examined after *in utero* exposure to 500 ppm benzene, a single pup exhibited exencephaly. In the same study, of 98 pups examined for skeletal effects after *in utero* exposures of 500 ppm, a single pup had angulated ribs and 2 others had nonsequential ossification of the forefeet. These anomalies were not statistically significant and may have resulted from maternal nutritional stress.

No significant skeletal malformations occurred in pups of rats exposed during gestation to 47 ppm for 8 days, 24 hours/day (Tatrai et al. 1980b) or 100 ppm for 10 days, 6 hours/day (Coate et al. 1984). Decreased fetal weights were seen at 47 ppm (Tatrai et al. 1980b) and 100 ppm (Coate et al. 1984), and increased fetal mortality was observed at 141 ppm (Tatrai et al. 1980b). In CFY rats exposed to pure air or 125 ppm benzene on Gd 7–14, there was a 17% decrease in placental weight, a decrease in mean fetal weight, and evidence of skeletal retardation (Tatrai et al. 1980a). Continuous exposure of female rats to 6 concentrations of benzene ranging from 0.3 to 210 ppm for 10–15 days before cohabitation with males and 3 weeks after did not affect newborn weight or induce malformations, but there were differences in the weights of individual organs of the dams at all exposure levels (Gofmekler 1968). There was a slight tendency toward decreased litter sizes at 20 ppm of benzene. A complete absence of litters resulted from exposure to 210 ppm. It is not known whether this was due to failure to mate, infertility, or early preimplantation losses of fertilized ova.

Alterations in hematopoiesis have also been observed in the fetuses and offspring of pregnant mice exposed to benzene (Keller and Snyder 1986). Administration of 20 ppm benzene to pregnant Swiss Webster mice for 6 hours/day on Gd 6–15 caused reductions in the levels of the CFU-E of the fetuses, whereas 5 and 10 ppm benzene caused enhancement of these colony-forming cells. In 2-day-old neonates, CFU-E numbers in the 5 ppm group returned to control values, but the 10 ppm neonates showed a bimodal response by litter. Granulocytic colony-forming cells were enhanced in neonates exposed *in utero* to 20 ppm benzene. Some of the mice exposed to 10 ppm prenatally were re-exposed to 10 ppm as adults. Their hematopoietic progenitor cell numbers were depressed compared with controls exposed for the first time as adults. No tests were conducted on the dams after benzene exposure.

In a follow-up study, pregnant Swiss Webster mice were exposed 6 hours/day on Gd 6–15 to 5, 10, or 20 ppm benzene (Keller and Snyder 1988). The results indicated that 16-day fetuses, when checked for erythrocyte and leukocyte counts, hemoglobin analysis, and the proliferating pool of differentiating hematopoietic cells, had no noteworthy change at any of the exposure levels. In contrast, 2-day neonates exposed *in utero* to all concentrations of benzene exhibited a reduced number of circulating erythroid precursor cells and, at 20 ppm, had increased numbers of hepatic hematopoietic blast cells and granulopoietic precursor cells accompanied by decreased numbers of erythropoietic precursor cells. Six-week-old adult mice exposed *in utero* to 20 ppm of benzene had a similar pattern of enhanced granulopoiesis. However, this effect was not clearly evident in 6-week-old adult mice exposed *in utero* to 5 or 10 ppm.

The results of inhalation studies conducted in experimental animals have been fairly consistent across species. It has been suggested that benzene fetotoxicity in animals is a function of maternal toxicity because the joint occurrence of a decrease in fetal weight and an increase in skeletal variants usually occurs when there is a decrease in maternal weight (Tatrai et al. 1980b). However, the mechanism underlying developmental toxicity has not been fully elucidated, and there are few data on the effect of benzene on maternal food consumption and on blood levels of benzene and its metabolites in the dams and their fetuses. There are apparently none of the usual fetotoxic findings after exposure *in utero* to low concentrations of benzene (10 ppm) (Coate et al. 1984; Kuna and Kapp 1981). As stated above, there is evidence for persistent hematopoietic anomalies in animals exposed *in utero* to benzene at 20 ppm (Keller and Snyder 1988). They may also exist at lower concentrations, but adequate testing has not been performed.

The highest NOAEL value and all reliable LOAEL values for developmental effects in each species following acute exposure are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.7 Cancer

Epidemiological studies and case reports provide clear evidence of a causal relationship between occupational exposure to benzene and benzene-containing solvents and the occurrence of acute nonlymphocytic leukemia (ANLL), particularly the myeloid cell type (acute myelogenous leukemia, AML) (EPA 1985b, 1998; Hayes et al. 1997; IARC 1982, 1987; IRIS 2007; Rinsky et al. 1987, 2002; Yin et al. 1996a, 1996b). Some of the studies also provide suggestive evidence of associations between benzene exposure and non-Hodgkin's lymphoma (NHL) and multiple myeloma (Hayes et al. 1997;

Rinsky et al. 1987). The epidemiological studies are generally limited by confounding chemical exposures and methodological problems, including inadequate or lack of exposure monitoring and low statistical power (due to small numbers of cases), but a consistent excess risk of leukemia across studies indicates that benzene is the causal factor. Many of the earlier studies are additionally limited by a lack of information on leukemia cell types other than AML, because leukemia used to be considered a single diagnostic category for epidemiological purposes, due in part to historical nomenclature, small numbers of deaths by cell type, and unavailability of cell-type-specific rates for comparison.

Two series of studies on workers exposed to benzene in Ohio (the Pliofilm study) (e.g., Rinsky et al. 1981, 1987, 2002) and China (the NCI/CAPM study) (e.g., Hayes et al. 1997; Yin et al. 1996a, 1996b) have yielded particularly strong data on the leukemogenic potential of benzene and are summarized below. The studies of the Pliofilm cohort provide the best set of data for evaluating human cancer risks from benzene exposure because, in comparison to other published studies, the Pliofilm workers had the fewest reported co-exposures to other potentially carcinogenic substances and experienced a greater range of estimated exposures to benzene (EPA 1998). The NCI/CAPM Chinese study is one of the largest of its type ever undertaken and evaluated many thousands of benzene-exposed workers, enabling detection of significantly elevated risks at unusually low levels of exposure.

A number of studies were performed on a cohort of workers exposed to benzene in three rubber hydrochloride ('Pliofilm') manufacturing plants in Ohio (Collins et al. 1997; Finkelstein 2000; Infante 1978; Infante et al. 1977; Ireland et al. 1997; Paxton et al. 1994a, 1994b; Rinsky et al. 1981, 1987, 2002; Schnatter et al. 1996a; Wong 1995). Analyses using various expansions and updates of the cohort, statistical methods, and exposure estimates have consistently shown a significant relationship between exposure to benzene and excess mortality from leukemia. In the report by Rinsky et al. (1987), a cohort of 1,165 white males employed between 1940 and 1965 and followed through 1981 experienced increased mortality from all leukemias (9 observed versus 2.7 expected; standardized mortality ratio [SMR]=3.37, 95% CI 1.54–6.41) and multiple myeloma (4 observed versus 1 expected; SMR=4.09, 95% CI 1.10–10.47). Death rates for age-matched U.S. white males during the same calendar period were used for comparison with death rates observed in the cohort. Assessment after an additional 15 years of follow-up showed declines in the SMRs for both leukemias (2.56, 95% CI 1.43–4.22) and multiple myeloma (2.12, 95% CI 0.69–4.96), suggesting that the excess risks diminished with time since exposure (Rinsky et al. 2002). Exposures in the most recent 10 years were most strongly associated with leukemia risk, and there was no significant relation between leukemia death and benzene exposures received more than 20 years previously (Finkelstein 2000). AML accounted for most of the increased leukemia

(SMR=5.03, 95% CI 1.84–10.97) (Schnatter et al. 1996a; Wong 1995). The risk of all leukemias and AML, but not multiple myeloma, significantly increased with increasing cumulative exposure above 200 ppm-years (Rinsky et al. 1987, 2002; Wong 1995). Analysis of 4,417 workers from one of the Pliofilm plants showed no clear evidence that the risks of all leukemias, ANLL, multiple myeloma, or other lymphohematopoietic cancers increased with increasing exposure at lower levels of cumulative exposure (1–72 ppm-years), and the number of peak exposures over 100 ppm for ≥40 days was a better predictor of risk for all leukemias and multiple myeloma (Collins et al. 2003; Ireland et al. 1997). No clear associations between exposure to benzene and mortality from NHL or Hodgkin's disease were reported (Collins et al. 2003; Ireland et al. 1997; Rinsky et al. 2002).

A collaborative study between the National Cancer Institute and the Chinese Academy of Preventive Medicine (NCI/CAPM) evaluated incidence rates (occurrence of disease and cause of death) for lymphohematopoietic malignancies and other hematologic disorders in a cohort of 74,828 benzene-exposed and 35,805 nonexposed workers employed in 672 factories in 12 cities in China (Hayes et al. 1996, 1997, 2001; Linet et al. 1996). The joint NCI/CAPM study is an expansion of earlier studies performed by CAPM alone (Yin et al. 1987a, 1987b, 1987c, 1989). The workers were employed from 1972–1987, followed for an average of nearly 12 years, and worked in various job categories using benzene as a solvent for paints, varnishes, glues, coatings, and other products. The derivation of the cohort from many different factories and job types suggests that the members were concurrently exposed to many other chemicals. Findings in the exposed workers included significantly increased relative risk (RR) for all hematologic neoplasms (RR=2.6, 95% CI 1.4–4.7), all leukemias (RR=2.5, 95% CI 1.2–5.1), ANLL (RR=3.0, 95% CI 1.0–8.9), and combined ANLL and precursor myelodysplastic syndromes (ANLL/MDS; RR=4.1, 95% CI 1.4–11.6) (Hayes et al. 1997). Increases were also observed for other (unspecified) leukemias (RR=2.0, 95% CI=0.7–5.4), but the results were not statistically significant. Analysis by level of average benzene exposure (<10, 10–24, and ≥25 ppm) and cumulative exposure (<40, 40–99, and ≥100 ppm-years) indicated that the risk for all hematologic neoplasms was significantly increased at <10 ppm (RR=2.2, 95% CI 1.1–4.2) and <40 ppm-years (RR=2.2, 95% CI 1.1–4.5), respectively. The risks for all leukemias, ANLL, and ANLL/MDS were increased at exposure levels of 10–24 ppm (average) and 40–99 ppm-years (cumulative). The elevated risks showed weak tendencies to increase with increasing average and cumulative levels of exposure. Analysis by duration of exposure (<5, 5–9, or ≥10 years) did not show increased risk with increasing exposure duration. Analysis by occupational group (coatings, rubber, chemical, shoe, other/mixed) showed that the increased risks for ANLL and ANLL/MDS were consistent across the spectrum of industries studied, implying that the associations were due to the common exposure to benzene rather than other industry-specific exposures.

The results of a study of shoe factory workers in Italy are similar to those of the Pliofilm and NCI/CAPM studies in showing that the risk of leukemia increases with increasing exposure to benzene (Costantini et al. 2003; Paci et al. 1989). The cohort was followed from 1950 to 1999 and consisted of 891 men and 796 women who were exposed to estimated benzene concentrations ranging from 0 to 92 ppm, and had mean cumulative exposures of 71.8 and 43.4 ppm-years, respectively (exposure durations not reported). Leukemia risk was significantly increased in both sexes in the highest of four exposure categories and most apparent in the men. For cumulative exposures of <40, 40–99, 100–199, and >200 ppm-years, the leukemia SMR values for the men were 1.4 (95% CI 0.2–5.0), 3.7 (95% CI 0.1–20.6), 3.0 (95% CI 0.4–10.9), and 7.0 (95% CI 1.9–18.0), respectively. Leukemia subtypes were not evaluated. The findings are consistent with earlier epidemiologic studies and case reports showing increased incidences of leukemia in shoe factory and rotogravure plant workers exposed to high benzene levels during its use as a solvent (Aksoy et al. 1974, 1987; IARC 1982; Vigliani and Forni 1976).

No significant increases in leukemia were found in chemical industry workers (Bloemen et al. 2004; Bond et al. 1986; Ott et al. 1978) or petroleum industry workers (Lewis et al. 1997; Raabe and Wong 1996; Rushton and Romaniuk 1997; Schnatter et al. 1993, 1996b, 1996c; Tsai et al. 1983) exposed to lower levels of benzene. Cause-specific mortality was determined in a prospective study of 2,266 chemical workers who were exposed to benzene in various Dow Chemical Company manufacturing processes between 1938 and 1970 (Bloemen et al. 2004). The workers were followed from 1940–1996 and had an average duration of exposure, intensity of exposure, and cumulative exposure of 4.8 years, 9.6 ppm, and 39.7 ppm-years, respectively. There were no significant increases in risk for any lymphohematopoietic malignancies, including all leukemias, ANLL, chronic lymphatic leukemia (CLL), NHL, and multiple myeloma. A previous investigation of this cohort found a significantly increased risk of 'myelogenous' leukemias (SMR=4.44, 95% CI not reported) (Bond et al. 1986; Ott et al. 1978), but this result is less reliable than those of the current study because it is based on a much smaller number of deaths.

A meta-analysis was conducted on 19 cohorts of petroleum workers in the United States and the United Kingdom that were pooled into a single database for cell-type-specific leukemia analysis (Raabe and Wong 1996). The combined cohort consisted of 208,741 workers, mainly refinery employees who contributed more than 4.6 million person-years of observation. Benzene exposures were mainly from handling gasoline and the estimated mean and cumulative exposures for the most exposed jobs were <1 ppm and <45 ppm-years, respectively. No increased risks were found for mortality from AML, chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), or CLL. Analyses limited to

studies of refinery workers or studies with at least 15 years of follow-up yielded similar results. The negative results of this meta-analysis are consistent with those of case-control studies of the common leukemia cell types in petroleum workers (Rushton and Romaniuk 1997; Schnatter et al. 1993, 1996b, 1996c). A case-control study of hairy cell leukemia, a rare B-lymphoid chronic leukemia, found no association between reported exposure to benzene and the risk of this cancer in workers from various occupations with generally low levels of exposure (Clavel et al. 1996).

A possible association between occupational exposure to benzene and NHL is suggested by results of the most recent analysis (Hayes et al. 1997) of the cohort from the NCI/CAPM Chinese study summarized above. The relative risk for mortality from NHL in the whole cohort was 3.0 (95% CI 0.9–10.5), an increase that was not statistically significant. However, the risk for NHL did significantly increase at the highest level and duration of benzene exposure. For exposure to average concentrations of <10, 10–24, and ≥25 ppm, the RR values were 2.7 (95% CI 0.7–10.6), 1.7 (95% CI 0.3–10.2), and 4.7 (95% CI 1.2–18.1), respectively (P for trend=0.04). For cumulative exposures of <40, 40–99, and ≥100 ppm-years, the RR values were 3.3 (95% CI 0.8–13.1), 1.1 (95% CI 0.1–11.1), and 3.5 (95% CI 0.9–13.2), respectively (P for trend=0.02). Additionally, the risk for NHL significantly increased with increasing duration of exposure among workers exposed for <5 years (RR=0.7, 95% CI 0.1–7.2), 5–9 years (RR=3.3, 95% CI 0.7–14.7), and >10 years (RR=4.2, 95% CI 1.1–15.9) (P for trend=0.01).

Although the results of the NCI/CAPM Chinese study (Hayes et al. 1997) indicated a possible association between exposure to benzene and NHL, other cohort mortality studies found no significant increases in NHL mortality. These studies include the entire Pliofilm cohort (Rinsky et al. 2002); a total of 4,417 workers from one of the three Pliofilm plants assessed for cumulative exposures of 0, <1, 1–6, and >6 ppm-years (Collins et al. 2003); a cohort of 2,266 chemical manufacturing workers exposed to a cumulative benzene level of 39.7 ppm-years (Bloemen et al. 2004); and a meta-analysis of 26 cohorts of petroleum workers from the United States and five other countries with expected low exposures to benzene (Wong and Raabe 2000). Furthermore, case-control studies provide no indications of an association between benzene exposure and risk of NHL (Schnatter et al. 1996b; Wong and Raabe 2000), and the adequacy of the data from the NCI/CAPM Chinese study (Hayes et al. 1997) has been questioned (Dosemeci et al. 1994; Wong 1999; Wong and Raabe 2000).

Other evidence suggests a possible association between occupational exposure to benzene and increased risk of multiple myeloma. Mortality from multiple myeloma was initially elevated in the Pliofilm cohort, but appeared to diminish with increasing duration of observation. Based on an evaluation of 1,165 white

male workers followed through 1981, Rinsky et al. (1987) found an SMR for multiple myeloma of
4.09 (95% CI 1.10–10.47), an increase that was statistically significant. Assessment of the cohort after an
additional 6 and 15 years of follow-up yielded lower SMRs of 2.91 (95% CI 0.79–7.45) (Wong 1995) and
2.12 (95% CI 0.69–4.96) (Rinsky et al. 2002), respectively, risks that were no longer statistically
significant. The risk of multiple myeloma did not increase with increasing level of cumulative exposure
to benzene or with duration of employment, but this finding is particularly limited by the small number of
deaths. No increases in mortality from multiple myeloma were found in other cohort mortality studies,
including the NCI/CAPM study of Chinese workers (no observed deaths) (Yin et al. 1996a, 1996b), the
Bloemen et al. (2004) study of chemical workers (SMR=0.72, 95% CI 0.15–2.10), and the Paci et al.
(1989) study of shoe factory workers (no observed deaths in males; SMR=1.11 in females, 95% CI not
calculated) summarized above. Additionally, mortality from multiple myeloma was not increased in a
meta-analysis of 22 other cohorts of petroleum workers with potential exposure to benzene or benzene-
containing petroleum products (Wong and Raabe 1997). The combined cohort consisted of
250,816 workers (from the United States, Canada, the United Kingdom, and Australia) who were
observed over a period of 55 years from 1937 to 1991. The overall SMR for multiple myeloma was
0.93 (95% CI=0.81–1.07), and analyses by type of facility/industrial process (refinery, distribution, and
crude oil production and pipeline workers) and duration of observation showed no differences in risk.
The individual cohorts used in the meta-analysis also had no increased risk of multiple myeloma.

As summarized above, one of the early assessments of the Pliofilm cohort found an increased risk of
mortality from multiple myeloma (Rinsky et al. 1987). The implications of this finding are unclear
because the risk declined to non-significant levels in subsequent follow-ups (Rinsky et al. 2002; Wong
1995), and was not supported by the findings of other cohort mortality studies (Bloemen et al. 2004; Paci
et al. 1989; Wong and Raabe 2000; Yin et al. 1996a, 1996b). Additionally, population-based and
hospital-based case-control studies indicate that benzene exposure is not likely to be causally related to
the risk of mulitple myeloma (Bezabeh et al. 1996; Heineman et al. 1992; Linet et al. 1987; Schnatter et
al. 1996b; Sonoda et al. 2001; Wong and Raabe 1997). A meta-analysis of case-control studies found no
significant association between occupational exposure to benzene and benzene-containing products and
risk of multiple myeloma from sources categorized as benzene and/or organic solvents (summary odds
ratio [OR]=0.74, 95% CI 0.60–0.90), petroleum (summary OR=1.11, 95% CI 0.96–1.28), or petroleum
products (summary OR=1.08, 95% CI 0.89–1.33) (Sonoda et al. 2001).

Studies in animals provide supporting evidence for the carcinogenicity of inhaled benzene. As summarized below, inhalation exposure to benzene induced tumors at multiple sites in rats and mice, with a tendency towards induction of lymphomas in mice.

Carcinomas of the Zymbal gland and oral cavity were clearly increased in Sprague-Dawley rats that were exposed to 200–300 ppm benzene for 4–7 hours/day, 5 days/week for up to 104 weeks (Maltoni et al. 1982a, 1982b, 1983, 1985, 1989). The Zymbal gland is a specialized sebaceous gland and a site for benzene-induced tumors. Marginal increases in nasal cavity carcinomas, mammary tumors, and hepatomas were also observed. The significance of these findings is unclear because statistical analyses were not performed and there were no clearly increased incidences of Zymbal gland carcinoma or tumors at other sites in Sprague-Dawley rats that were similarly exposed to 100 or 300 ppm benzene for 6 hours/day, 5 days/week for life (Snyder et al. 1978b, 1984).

Benzene was carcinogenic in mice exposed to 100 or 300 ppm benzene for 6 hours/day, 5 days/week for 16 weeks and observed for 18 months or life (Cronkite 1986; Cronkite et al. 1984, 1985, 1989; Farris et al. 1993). These exposures consistently induced a variety of tumors, including leukemia (mainly thymic lymphoma) and Zymbal gland and ovarian tumors in C57BL/6 mice, and myelogenous leukemias, malignant lymphomas, and Zymbal gland and lung tumors in CBA/Ca mice. Incidences of lymphocytic lymphoma with thymic involvement were significantly increased in C57BL/6 mice similarly exposed to 300 ppm benzene for life (Snyder et al. 1980). Intermittent lifetime exposure to 300 ppm benzene (6 hours/day, 5 days/week on every third week) was more tumorigenic (in the Zymbal gland and lungs) to CD-1 and C57BL/6 mice than short-term exposure to 1,200 ppm (6 hours/day, 5 days/week for 10 weeks) followed by lifetime observation, although neither of these exposures induced leukemia (Snyder et al. 1988).

The cancer effect levels (CELs) for each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

EPA, IARC, and the Department of Health and Human Services have concluded that benzene is a human carcinogen. The Department of Health and Human Services (NTP 2005) determined that benzene is a known carcinogen based on human evidence showing a causal relationship between exposure to benzene and cancer. IARC (1987, 2004, 2007) classified benzene in Group 1 (carcinogenic to humans) based on sufficient evidence in both humans and animals. EPA (IRIS 2007) classified benzene in Category A (known human carcinogen) based on convincing evidence in humans supported by evidence from animal

studies. Under EPA's most recent guidelines for carcinogen risk assessment, benzene is characterized as a known human carcinogen for all routes of exposure based on convincing human evidence as well as supporting evidence from animal studies (IRIS 2007). Based on the Rinsky et al. (1981, 1987) human leukemia data, EPA derived a range of inhalation unit risk values of 2.2×10^{-6}–7.8×10^{-6} $(\mu g/m^3)^{-1}$ for benzene (IRIS 2007). For risks ranging from 1×10^{-4} to 1×10^{-7}, the corresponding air concentrations for lifetime exposure range from 13.0–45.0 $\mu g/m^3$ (4–14 ppb) to 0.013–0.045 $\mu g/m^3$ (0.004–0.014 ppb), respectively. These risk levels are presented in Figure 3-1.

3.2.2 Oral Exposure

3.2.2.1 Death

Individual case reports of death from acute oral exposure to benzene have appeared in the literature since the early 1900s. The benzene concentrations encountered by the victims were often not known. However, lethal oral doses for humans have been estimated at 10 mL (8.8 g or 125 mg/kg for a 70-kg person) (Thienes and Haley 1972). Lethality in humans has been attributed to respiratory arrest, central nervous system depression, or cardiac collapse (Greenburg 1926). Accidental ingestion and/or attempted suicide with lethal oral doses of benzene have produced the following signs and symptoms: staggering gait; vomiting; shallow and rapid pulse; somnolence; and loss of consciousness, followed by delirium, pneumonitis, collapse, and then central nervous system depression, coma, and death (Thienes and Haley 1972). Ingestion of lethal doses may also result in visual disturbances and/or feelings of excitement and euphoria, which may quite suddenly change to weariness, fatigue, sleepiness, convulsion, coma, and death (Von Oettingen 1940).

Animal lethality data indicate that benzene is of low toxicity following acute oral exposure (O'Bryan and Ross 1988). Oral LD_{50} values for rats ranged from 930 to 5,600 mg/kg; the values varied with age and strain of the animals (Cornish and Ryan 1965; Wolf et al. 1956). Male Sprague-Dawley rats were given various doses of benzene to determine the LD_{50} (Cornish and Ryan 1965). The LD_{50} for nonfasted rats was found to be 930 mg/kg. In 24-hour fasted rats, the LD_{50} was 810 mg/kg. No increase in mortality was reported in Fischer 344 rats or B6C3F$_1$ mice treated with 600 mg/kg/day for up to 17 weeks (Huff et al. 1989; NTP 1986).

Sprague-Dawley rats (30–35 males, 30–35 females) were exposed to benzene by ingestion (stomach tube), in olive oil, at 0, 50, or 250 mg/kg/day for 4–5 days weekly for 52 weeks and then kept under supervision until spontaneous death (Maltoni et al. 1983). Exposure to 50 mg/kg/day benzene after

52 weeks resulted in deaths in 9 of 30 male (same as controls) and 2 of 30 female rats. At 250 mg/kg/day exposure, 13 of 35 males and 9 of 35 females died. For rats receiving only olive oil, 9 of 30 males and 0 of 30 females died. In a companion study, Sprague-Dawley rats were exposed to 500 mg/kg/day benzene by ingestion (stomach tube), in olive oil, 4–5 days/week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Mortality rates were the same as the controls.

In a chronic-duration oral study conducted by the NTP (1986), increased mortality was observed in male Fischer 344 rats exposed to 200 mg/kg/day benzene in corn oil, and in female Fischer 344 rats exposed to 50 mg/kg/day benzene. B6C3F$_1$ mice given 100 mg/kg/day also had increased mortality compared to control mice.

The LD$_{50}$ values and all reliable LOAEL values for death in each species following acute and chronic exposure are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, musculoskeletal, hepatic, renal, endocrine, ocular, metabolic, or body weight effects in humans. Human and animal data pertaining to other systemic effects are presented below.

The highest NOAEL value and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects.   Male and female Fischer 344 rats and B6C3F$_1$ mice were given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). No histopathological lesions were observed in lungs, trachea, or mainstream bronchi. After chronic-duration exposure to 50, 100, or 200 mg/kg/day (male rats) or 25, 50, or 100 mg/kg/day (female rats, male and female mice), no histopathological lesions were observed in trachea, lungs, or mainstream bronchi in rats (NTP 1986). In mice, a significantly increased incidence of alveolar hyperplasia was observed at 50 and 100 mg/kg/day in females and at 100 mg/kg/day in males.

Cardiovascular Effects.   No histopathological lesions were observed in cardiac tissue from male and female Fischer 344 rats or B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). After chronic-duration exposure to ≤200 mg/kg/day (male

Table 3-2 Levels of Significant Exposure to Benzene - Oral

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
ACUTE EXPOSURE								
Death								
1	Human	once				126 (death)	Thienes and Haley 1972	
2	Rat (Sprague-Dawley)	once (G)				930 M (LD50)	Cornish and Ryan 1965	
3	Rat (Wistar)	once (GO)				5600 M (LD50)	Wolf et al. 1956	
Systemic								
4	Rat (Sprague-Dawley)	Gd 6-15 daily (G)	Renal	1000 F			Exxon 1986	
			Dermal		50 F (alopecia of hindlimbs and trunk)			
			Bd Wt	500 F	1000 F (body weight decreased 11%)			
			Other	50 F	250 F (decreased food consumption)			
5	Rat (CF)	1-3 d	Hepatic		1402 M (increased liver weight, biochemical changes)		Pawar and Mungikar 1975	
Neurological								
6	Human	once				126 (muscular incoordination, unconsciousness)	Thienes and Haley 1972	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
7	Rat (Sprague-Dawley)	1 d			88 M (slight CNS depression)	1870 M (tremors)	Cornish and Ryan 1965	
8	Rat (Sprague-Dawley)	once (G)			950 M (altered neurotransmitter concentrations)		Kanada et al. 1994	
Reproductive								
9	Rat (Sprague-Dawley)	Gd 6-15 daily (G)		1000 F			Exxon 1986	
Developmental								
10	Rat (Sprague-Dawley)	Gd 6-15 daily (G)		1000 F			Exxon 1986	
11	Mouse (ICR/SIM)	Gd 8-12 (GO)			1300 F (decreased pup weight on neonatal days 1-3)		Seidenberg et al. 1986	

INTERMEDIATE EXPOSURE
Death

12	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/d (GO)				250 F (9/35 died)	Maltoni et al. 1983, 1985	

Table 3-2 Levels of Significant Exposure to Benzene - Oral   **(continued)**

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form	Comments
					Less Serious (mg/kg/day)		Serious (mg/kg/day)		

Systemic

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	Reference Chemical Form	Comments
13	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/d (GO)	Bd Wt	50	250 (body weight decreased 19%)		Maltoni et al. 1983, 1985	
14	Rat (F-344/N)	<1 yr 5 d/wk (GO)	Hemato		50 M (lymphocytopenia and leukocytopenia)		NTP 1986	
					25 [b] F (lymphocytopenia and leukocytopenia)			
			Bd Wt	100	200 M (body weight decreased 11% or more in 25 weeks)			

Table 3-2 Levels of Significant Exposure to Benzene - Oral   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form	Comments
					Less Serious (mg/kg/day)		Serious (mg/kg/day)			
15	Rat (F-344/N)	60-120 d 5 d/wk (GO)	Resp	600					NTP 1986	
			Cardio	600						
			Gastro	600						
			Hemato		200	(dose-related leukopenia at 60 days)				
					25 [b]	(dose-related leukopenia at 120 days)				
			Musc/skel	600						
			Hepatic	600						
			Renal	600						
			Endocr	600						
			Bd Wt		200	(body weight decreased 14% in males and 16% in females)				
16	Rat (Fischer- 344)	6 wk 5 d/wk (GO)	Hepatic	400 M					Taningher et al. 1995	
			Bd Wt	400 M						
17	Rat (Wistar)	6 mo 5 d/wk (GO)	Hemato	1 F			50 F	(leukopenia, erythrocytopenia)	Wolf et al. 1956	

Table 3-2 Levels of Significant Exposure to Benzene - Oral   (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
18	Mouse (C57BL/6)	28 d (W)	Bd Wt	1000 M			Fan 1992	
19	Mouse (CD-1)	4 wk ad lib (W)	Hemato			8 M (erythrocytopenia, increased mean corpuscular volume; leukopenia)	Hsieh et al. 1988b	
20	Mouse (CD-1)	4 wk (W)	Hemato			31.5 M (decreased erythrocyte, leukocyte counts)	Hsieh et al. 1990	
			Hepatic	31.5 M				
			Renal	31.5 M				
			Bd Wt	31.5 M				
21	Mouse (B6C3F1)	<1 yr 5 d/wk (GO)	Hemato		25 (lymphocytopenia)		NTP 1986	
			Bd Wt	100				

Table 3-2 Levels of Significant Exposure to Benzene - Oral   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
22	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)	Resp	600			NTP 1986	
			Cardio	600				
			Gastro	600				
			Hemato	200 F	50 M [b] (dose-related leukopenia)			
				25	400 F (dose-related leukopenia)			
			Musc/skel	600				
			Hepatic	600				
			Renal	600				
			Endocr	600				
			Bd Wt	600				
23	Mouse (B6C3F1)	30 d ad lib (W)	Hemato	12 F	195 F (decreased leukocytes)	350 F (decreased hemoglobin, hematocrit, leukocytes, MCV, and MCH)	Shell 1992	
			Hepatic	350 F				
			Renal	350 F				
			Bd Wt	350 F				
			Other		12 F (decreased fluid intake)			

Table 3-2 Levels of Significant Exposure to Benzene - Oral   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		

Immuno/ Lymphoret

24	Rat (F-344/N)	60-120 d 5 d/wk (GO)			200 (lymphopenia at 60 days, lymphoid depletion in B-cell of the spleen)		Huff et al. 1989; NTP 1986	
					25 F dose-related lymphopenia at 120 days)			
25	Rat (Wistar)	6 mo 5 d/wk (GO)		1 F	50 F (leukopenia)		Wolf et al. 1956	
26	Mouse (C57BL/6)	28 d (W)			27 M (decreased number of splenocytes and IL-2 production)		Fan 1992	
27	Mouse (CD-1)	4 wk ad lib (W)				8 M (leukopenia and lymphopenia; enhanced splenic lymphocyte proliferation)	Hsieh et al. 1988b	
28	Mouse (CD-1)	4 wk (W)				31.5 M (reduction in thymus mass; suppresion of both B- and T-cell mitogeneses; suppressed IL-2 secretions; leukopenia, lymphopenia)	Hsieh et al. 1990	

Table 3-2 Levels of Significant Exposure to Benzene - Oral   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
29	Mouse (CD-1)	4 wk ad lib (W)				40 M (elevated corticosterone levels; T-lymphocyte suppression)	Hsieh et al. 1991	
30	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)		25 M [b] 200 F	50 M [b] (dose-related lymphopenia) 400 F		Huff et al. 1989; NTP 1986	
31	Mouse (B6C3F1)	30 d ad lib (W)			12 F (decreased leukocytes)		Shell 1992	
Neurological								
32	Rat (F-344/N)	60-120 d 5 d/wk (GO)		600			NTP 1986	
33	Mouse (CD-1)	4 wk ad lib (W)			8 M (fluctuation of neurotransmitter levels)		Hsieh et al. 1988a	
34	Mouse (CD-1)	4 wk (W)			31.5 M (decreased NE, DA, 5-HT)		Hsieh et al. 1990	
35	Mouse (CD-1)	4 wk ad lib (W)		8 M	40 M (increased hypothalmic NE and VMA)		Hsieh et al. 1991	

			LOAEL					

Table 3-2 Levels of Significant Exposure to Benzene - Oral   (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	Reference Chemical Form	Comments
36	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)		200	400 (intermittent tremors)		NTP 1986	
37	Mouse (B6C3F1)	30 d ad lib (W)		195 F	350 F (decreased brain weight)		Shell 1992	
Reproductive								
38	Rat (F344/N)	17 wk 5 d/wk (GO)		600			NTP 1986	
39	Mouse (B6C3F1)	17 wk 5 d/wk (GO)		600			NTP 1986	
Cancer								
40	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/d (GO)				50 F (CEL: Zymbal gland carcinoma in 2/30; oral cavity carcinoma)	Maltoni et al. 1983, 1985	
41	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/d (GO)				50 (CEL: Zymbal gland carcinoma) 250 (CEL: nasal cavity carcinoma, fore-stomach liver angiosarcoma; Zymbal gland carcinoma)	Maltoni et al. 1989	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
42	Mouse (RF/J)	52 wk 4-5 d/wk (GO)				500 (CEL: mammary pulmonary leukemias)	Maltoni et al. 1989	
CHRONIC EXPOSURE								
Death								
43	Rat (F-344/N)	2 yr 5 d/wk (GO)				200 M (30/50 died) 50 F [b] (14/50 died)	NTP 1986	
44	Mouse (B6C3F1)	2 yr 5 d/wk (GO)				100 (41/50 males died, 35/50 females died)	NTP 1986	
Systemic								
45	Human	6.1 yr (avg) (Occup)	Hemato		0.29 [c] (reduced WBC and platelet counts, approximately 7-18% lower than control values)		Lan et al. 2004a	Route-to-route extrapolation from the reported LOAEL of 0.57 ppm for occupational exposure was used by ATSDR to estimate equivalent oral dose.

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
46	Rat (F-344/N)	2 yr 5 d/wk (GO)	Resp	200 M 100 F[b]			Huff et al. 1989; NTP 1986	
			Cardio	200 M 100 F[b]				
			Gastro	100	200 M (hyperkeratosis and acanthosis in nonglandular forestomach)			
			Hemato		50 M (lymphocytopenia and leukocytopenia)			
					25 F[b] (lymphocytopenia and leukocytopenia)			
			Musc/skel	200 M 100 F[b]				
			Hepatic	200 M 100 F[b]				
			Renal	200 M 100 F[b]				
			Endocr	200 M 100 F[b]				
			Dermal	200 M 100 F[b]				
			Ocular	200 M 100 F[b]				

Table 3-2 Levels of Significant Exposure to Benzene - Oral   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
47	Rat (F-344/N)	2 yr 5 d/wk (GO)	Bd Wt	100 M		200 M (body weights decreased 23% in 103 weeks)	Huff et al. 1989; NTP 1986	
48	Rat (Sprague-Dawley)	92 wk 4-5 d/wk 1 x/d (GO)	Hemato			500 (decreased RBCs and WBCs after 84 weeks)	Maltoni et al. 1983, 1985	
			Bd Wt		500 (decreased body weights in 92 weeks)			

Table 3-2 Levels of Significant Exposure to Benzene - Oral   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
49	Mouse (B6C3F1)	2 yr 5 d/wk (GO)	Resp	50 M 25 F [b]	100 M (alveolar hyperplasia) 50 F [b]		NTP 1986	
			Cardio	100				
			Gastro		25 (epithelial hyperplasia and hyperkeratosis of forestomach)			
			Hemato		25 (lymphocytopenia; increased frequency of micronucleated normochromatic peripheral erythrocytes)			
			Musc/skel	100				
			Hepatic	100				
			Renal	100				
			Endocr		25 (hyperplasia of adrenal gland and harderian gland)			
			Dermal	100				
			Bd Wt	50	100 (mean body weight decreased 10% in 47 weeks to 19% in 103 weeks in males; decreased 14-15% in week 99-103 in females)			

Table 3-2 Levels of Significant Exposure to Benzene - Oral   **(continued)**

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
Immuno/ Lymphoret								
50	Rat (F-344/N)	2 yr 5 d/wk (GO)			50 M (lymphoid depletion of spleen and thymus)		Huff et al. 1989; NTP 1986	
					25 F [b] (lymphoid depletion of spleen and thymus)			
51	Rat (Sprague-Dawley)	92 wk 4-5 d/wk 1 x/d (GO)			500 (decreased WBCs after 84 weeks)		Maltoni et al. 1983, 1985	
52	Mouse (B6C3F1)	2 yr 5 d/wk (GO)			25 (lymphopenia, hematopoietic hyperplasia in the bone marrow, splenic hematopoiesis)		Huff et al. 1989; NTP 1986	
Neurological								
53	Rat (F-344/N)	2 yr 5 d/wk (GO)		200 M 100 F [b]			NTP 1986	
54	Mouse (B6C3F1)	2 yr 5 d/wk (GO)		100			NTP 1986	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		

Reproductive

| 55 | Rat (F-344/N) | 2 yr 5 d/wk (GO) | | 200 M

50 F[b] | | 100 F (endometrial polyps) | NTP 1986 | |
| 56 | Mouse (B6C3F1) | 2 yr 5 d/wk (GO) | | | 25 (preputial gland hyperplasia in males; ovarian hyperplasia and senile atrophy in females) | | NTP 1986 | |

Cancer

| 57 | Rat (F-344/N) | 2 yr 5 d/wk (GO) | | | | 50 M (CEL: squamous cell papillomas and carcinomas of the oral cavity)

25 F[b] (CEL: Zymbal gland carcinomas) | Huff et al. 1989; NTP 1986 | |
| 58 | Rat (Sprague-Dawley) | 92 wk 4-5 d/wk 1 x/d (GO) | | | | 500 (CEL: Zymbal gland carcinoma; oral and nasal cavity carcinoma; angiosarcoma of the liver) | Maltoni et al. 1983, 1985 | |

					LOAEL			
Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	Reference Chemical Form	Comments
59	Rat (Wistar)	104 wk 4-5 d/wk 1 x/d (GO)				500 (CEL: Zymbal gland and oral and nasal cavity carcinoma; angiosarcoma of the liver)	Maltoni et al. 1989	
60	Rat (Sprague-Dawley)	104 wk 5 d/wk (GO)				50 M (CEL: Zymbal gland carcinoma)	Maltoni et al. 1989	
61	Mouse (B6C3F1)	2 yr 5 d/wk (GO)				25 (CEL: harderian gland adenoma, lymphoma in males; CEL: lymphoma in females)	Huff et al. 1989; NTP 1986	
62	Mouse (Swiss)	78 wk 4-5 d/wk 1 x/d (GO)				500 (CEL: mammary tumors, lung tumors, Zymbal gland)	Maltoni et al. 1989	

Table 3-2 Levels of Significant Exposure to Benzene - Oral   (continued)

a The number corresponds to entries in Figure 3-2.

b Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

c Study results used to derive a chronic-duration oral minimal risk level (MRL) of 0.0005 mg/kg/day based on route-to-route extrapolation, as described in detail in Chapter 2 and Appendix A. Benchmark dose (BMD) analysis was performed on B-lymphocyte counts to select a point of departure, which was adjusted for intermittent exposure. An equivalent oral dose was estimated based on route-to-route extrapolation to determine a point of departure for deriving a chronic-duration oral MRL for benzene, which was divided by an uncertainty factor of 30 (10 for human variability and 3 for uncertainty in route-to-route extrapolation).

ad lib = ad libitum; Bd Wt = body weight; CEL = cancer effect level; CNS = central nervous system; d = day(s); DA = dopamine; F = female; (F) = feed; (G) = gavage; Gd = gestational day; (GO) = gavage in oil; Hemato = hematological; 5-HT = 5-hydroxystryptamine; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; mo = month(s); NE = norepinephrine; NOAEL = no-observed-adverse-effect level; NS = not specified; RBC = red blood cells; VMA = vanillin mandelic acid; (W) = water; WBC = white blood cells; wk = week(s); yr = year(s); x = times

Figure 3-2 Levels of Significant Exposure to Benzene - Oral

Acute (≤14 days)
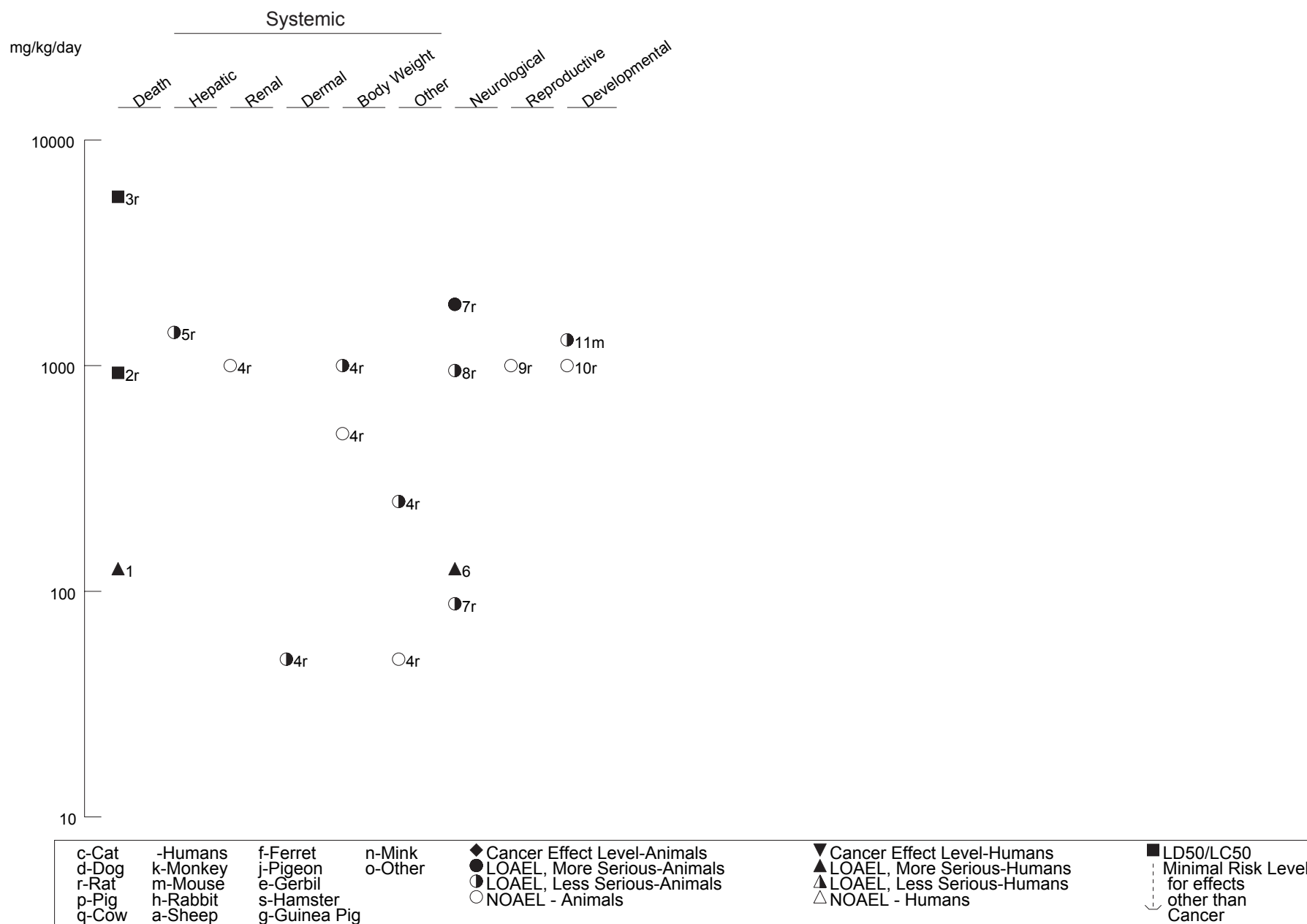
Figure 3-2 Levels of Significant Exposure to Benzene - Oral *(Continued)*

Intermediate (15-364 days)

Figure 3-2 Levels of Significant Exposure to Benzene - Oral *(Continued)*
Intermediate (15-364 days)

Figure 3-2 Levels of Significant Exposure to Benzene - Oral *(Continued)*
Chronic (≥365 days)

Figure 3-2 Levels of Significant Exposure to Benzene - Oral *(Continued)*
Chronic (≥365 days)

*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◖ LOAEL, Less Serious-Animals	▲ LOAEL, Less Serious-Humans	for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer
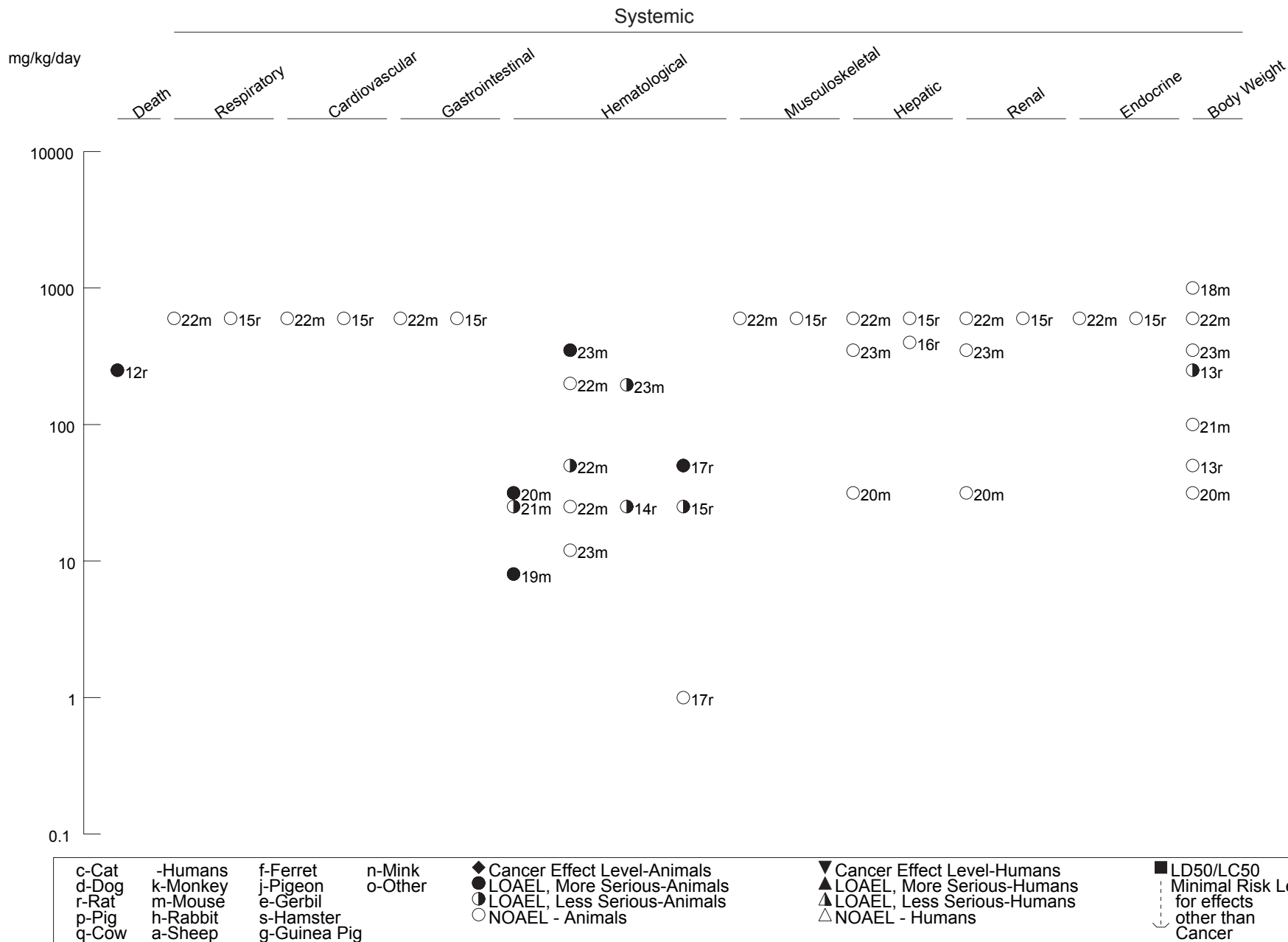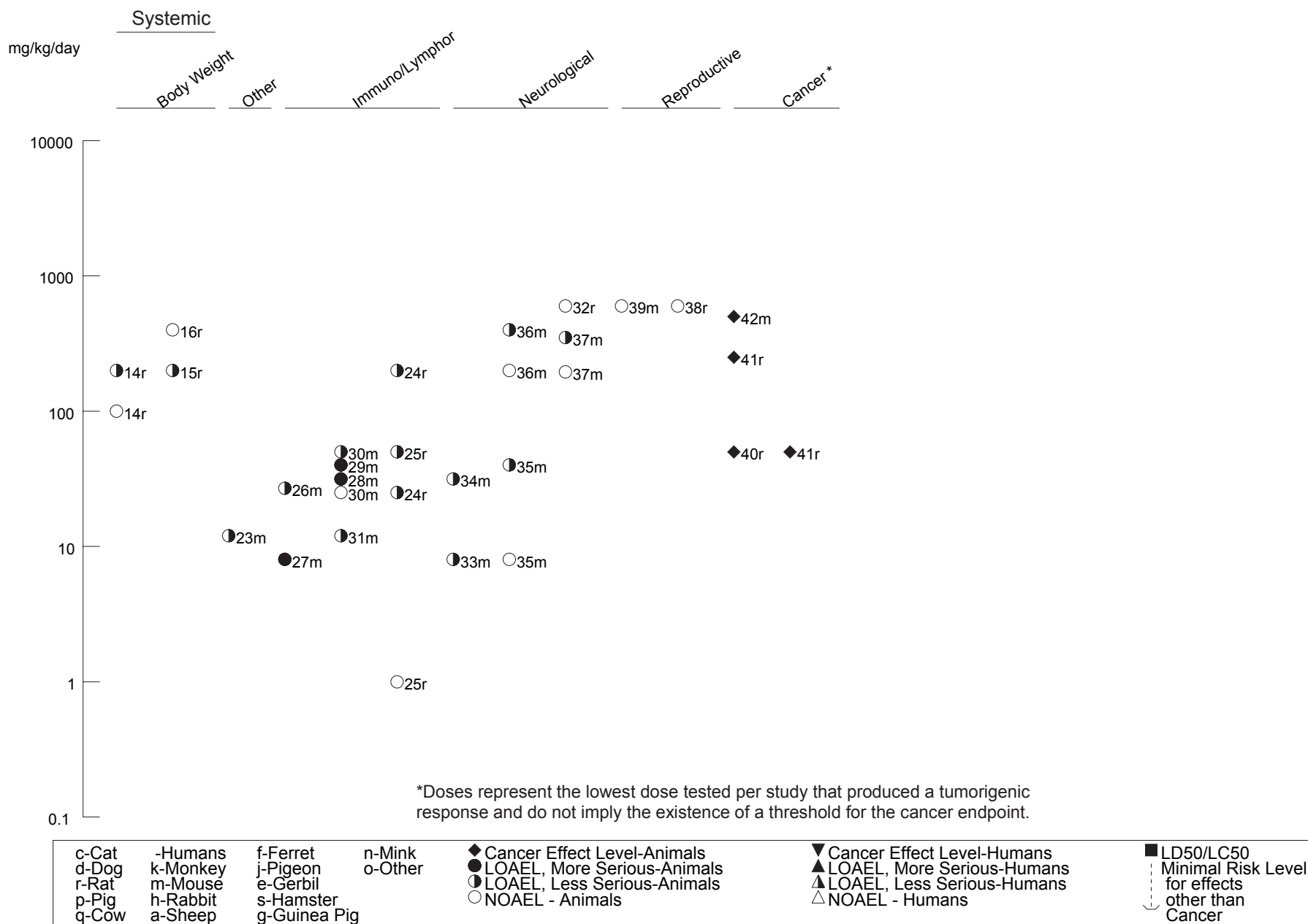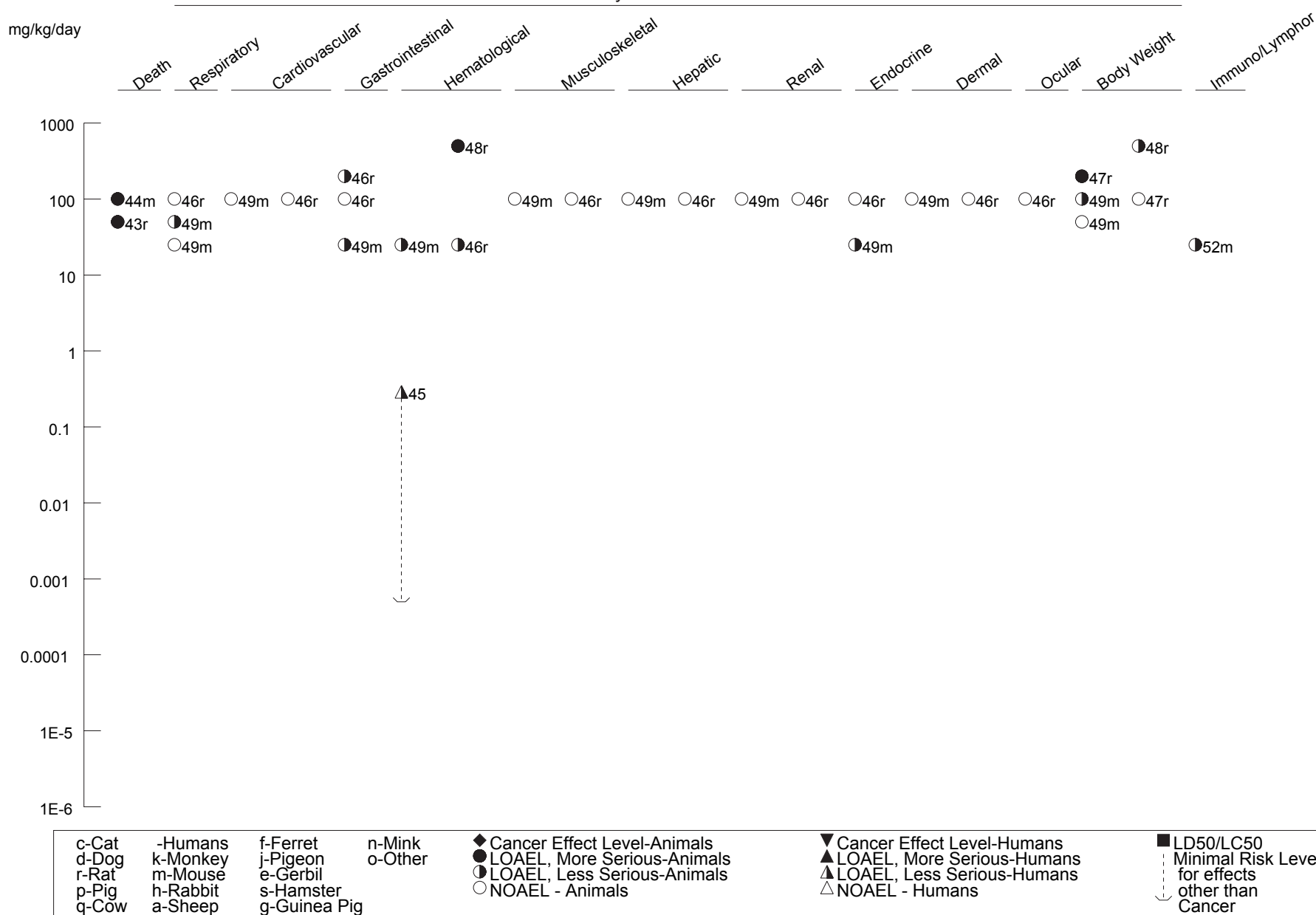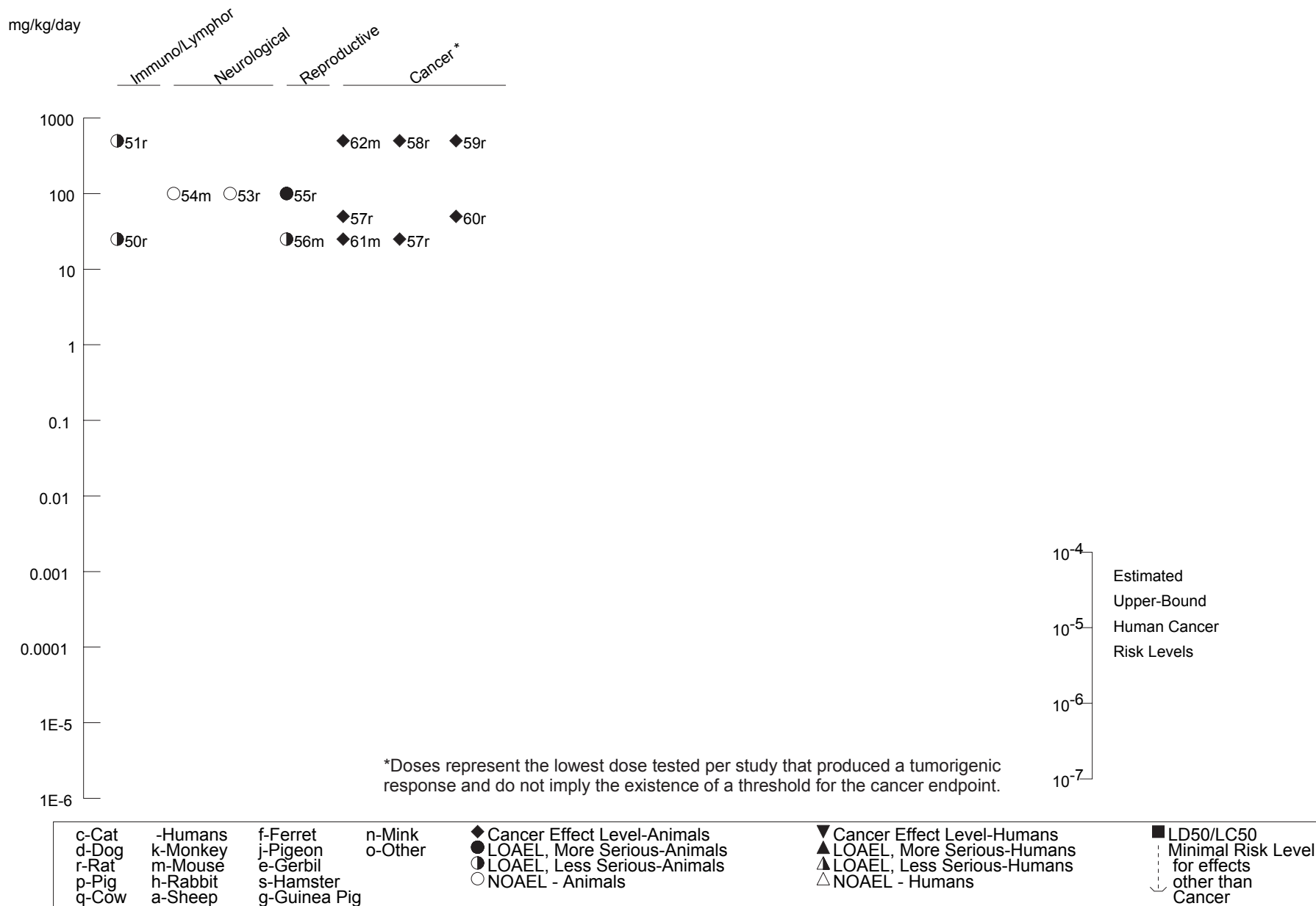
rats) or ≤100 mg/kg/day (female rats, male and female mice) no histopathological lesions were observed in the heart (NTP 1986).

Gastrointestinal Effects.   A man swallowed an unspecified amount of benzene and survived, but developed an intense toxic gastritis and later pyloric stenosis (Greenburg 1926).

No histopathological lesions were observed in esophageal and stomach tissue or in the small intestine and colon from male and female Fischer 344 rats or B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). After chronic-duration exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats, male and female mice), male rats exhibited hyperkeratosis and acanthosis in the nonglandular forestomach at 200 mg/kg/day, and mice exhibited epithelial hyperplasia and hyperkeratosis in the forestomach at 25 mg/kg/day (NTP 1986).

Hematological Effects.   Prior to 1913, benzene was used as a treatment for leukemia. Benzene was given in gelatin capsules starting with 43 mg/kg/day and increasing to 71 mg/kg/day for unspecified durations (Selling 1916). Leukemia patients showed a great reduction in leukocyte count and multiple hemorrhages with advanced anemia. However, it is difficult to determine which effects were due to the leukemia and which were due to the benzene treatment.

Intermediate-duration studies in animals have revealed decreases in numbers of erythrocytes and leukocytes following exposure to benzene. Male and female Fischer 344 rats and B6C3F$_1$ mice were given oral doses of 0, 25, 50, 100, 200, 400, and 600 mg/kg/day benzene in corn oil for 120 days. Dose-related leukopenia and lymphopenia were observed at 200 and 600 mg/kg/day for both male and female rats killed on day 60, and at all doses in female rats killed on day 120. Dose-related leukopenia and lymphopenia were observed in male mice at 50 mg/kg/day and female mice at 400 mg/kg/day for 120 days, but not for 60 days. Mice exposed to 8 mg/kg/day in the drinking water for 4 weeks had decreased numbers of erythrocytes, increased mean corpuscular volumes, and decreased numbers of lymphocytes (Hsieh et al. 1988b, 1990). Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased hemoglobin, hematocrit, leukocytes, MCV, and MCH were observed at 350 mg/kg/day. Decreased leukocytes also occurred at 195 mg/kg/day. Decreased leukocytes also occurred at 195 mg/kg/day. Rats gavaged with 50 mg/kg/day of benzene 5 days/week for 6 months also had decreased numbers of erythrocytes and leukocytes (Wolf et al. 1956). One chronic-duration study showed that gavage doses of 25 mg/kg/day resulted in leukopenia and/or lymphocytopenia in both rats and mice, both at the interim sacrifices at 3–18 months, and at the

end of 2 years (Huff et al. 1989; NTP 1986). Increased frequency of micronucleated normochromatic peripheral erythrocytes was observed in mice at 25 mg/kg/day after 2 years. Sprague-Dawley rats were exposed to 500 mg/kg benzene by ingestion (stomach tube), in olive oil, once daily, 4–5 days/week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Decreased erythrocytes and leukocytes were observed after 84 weeks.

Musculoskeletal Effects. No histopathological lesions were observed in femoral tissue from male and female Fischer 344 rats or B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days, or in the sternebrae, femur, or vertebrae from Fischer 344 rats and mice exposed to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats, male and female mice) for 2 years (NTP 1986).

Hepatic Effects. Acute oral administration of 1,402 mg/kg/day benzene for 3 days induced hepatic changes in rats evidenced by increased liver weight, decreased protein in the postmitochondrial supernatant fractions (9,000 times specific gravity), and changes in hepatic drug metabolism and lipid peroxidation (Pawar and Mungikar 1975). The initiation-promotion-progression (IPP) model for the induction of malignant neoplasms in the liver was evaluated for benzene in male and female Sprague-Dawley rats (Dragan et al. 1993). Initiation was begun in 5-day-old rats with administration of a single intraperitoneal injection of diethylnitrosamine during the time when the liver is undergoing rapid growth. Promotion began at 6 months of age with phenobarbital in the feed, and continued into young adulthood. Partial hepatectomy was performed, and at the height of the regenerative proliferation phase following the hepatectomy, benzene (1 g/kg) was administered by gavage; phenobarbital treatment was maintained after the administration of benzene. A slight increase in the incidence of altered hepatic foci was observed after initiation and promotion. Few hepatic foci were observed in the livers of male Fischer 344 rats treated by gavage with 400 mg/kg/day benzene in corn oil for 5 days/week for 6 weeks (Taningher et al. 1995). No histopathological non-neoplastic lesions were observed in hepatic tissue from male and female Fischer 344 rats given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days or in male rats exposed to 50–200 mg/kg/day and female rats exposed to 25–100 mg/kg/day for 2 years (NTP 1986).

Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). No adverse liver effects, as evidenced by gross necropsy, liver weight determination, and serum levels of hepatic enzymes, were observed. Oral administration of 31.5 mg/kg/day benzene continuously in drinking water for 4 weeks did not affect liver weight in CD-1

mice (Hsieh et al. 1990). No histopathological non-neoplastic lesions effects were observed in hepatic tissue from male and female B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days, or in male and female mice exposed to 25–100 mg/kg/day for 2 years (NTP 1986).

Renal Effects. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day benzene on Gd 6–15 and killed on Gd 20 (Exxon 1986). No adverse effects were noted in the kidneys based on gross necropsy. No adverse effects based on histological examination were observed on renal tissue or the urinary bladder from male and female Fischer 344 rats given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days or in male rats exposed to 50–200 mg/kg/day and female rats exposed to 25–100 mg/kg/day for 2 years (NTP 1986). Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg benzene in drinking water for 30 days (Shell 1992). No adverse effects were observed in the kidneys, based on kidney weights, gross examination, and blood urea nitrogen and creatinine determinations. Oral administration of 31.5 mg/kg/day benzene continuously in drinking water for 4 weeks did not affect kidney weight in CD-1 mice (Hsieh et al. 1990). No adverse effect based on histological examination was observed on renal tissue or the urinary bladder from male and female B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days, or in male and female mice exposed to 25–100 mg/kg/day for 2 years (NTP 1986).

Endocrine Effects. No histopathological lesions were observed in salivary, thyroid, parathyroid, pancreas, adrenal, or pituitary glands from male and female Fischer 344 rats or B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). In the companion chronic-duration oral study, male Fischer 344 rats were exposed to 25, 50, 100, or 200 mg/kg/day benzene, while female rats received 25, 50, or 100 mg/kg/day benzene. Hyperplasia of the Zymbal gland was increased in low-dose males and in mid-dose females. In the adrenal gland, hyperplasia was observed in both sexes (males: 27 and 4% at 50 and 200 mg/kg, respectively; females: 34% at 25 mg/kg). In the thyroid gland, incidences of C-cell hyperplasia were 14, 26, 15, and 15% in males treated with 0, 50, 100, and 200 mg/kg, respectively. Analysis of the pituitary gland showed incidence of hyperplasia in males treated with 0, 50, 100, and 200 mg/kg at 6, 16, 20, and 10%, respectively; in females treated with 0, 25, 50, and 100 mg/kg at 11, 20, 10, and 14%, respectively. None of the increased incidences of hyperplasia in these glands were considered to be treatment-related by NTP. The non-dose-related increase of hyperplasia of the Zymbal gland could represent a progression to the neoplasms (see Section 3.2.2.7). In mice, Zymbal gland lesions showed epithelial hyperplasia in

males (0, 9, 30, and 26%) and in females (2, 3, 5, and 19%) exposed to 0, 25, 50, or 100 mg/kg, respectively. Hyperplasia of the adrenal cortex occurred at incidences of 4, 67, 29, and 9% in males and 10, 43, 68, and 13% in females, respectively. Hyperplasia of the harderian gland occurred at incidences of 0, 11, 22, and 15% in males and 13, 23, 22, and 21% in females, respectively (NTP 1986).

Dermal Effects.   A case of accidental poisoning in which the patient survived but developed an odd skin condition consisting of swelling and edema has been reported (Greenburg 1926).

Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day benzene daily on Gd 6–15 and killed on Gd 20 (Exxon 1986). Alopecia of the hind limbs and trunk was noted in all dose groups.

No histopathological lesions were observed in the skin of male and female Fischer 344 rats and B6C3F$_1$ mice after chronic oral exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice) (NTP 1986).

Ocular Effects.   No histopathological lesions were noted in the eyes of male and female Fischer 344 rats and B6C3F$_1$ mice after chronic-duration oral exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice) (NTP 1986).

Body Weight Effects.   No significant change in body weight was observed in male Fischer 344 rats treated by gavage with 400 mg/kg/day benzene in corn oil for 5 days/week for 6 weeks (Taningher et al. 1995). Body weight was unaffected in male and female Fischer 344 rats given oral doses of 0, 25, 50, or 100 mg/kg/day benzene in corn oil for 120 days (NTP 1986). However, animals receiving 200, 400, or 600 mg/kg/day benzene exhibited a 14–22% decrease in body weight after 120 days. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg benzene daily on Gd 6–15 and killed on Gd 20 (Exxon 1986). Maternal body weight decreased 11% at the high dose. C57BL/6 male mice were given benzene at concentration levels of 200 and 1,000 mg/L (assumed benzene intake of 27 and 154 mg/kg/day) in drinking water for 28 days (Fan 1992). Control groups were given untreated tap water. Groups of mice were killed on day 7, 14, 21, and 28 of administration, and on day 7, 14, 21, and 28 after the last administration of benzene at 200 mg/L. There was no effect of treatment on body weight. Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). There was no significant effect on body weight at the highest treatment level. Oral administration of 31.5 mg/kg/day benzene continuously in drinking water for 4 weeks did not affect

body weight in CD-1 mice (Hsieh et al. 1990). No adverse effect was observed on body weight of male and female B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986).

Sprague-Dawley rats were exposed to benzene by gavage in olive oil, at 0, 50, or 250 mg/kg/day body weight for 4–5 days/week for 52 weeks, and then kept under supervision until spontaneous death (Maltoni et al. 1983, 1985). A 19% decrease in body weight was reported in animals exposed to 250 mg/kg benzene for 52 weeks. In a companion study, Sprague-Dawley rats were exposed to 500 mg/kg benzene by ingestion (stomach tube), in olive oil, once daily, 4–5 days/week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Decreased body weight was observed after 92 weeks. In a chronic-duration oral study, male Fischer 344 rats exhibited a decrease in body weight of 11% or more after 25 weeks exposure to doses of 200 mg/kg/day benzene in corn oil (NTP 1986). Females rats and male and female B6C3F$_1$ mice in the same study exposed to doses up to 100 mg/kg/day benzene did not show any change in body weight after 12 months of exposure, or after 2 years exposure (female rats). Male and female mice exhibited body weight effects after chronic exposure. In male mice given 100 mg/kg, mean body weights decreased from 10% after 47 weeks to 19% in 103 weeks of exposure relative to controls. In female mice given 100 mg/kg, mean body weights decreased 14–15% in weeks 99–103 of exposure (NTP 1986).

Other Systemic Effects.   C57BL/6 male mice were given benzene at concentration levels of 200 and 1,000 mg/L (assumed benzene intake of 27 and 154 mg/kg/day) in drinking water for 28 days (Fan 1992). Control groups were given untreated tap water. Groups of mice were killed on day 7, 14, 21, and 28 of administration, and on day 7, 14, 21, and 28 after the last administration of benzene at 200 mg/L. There was no effect of treatment on food or water consumption. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day on Gd 6–15 and killed on Gd 20 (Exxon 1986). Decreased feed consumption was noted at doses of 250 mg/kg and above, and body weight decreased 11% at the high dose. Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased fluid consumption was observed at >12 mg/kg.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after oral exposure to benzene.

Oral administration of benzene to CD-1 mice produced an immunotoxic effect on both the humoral and cellular immune responses (Hsieh et al. 1988b). Exposure to benzene at 8, 40, or 180 mg/kg/day for 4 weeks caused a significant dose-response reduction of total peripheral blood leukocytes and erythrocytes. Lymphocytes, but not neutrophils or other leukocytes, were decreased in number. Splenic lymphocyte proliferative response to B- and T-cell mitogens was biphasic—enhanced in the 8 mg/kg/day dosage group and depressed in the 40 and 180 mg/kg/day dosage groups. Cell-mediated immunity evaluated in mixed-lymphocyte reaction and in the ^{51}Cr-release assay showed a similar biphasic response. Antibody production was significantly suppressed in mice dosed at 40 and 180 mg/kg/day. The results indicate that administration of 40 mg/kg/day benzene has an immunosuppressive effect evident in decreased immune functions evaluated in *in vitro* assays for cell-mediated immunity and antibody production. A dose-related decrease in spleen weight was observed, which was significant only in the 180 mg/kg/day group.

C57BL/6 male mice were given benzene at concentration levels of 200 and 1,000 mg/L (assumed benzene intake of 27 and 154 mg/kg/day) in drinking water for 28 days (Fan 1992). Control groups were given untreated tap water. Mice were sacrificed on days 7, 14, 21, and 28 of administration. Selected mice of the 27 mg/kg/day dose group were sacrificed on postexposure days 7, 14, and 21 in order to assess the postexposure time course of the benzene toxicity. At 27 mg/kg/day, a decreased number of splenocytes was observed on day 21 and 28 of exposure. At 154 mg/kg/day, spleen cell numbers decreased significantly as a function of time in mice treated for 14, 21, and 28 days. Benzene treatment for 3 weeks raised natural killer (NK) cell activity significantly at both doses. However, after another week of benzene treatment, NK cell activity resumed to control levels. Significant depression of interleukin-2-(IL-2) production was detected in both levels for 28 days. The NK cell activity showed normal levels on day 7, 14, and 21 after the last administration of benzene exposure for 28 days at 27 mg/kg/day. IL-2 production decreased significantly on day 7 and 14 after cessation of benzene administration, but recovered with time (43, 71, and 79% of control on day 7, 14, and 21, respectively). Spleen cell number decreased significantly on the 7th day, but recovered on day 14 and 21. Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased leukocytes were observed at 12 mg/kg/day, and decreased spleen cell number was observed at 195 mg/kg/day.

In the NTP-sponsored intermediate-duration oral study using Fischer 344 rats and B6C3F$_1$ mice, dose-related leukopenia and lymphopenia were observed for both male and female Fischer 344 rats at 200 and 600 mg/kg/day killed on day 60, and at all doses in female rats killed on day 120 (NTP 1986). Decreased

leukocytes were observed in male and female rats exposed for 60 days to 200 and 600 mg/kg/day benzene. Lymphoid depletion in the B-cells of the spleen was observed in animals exposed to 200 mg/kg/day (3 of 5 males, 4 of 5 females) and 600 mg/kg/day (5 of 5 males, 5 of 5 females) benzene for 60 days and in animals that received 600 mg/kg/day (10 of 10 males, 10 of 10 females) benzene for 120 days. At 600 mg/kg/day benzene exposure, increased extramedullary hematopoiesis was observed in the spleen of 4 of 5 male and 3 of 5 female rats. Dose-related leukopenia and lymphopenia were observed for both male and female mice exposed for 120 days, but not for 60 days. Leukocytes and lymphocytes were significantly decreased in male mice exposed for 120 days to 50, 100, 200, 400, and 600 mg/kg/day benzene. At 120 days of exposure, leukocytes were significantly decreased in female mice at 600 mg/kg/day and lymphocytes at 400 and 600 mg/kg/day. Histological examination revealed no adverse effects in mandibular lymph node or the thymus for either rats or mice (Huff et al. 1989; NTP 1986). Rats exposed to benzene at 50 and 100 mg/kg/day for 6 months had significant leukopenia (Wolf et al. 1956).

Leukopenia and lymphopenia were observed in mice at 31.5 mg/kg/day after 4 weeks of oral exposure (Hsieh et al. 1990). Reduction in thymus mass, suppression of B- and T-cell mitogenesis, and suppressed IL-2 release were also noted. Similar results were noted at 40 mg/kg/day (Hsieh et al. 1991). Oral administration of benzene to B6C3F$_1$ mice and Fischer 344 rats at doses of 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice), 5 days/week for 103 weeks resulted in significant leukocytopenia and lymphocytopenia in both species (Huff et al. 1989; NTP 1986). In the thymus, lymphoid depletion was observed at 0, 10, 20, or 28% in male rats treated with 0, 50, 100, or 200 mg/kg/day, respectively. Increased incidences of lymphoid depletion of the spleen were observed in male rats treated with 0 (0%), 50 (40%), 100 (17%), and 200 (49%) mg/kg/day and in female rats treated with 0 (0%), 25 (22%), 50 (16%), and 100 (20%) mg/kg/day. In mice, an increased incidence of hematopoietic hyperplasia was observed in the bone marrow of dosed animals in both sexes. Splenic hematopoiesis was increased in dosed animals of both sexes of mice (Huff et al. 1989; NTP 1986). Maltoni et al. (1983, 1985) observed decreased leukocytes in Sprague-Dawley rats dosed with 500 mg/kg/day benzene for 84 weeks or more.

The highest NOAEL values and all reliable LOAEL values for immunologic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

In humans, symptoms of central nervous system toxicity (including euphoria, vertigo, muscular incoordination, and unconsciousness) have been reported following one-time ingestion of benzene at 125 mg/kg (Thienes and Haley 1972).

Neurochemical profiles were conducted on rats after oral exposure to benzene (Kanada et al. 1994). Sprague-Dawley rats received a single dose of 950 mg/kg benzene by gavage and were sacrificed 2 hours after treatment. The control group received nothing. Brains were dissected into small-brain areas and stored until analysis. Acetylcholine, 3,4-dihydroxyphenylalanine (DOPA), dopamine, 3,4-dihydroxy-phenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine, 3-methoxy-4-hydroxy-phenylglycol (MHPG), serotonin, and 5-hydroxyindoleacetic acid (5HIAA) contents in the small-brain regions were measured. Results showed that benzene decreased acetylcholine content of rat hippocampus. DOPA and norepinephrine content decreased in the rat midbrain. Dopamine, serotonin and 5HIAA content increased in the rat midbrain. Dopamine, DOPAC, norepinephrine, and 5HIAA content increased and serotonin content decreased in the rat hypothalmus after oral administration of benzene. Increased dopamine, HVA, MHPG, and serotonin content of rat medulla oblongata was observed. Decreased norepinephrine and 5HIAA content of rat medulla oblongata by benzene treatment was observed.

Oral exposure to benzene induced both synthesis and catabolism of monoamine neurotransmitters in CD-1 mice (Hsieh et al. 1988a). Mice given 8, 40, or 180 mg/kg/day of benzene for 4 weeks in drinking water exhibited changes in the levels of norepinephrine, dopamine, serotonin, and catecholamine metabolites in several brain regions but no treatment-related behavioral changes. Similar results were seen at 31.5–40 mg/kg/day (Hsieh et al. 1990, 1991). Because of the lack of association with behavioral changes, the effects on the neurotransmitters cannot be adequately assessed. Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased brain weight was observed at 350 mg/kg/day. Sprague-Dawley rats given one oral dose of 88 mg/kg benzene exhibited slight central nervous system depression, whereas at 1,870 mg/kg/day, tremors were observed (Cornish and Ryan 1965). Histological examination of the brain revealed no treatment-related lesions after gavage treatment of male and female Fischer 344 rats and B6C3F$_1$ mice with doses up to 600 mg/kg/day for 120 days (NTP 1986). In the same experiment, B6C3F$_1$ mice exhibited tremors intermittently at doses of 400 mg/kg/day, which were more pronounced in males during the last 3 weeks of the study. No adverse effects based on histological examination of brain or spinal cord were observed

in male and female Fischer 344 rats and B6C3F$_1$ mice after chronic oral exposure to 50–200 mg/kg/day
(male rats) or 25–100 mg/kg/day (female rats and male and female mice) (NTP 1986).

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and
duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to benzene.

Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg benzene daily
on Gd 6–15 and killed on Gd 20 (Exxon 1986). No adverse effects were noted on reproductive
competency. No histological changes were reported in the prostate, testes, ovaries, mammary gland, or
uterus of male and female Fischer 344 rats and B6C3F$_1$ mice dosed by gavage with up to 600 mg/kg/day
benzene for 17 weeks (NTP 1986). In male and female Fischer 344 rats and B6C3F$_1$ mice after chronic
oral exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female
mice), endometrial stromal polyps occurred with a significant positive trend in female rats (NTP 1986).
The incidence in high dose group (14/50) was significantly greater than that in the control (7/50). In
mice, analysis of preputial gland lesions in male mice dosed at 0, 25, 50, or 100 mg/kg showed increased
incidences of focal, diffuse or epithelial hyperplasia (5, 65, 31, and 3%, respectively). The lower
incidences of hyperplasia in the higher dose groups were probably due to the progression of the preputial
gland lesions to neoplasias (see Section 3.2.2.7). Various non-neoplastic and neoplastic ovarian lesions
were observed in dosed female mice, including epithelial hyperplasia and senile atrophy (NTP 1986).

The NOAEL and LOAEL values for reproductive effects in rats and mice are recorded in Table 3-2 and
plotted in Figure 3-2.

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to benzene.

Benzene was embryotoxic as evidenced by reduced pup body weights when mice were administered
1,300 mg/kg/day of benzene by gavage on Gd 8–12 (Seidenberg et al. 1986). No maternal toxicity was
observed. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg

benzene daily on Gd 6–15 and killed on Gd 20 (Exxon 1986). No adverse effects were noted on morphological development.

The NOAEL value for rats and the LOAEL value for mice for developmental effects following acute oral exposure are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

Essentially no information was located regarding the oral carcinogenicity of benzene in humans. Lymphatic and hematopoietic cancers were increased in vehicle maintenance workers who occasionally siphoned gasoline by mouth (Hunting et al. 1995), but the skin and lungs were the main routes of exposure (see Section 3.2.3.7).

Benzene has been shown to be a multiple site carcinogen by the oral route in animals (Huff et al. 1989; Maltoni et al. 1983, 1985, 1989; NTP 1986). In bioassays conducted by the NTP, benzene in corn oil was administered groups of 50 F344/N rats and 50 B6C3F$_1$ of each sex by gavage on 5 days/week for 103 weeks (Huff et al. 1989; NTP 1986). The male rats were exposed to dose levels of 0, 50, 100, or 200 mg/kg/day, and female rats and mice of both sexes were exposed to 0, 25, 50, or 100 mg/kg/day. In the rats, benzene caused significantly increased incidences of Zymbal gland carcinomas in males at ≥100 mg/kg/day and females at ≥25 mg/kg/day, oral cavity squamous cell papillomas and carcinomas in males at ≥50 mg/kg/day and females at ≥25 mg/kg/day, and skin squamous cell papillomas and carcinomas in males at 200 mg/kg/day. In the mice, benzene mainly caused significantly increased incidences of malignant lymphomas in both sexes at ≥25 mg/kg/day, Zymbal gland carcinomas in males at ≥50 mg/kg/day and females at 100 mg/kg/day, lung alveolar/bronchiolar adenomas and carcinomas in males at ≥100 mg/kg/day and females at ≥50 mg/kg/day, Harderian gland adenomas in males at ≥25 mg/kg/day, preputial gland squamous cell carcinomas in males at ≥50 mg/kg/day, and mammary gland carcinomas in females at ≥50 mg/kg/day. NTP (1986) concluded that there was clear evidence of carcinogenicity of benzene in male and female F344/N rats and B6C3F$_1$ mice under the conditions of these studies.

Maltoni et al. (1983, 1985, 1989) assessed carcinogenicity in groups of 30–50 rats and mice of each sex that were exposed to benzene in olive oil by gavage on 4–5 days/week for up to 104 weeks and observed for life. Sprague-Dawley rats were exposed to 0, 50, or 250 mg/kg/day for 52 weeks or 0 or 500 mg/kg/day for 104 weeks; effects included increased incidences of Zymbal gland carcinomas in

females at ≥50 mg/kg/day, oral cavity carcinomas in females at 250 mg/kg/day and both sexes at
500 mg/kg/day, forestomach carcinomas in females at 500 mg/kg/day, nasal cavity and skin carcinomas
in males at 500 mg/kg/day, and liver angiosarcomas in both sexes at 500 mg/kg/day. Wistar rats were
exposed to 0 or 500 mg/kg/day for 104 weeks; effects included increased incidences of Zymbal gland,
nasal cavity, and oral cavity carcinomas. Swiss mice were exposed to 0 or 500 mg/kg/day for 78 weeks;
effects included increased incidences Zymbal gland carcinomas in males, mammary carcinomas in
females, and lung adenomas in both sexes. RF/J mice were exposed to 0 or 500 mg/kg/day for 52 weeks;
effects included increased incidences of mammary carcinomas in females and lung adenomas in both
sexes.

As discussed in Section 3.2.1.7, there is a consensus that benzene is a human carcinogen (IARC 1987,
2004, 2007; IRIS 2007; NTP 2005). This conclusion is based on sufficient inhalation data in humans
supported by animal evidence, including the oral studies summarized above. The human cancer induced
by inhalation exposure to benzene is predominantly acute nonlymphocytic leukemia, whereas benzene is
a multiple site carcinogen in animals by both the inhalation and oral routes. Due to the lack of oral
carcinogenicity data in humans, as well as the lack of a well-demonstrated and reproducible animal model
for leukemia from benzene exposure, EPA extrapolated an oral slope factor from the inhalation unit risk
range (IRIS 2007). The oral slope factor ranges from $1.5x10^{-2}$ to $5.5x10^{-2}$ $(mg/kg/day)^{-1}$, and for cancer
risks of $1x10^{-4}$–$1x10^{-7}$, the corresponding dose levels range from $6.7x10^{-3}$–$1.8x10^{-3}$ to $6.7x10^{-6}$–$1.8x10^{-6}$
mg/kg/day, respectively. These risk levels are presented in Figure 3-1.

3.2.3   Dermal Exposure

3.2.3.1   Death

No studies were located regarding deaths in animals after dermal exposure to benzene.

A cohort of 338 men was investigated as to causes of death among employees of the fleet maintenance
division of Washington DC's Department of Public Works (Hunting et al. 1995). This mortality study
was undertaken because of three cases of leukemia among car and mobile equipments mechanics.
Preliminary evaluation showed that the garage mechanics regularly used gasoline to clean parts and wash
their hands; these workers also experienced dermal and inhalation exposure to gasoline during
maintenance of vehicles. The men were employed for at least 1 year between January 1, 1977, and
December 31, 1989. Cause-specific SMRs were calculated. Increased risk of death was found in some
categories.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, or body weight effects in humans or animals after dermal exposure to benzene. Available data pertaining to hematological, dermal, and ocular effects are presented below. All reliable LOAEL values for systemic effects in humans and rabbits for acute- and chronic-duration dermal exposure are recorded in Table 3-3.

Hematological Effects.   Tondel et al. (1995) reported a case of myelofibrosis that was diagnosed in a 46-year-old man who had worked from 1962 to 1979 as a gasoline station attendant. Although the exposure was primarily by inhalation, it is likely that dermal exposure also occurred.

Dermal Effects.   In humans, benzene is a skin irritant. By defatting the keratin layer, it may cause erythema, vesiculation, and dry and scaly dermatitis (Sandmeyer 1981). Acute fatal exposure to benzene vapors caused second degree burns on the face, trunk, and limbs of the victims (Avis and Hutton 1993). Fifteen male workers were exposed to benzene vapors (>60 ppm) over several days during the removal of residual fuel from shipyard fuel tanks (Midzenski et al. 1992). Exposures to benzene range from 1 day to 3 weeks (mean of 5 days), 2.5–8 hours/day (mean of 5.5 hours). Workers with more than 2 days (16 hours) exposure reported mucous membrane irritation (80%), and skin irritation (13%) after exposure to the vapor.

Benzene was slightly irritating to the skin of rabbits (Wolf et al. 1956). The skin showed moderate erythema, edema, and moderate necrosis following application 1 time/day for 4 weeks.

Ocular Effects.   Solvent workers who were exposed to 33 (men) or 59 (women) ppm benzene exhibited eye irritation while being exposed to the vapors (Yin et al. 1987b).

A transient increase in lacrimation was observed in male rats exposed to 10–300 ppm benzene for 6 hours/day, 5 days/week (Shell 1980). Moderate conjunctival irritation and transient corneal damage were observed in rabbits subsequent to placement of 2 drops of benzene onto the eyeball (Wolf et al. 1956).

Table 3-3 Levels of Significant Exposure to Benzene - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL			Reference Chemical Form	Comments
				Less Serious		Serious		
ACUTE EXPOSURE								
Systemic								
Human	1-21 d 2.5-8 hr/d	Dermal		60 M ppm	(mucous membrane and skin irritation)		Midzenski et al. 1992	
Rabbit (NS)	once	Ocular		2 Unknown	(moderate conjunctival irritations; light corneal injury)		Wolf et al 1956	
INTERMEDIATE EXPOSURE								
Systemic								
Rat (CD)	10 wk 5 d/wk 6 hr/d	Ocular	1 M ppm	10 M ppm	(lacrimation)		Shell 1980	
CHRONIC EXPOSURE								
Systemic								
Human	>1 yr (occup)	Ocular		33 M ppm	(eye irritation)		Yin et al. 1987b	
				59 F ppm	(eye irritation)			

d = day(s); F = female; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; yr = year

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans or animals after dermal exposure to benzene.

3.2.3.4 Neurological Effects

Tondel et al. (1995) reported the case of a gasoline station attendant who had worked from 1962 to 1979 and who developed myelofibrosis. The patient described symptoms of fatigue for 3 weeks and night sweats, among other symptoms. Although the exposure was primarily by inhalation, it is probable that dermal exposure also occurred.

No studies were located regarding neurological effects in animals after dermal exposure to benzene.

3.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after dermal exposure to benzene.

3.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after dermal exposure to benzene.

3.2.3.7 Cancer

A cohort mortality study was conducted to estimate the relative risk of hematological cancer among 335 male vehicle maintenance workers who were employed for at least 1 year between 1977 and 1989 and followed through 1991 (Hunting et al. 1995). The workers were car and mobile equipment mechanics who regularly used gasoline to clean parts and wash their hands. Exposure to gasoline also occurred via inhalation, and some of the workers occasionally siphoned gasoline by mouth. Since most of the cohort consisted of non-white (race data were missing for 9.5% of the cohort) vehicle maintenance workers who lived within the District of Columbia, death rates for age- and calendar-matched non-white males in the District of Columbia were used to calculate expected deaths for the cohort. Three deaths due to lymphatic and hematopoietic cancer were observed, yielding SMRs of 3.63 (95% CI 0.75–10.63) in the whole cohort and 4.22 (95% CI 0.87–12.34) in a subgroup of 297 workers with the highest potential for

exposure. Neither of these SMRs were significant, although analysis by cancer subtype showed a significantly elevated risk for leukemia and aleukemia in the high exposure subgroup (two cases, SMR=9.26, 95% CI 1.12–33.43). Aleukemia refers to leukemias in which the blood has normal or near-normal white blood cell counts, but few numbers of young leukocytes. Two additional cases of leukemia were identified among workers who were not included in the cohort (one died after the end of the follow-up period and another was still alive). Mortalities from causes other than lymphatic/hematopoietic cancer were not significantly increased.

Application of benzene to the skin of animals has not produced evidence of carcinogenicity, although most studies were inadequate for evaluation. As summarized by IARC (1982, 1987), many dermal carcinogenicity studies of chemicals other than benzene used benzene as a vehicle, and treated large numbers of control animals (mice) with benzene alone. None of these studies indicated that benzene induced skin tumors; however, all possible tumor sites usually were not examined.

3.3   GENOTOXICITY

The genotoxic effects of benzene have been studied extensively. The *in vivo* and *in vitro* data are summarized in Tables 3-4 and 3-5, respectively. In chronically-exposed humans, benzene and/or its metabolites primarily cause chromosomal aberrations (Andreoli et al. 1997; Bogadi-Šare et al. 1997; Ding et al. 1983; Forni and Moreo 1967, 1969; Forni et al. 1971a; Hartwich et al. 1969; Hedli et al. 1991; Karacic et al. 1995; Kašuba et al. 2000; Major et al. 1992, 1994; Picciano 1979; Popp et al. 1992; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Sellyei and Kelemen 1971; Smith et al. 1998; Sul et al. 2002; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Türkel and Egeli 1994; Van den Berghe et al. 1979; Yardley-Jones et al. 1990; Zhang et al. 1998b, 1999). Chromosomal aberrations in humans are frequently demonstrated in peripheral blood lymphocytes and bone marrow. Although inhalation, oral, and dermal routes are all potential pathways of exposure relevant to humans, available *in vivo* human data are usually drawn from occupational settings in which inhalation and dermal exposure routes are most prevalent. In most of these studies, chromosome abnormalities were detected in workers exposed to high concentrations of benzene, sufficient to produce blood dyscrasias. However, Qu et al. (2003a, 2003b) noted a concentration-related increase in chromosomal aberrations across a wide range of exposure concentrations, including workers with relatively low-level benzene exposure. Limitations of many of the occupational studies include lack of accurate exposure data, possible coexposure to other chemicals, and lack of appropriate control groups.

Table 3-4. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Result	Reference
Prokaryotic cells:			
Escherichia coli (host mediated DNA repair)	DNA synthesis	–	Hellmér and Bolcsfoldi 1992a
Invertebrate animal cells:			
Drosophila melanogaster	Sex-linked recessive lethal	–	Kale and Baum 1983
Spermatocytes	Recombination	–	
Spermatogonia	Recombination	+	
Spermatocytes	Heritable translocation	–	
Mammalian cells:			
Mouse (bone marrow)	Chromosomal aberrations	+	Giver et al. 2001; Shelby and Witt 1995; Siou et al. 1981
Mouse (bone marrow)	Chromosomal aberrations	+[a]	Meyne and Legator 1980
Mouse (bone marrow)	Chromosomal aberrations	(+)	Tice et al. 1980; 1982
Mouse (spleen lymphocytes)	Chromosomal aberrations	+	Au et al. 1991; Rithidech et al. 1987
Mouse (lymphoid cells, myeloid cells)	Chromosomal aberrations	+	Giver et al. 2001
Rat (bone marrow)	Chromosomal aberrations	+	Fujie et al. 1990; Hoechst 1977; Philip and Jensen 1970; Styles and Richardson 1984
Rat (bone marrow)	Chromosomal aberrations	+[b]	Anderson and Richardson 1981[b]
Rat (bone marrow)	Chromosomal aberrations	–	Hoechst 1977
Chinese hamster (bone marrow)	Chromosomal aberrations	+	Siou et al. 1981
Rabbit (bone marrow)	Chromosomal aberrations	+	Kissling and Speck 1972; 1973
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Bogadi-Šare et al. 1997; Ding et al. 1983; Forni et al. 1971a; Kašuba et al. 2000; Picciano 1979; Sasiadek 1992; Sasiadek and Jagielski 1990; Sasiadek et al. 1989; Smith et al. 1998; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Zhang et al. 1998a, 1999
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	(+)	Yardley-Jones et al. 1990
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	–	Bogadi-Šare et al. 1997; Jablonická et al. 1987
Mouse (bone marrow)	Micronuclei	+	Shelby and Witt 1995; Shelby et al. 1993
Mouse (bone marrow PCEs)	Micronuclei	+[c]	Ciranni et al. 1988
Mouse[d] (bone marrow PCEs)	Micronuclei	+[e]	Suzuki et al. 1989

Table 3-4. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Result	Reference
Mouse (bone marrow PCEs)	Micronuclei	+	Au et al. 1990; Barale et al. 1985; Chen et al. 1994; Diaz et al. 1980; Erexson et al. 1986; Farris et al. 1996; Harper et al. 1984; Hite et al. 1980; Siou et al. 1981; Toft et al. 1982
Mouse (bone marrow PCEs)	Micronuclei	+[a]	Meyne and Legator 1980
Mouse (bone marrow PCEs)	Micronuclei	+[f]	Eastmond et al. 2001
Mouse (bone marrow NCEs)	Micronuclei	+	Farris et al. 1996
Mouse (bone marrow NCEs)	Micronuclei	+[f]	Eastmond et al. 2001
Mouse (pregnant/bone marrow PCEs)	Micronuclei	(+)	Ciranni et al. 1988
Mouse (peripheral blood)	Micronuclei	+	Hayashi et al. 1992; Healy et al. 2001
Mouse (peripheral blood PCEs)	Micronuclei	+	Farris et al. 1996
Mouse (peripheral blood PCEs)	Micronuclei	+[c,g]	Luke et al. 1988a
Mouse (peripheral blood NCEs)	Micronuclei	+	Barale et al. 1985; Choy et al. 1985; Farris et al. 1996; Rithidech et al. 1988
Mouse (peripheral blood NCEs)	Micronuclei	+[c,f]	Luke et al. 1988a
Mouse (lung fibroblasts)	Micronuclei	+	Ranaldi et al. 1998
Mouse (fetus/liver cells)	Micronuclei	+	Ciranni et al. 1988
Rat (lymphocytes)	Micronuclei	+	Erexson et al. 1986
Chinese hamster (bone marrow)	Micronuclei	+	Siou et al. 1981
Human (lymphocytes)	Micronuclei	+	Robertson et al. 1991
Human (occupational exposure/lymphocytes)	Micronuclei	+	Liu et al. 1996
Human (occupational exposure/lymphocytes)	Micronuclei	–	Pitarque et al. 1996; Surrallés et al. 1997
Mouse (bone marrow)	Sister chromatid exchange	+	Tice et al. 1980; 1982
Mouse (pregnant/bone marrow)	Sister chromatid exchange	+	Sharma et al. 1985
Mouse (lymphocytes)	Sister chromatid exchange	+	Erexson et al. 1986
Mouse (fetus/liver cells)	Sister chromatid exchange	+	Sharma et al. 1985
Rat (lymphocytes)	Sister chromatid exchange	+	Erexson et al. 1986
Human (occupational exposure/lymphocytes)	Sister chromatid exchange	+	Popp et al. 1992
Human (occupational exposure/lymphocytes)	Sister chromatid exchange	–	Kašuba et al. 2000; Pitarque et al. 1997; Seiji et al. 1990; Yardley-Jones et al. 1988
Mouse (spleen lymphocytes)	Mutations	+	Ward et al. 1992
Mouse (lung tissue)	Mutations	+	Mullin et al. 1998
Mouse embryo (premelanocytes)	Mutations (deletions)	+	Schiestl et al. 1997
Human (bone marrow)	Mutations (gene-duplicating)	+	Rothman et al. 1995

Table 3-4. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Result	Reference
Human (bone marrow)	Mutations (gene-inactivating)	–	Rothman et al. 1995
Mouse (bone marrow)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Pathak et al. 1995; Turteltaub and Mani 2003
Mouse (white blood cells)	DNA adducts	+	Lévay et al. 1996
Mouse (liver)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Mani et al. 1999; Turteltaub and Mani 2003
Rat (bone marrow)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Pathak et al. 1995; Turteltaub and Mani 2003
Rat (liver)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lutz and Schlatter 1977; Mani et al. 1999; Mazzullo et al. 1989; Turteltaub and Mani 2003
Mouse (peripheral blood lymphocytes)	DNA strand breaks	+	Tuo et al. 1996
Rat (lymphocytes, bone marrow, spleen, liver)	DNA strand breaks	+	Lee et al. 2005
Human (occupational exposure/lymphocytes)	DNA strand breaks	+	Andreoli et al. 1997; Nilsson et al. 1996; Sul et al. 2002
Mouse (peripheral blood lymphocytes, bone marrow)	DNA damage	+	Chang et al. 2005
Human (occupational exposure/lymphocytes)	DNA repair efficiency	_	Hallberg et al. 1996
Rat (bone marrow)	DNA oxidative damage	+	Kolachana et al. 1993
Human (occupational exposure/lymphocytes)	DNA oxidative damage	+	Liu et al. 1996
Mouse (bone marrow)	DNA synthesis inhibition	+	Lee et al. 1988
Rabbit (bone marrow)	DNA synthesis inhibition	+	Kissling and Speck 1972
Mouse (bone marrow)	RNA synthesis inhibition	+	Kissling and Speck 1972
Rat (liver mitochondria)	RNA synthesis inhibition	+	Kalf et al. 1982

3. HEALTH EFFECTS

Table 3-4. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Result	Reference
Mouse (spermatogonia)	Sperm head abnormality	+	Topham 1980

[a]This result was observed following both oral and intraperitoneal exposure.
[b]This result was observed following both inhalation and intraperitoneal exposure.
[c]Males affected to a significantly greater degree than females.
[d]Two strains of mouse were tested; Ms/Ae and CD-1. The result applies to both strains.
[e]This result was observed following both oral and intraperitoneal exposure; however, oral exposure produced the greater effect.
[f]Increase in micronuclei was exposure duration-dependent.
[g]Increase in micronuclei was exposure duration-independent.

+ = Positive result; – = negative result; (+) = weakly positive result; DNA = deoxyribonucleic acid;
NCEs = normochromatic erythrocytes; PCEs = polychromatic erythrocytes; RNA = ribonucleic acid

Table 3-5. Genotoxicity of Benzene *In Vitro*

Species (test system)	End point	Result With activation	Result Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium (Ames test)	Gene mutation	–	–	De Flora et al. 1984
S. typhimurium (histidine reversion)	Gene mutation	+	–	Glatt et al. 1989
S. typhimurium (azaquanine reversion)	Gene mutation	+	No data	Kaden et al. 1979; Seixas et al. 1982
Bacillus subtilis (histidine reversion)	Gene mutation	–	–	Tannoka 1977
Escherichia coli (DNA polymerase 1/cell-free DNA synthetic system)	DNA synthesis inhibition	No data	–	Lee et al. 1988
E. coli (host meditated DNA repair)	DNA synthesis	No data	No data	Hellmér and Bolcsfoldi 1992b
Plasmid DNA ΦX-174 RF I	DNA degradation	No data	+	Li et al. 1995
Eukaryotic organisms:				
Fungi:				
Aspergillus nidulans (methionine supressors)	Gene mutation	No data	–	Crebelli et al. 1986
Mammalian cells:				
Mouse (L5178Y cells/TK test)	Gene mutation	–	–	Oberly et al. 1984
Chinese hamster (ovary cell culture)	Chromosomal aberrations	–	–	Gulati et al. 1989
Human (lymphocyte cell culture)	Chromosomal aberrations	No data	+	Eastmond et al. 1994; Morimoto 1976
Human (lymphocyte cell culture)	Chromosomal aberrations	No data	–	Gerner-Smidt and Friedrich 1978
Human (lymphoblastoid culture)	Intrachromosomal recombination	No data	+	Aubrecht et al. 1995
Chinese hamster (ovary cell culture)	Micronuclei	–	–	Douglas et al. 1985
Human (whole blood cells)	Micronuclei	–	–	Zarani et al. 1999
Chinese hamster (ovary cell culture)	Sister chromatid exchange	–	–	Douglas et al. 1985; Gulati et al. 1989
Human (lymphocyte cell culture)	Sister chromatid exchange	+	No data	Morimoto 1983
Human (lymphocyte cell culture)	Sister chromatid exchange	No data	–	Gerner-Smidt and Friedrich 1978
Rabbit (bone marrow mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984

Table 3-5. Genotoxicity of Benzene *In Vitro*

Species (test system)	End point	Result With activation	Without activation	Reference
Rat (liver mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984
Calf thymus DNA	DNA adducts	No data	+	Chenna et al. 1995
Human (bone marrow)	DNA adducts	No data	+	Bodell et al. 1993; Lévay and Bodell 1992
Human (leukemia cells)	DNA adducts	No data	+	Bodell et al. 1993; Lévay and Bodell 1992
Rat (hepatocytes)	DNA breaks	No data	–	Bradley 1985
Chinese hamster (ovary cell culture)	DNA breaks	+	+	Douglas et al. 1985
Chinese hamster (ovary cell culture)	DNA breaks	+	+[a]	Lakhanisky and Hendricks 1985
Chinese hamster (V79 cell culture)	DNA breaks	–	–	Swenberg et al. 1976
Mouse (L5178Y cell culture)	DNA breaks	No data	–	Pellack-Walker and Blumer 1986
Human (leukemia cells)	DNA oxidative damage	No data	+	Kolachana et al. 1993
Human (lymphocyte cell culture)	DNA repair	No data	_	Hallberg et al. 1996
Rat liver epithelial cells	DNA hyperphos-phorylatioin	No data	+	Dees and Travis 1994
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	(+)	Glauert et al. 1985
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	–	Probst and Hill 1985; Williams et al. 1985
Human (HeLa S3 cells)	Unscheduled DNA synthesis	–	–	Barrett 1985
Mouse (bone marrow cell culture)	DNA synthesis inhibition	No data	+	Lee et al. 1988
Mouse (bone marrow cell culture)	DNA synthesis inhibition	+	(+)	Lee et al. 1989
Calf (thymus DNA polymerase α/cell-free DNA synthetic system)	DNA synthesis inhibition	No data	+	Lee et al. 1988
Human (HeLa cells)	DNA synthesis inhibition	–	–	Painter and Howard 1982
Mouse (spleen lymphocytes)	RNA synthesis inhibition	No data	+	Post et al. 1985

Table 3-5. Genotoxicity of Benzene *In Vitro*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Rat (liver mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982
Cat, rabbit (bone marrow mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982

[a]Benzene's effect on DNA breaks was reduced when metabolic activators were used.

– = negative results; + = positive results; (+) = weakly positive result; DNA = deoxyribonucleic acid; RNA = ribonucleic acid

Chromosomal aberrations observed in workers chronically exposed to benzene include hypo- and hyperdiploidy, deletions, breaks, and gaps. For example, analysis of peripheral lymphocytes of workers exposed to benzene vapors at a mean concentration of 30 ppm revealed significant increases in monosomy of chromosomes 5, 7, and 8 (but not 1), and tri- and/or tetrasomy of chromosomes 1, 5, 7, and 8 (Zhang et al. 1998b, 1999). In another series of epidemiological studies in workers chronically exposed to benzene, nonrandom effects were apparent in chromosomes 1, 2, 4, and 9; nonrandom breaks in chromosomes 2, 4, and 9 were twice as prevalent in benzene-exposed workers versus controls; and chromosomes 1 and 2 were nearly twice as prone to gaps (Sasiadek and Jagielski 1990; Sasiadek et al. 1989). Twenty-one people with hematological signs of chronic benzene poisoning exhibited significantly more chromosomal abnormalities than controls (Ding et al. 1983). A significant increase in dicentric chromosomes and unstable aberrations was noted in 36 female workers exposed to benzene in a shoe factory for up to 32 years (Kašuba et al. 2000). Significant increases in hyperploidy of chromosomes 8 and 21 and translocations between chromosomes 8 and 21 were observed in workers exposed to benzene vapors at a mean TWA of 31 ppm (Smith et al. 1998).

DNA repair efficiency was evaluated in blood lymphocytes collected from exposed or unexposed workers in a petrochemical plant (Hallberg et al. 1996). Plasmids ($pCMV_{CAT}$) were irradiated with UV light (254 nm) to induce thymidine dimers and were then transfected into blood lymphocytes from workers. Transfected plasmids that were repaired in the lymphocytes would express the chloramphenicol actetyltransferase reporter gene (CAT) product whereas unrepaired plasmids would not. Lymphocytes from exposed or unexposed workers did not show significant differences in their ability to repair light-damaged DNA; however, the authors suggest that the sample population was too small to detect any differences given the large individual variations in repair capacity (Hallberg et al. 1996).

Oxidative DNA damage was assessed in workers (n=87) exposed to benzene by conducting measurements of 8-hydroxy-2-deoxyguanosine (8-OHdG) in peripheral blood lymphocytes (Liu et al. 1996). 8-OHdG is formed by hydroxy radical (OH·) addition at the C-8 position of deoxyguanosine (Kasai and Nishimura 1986) and appears to be a biomarker of oxidative DNA damage (Liu et al. 1996). The exposure to benzene was classified as low, medium, or high (mean benzene levels of 2.46, 103, or 424 mg/m^3, respectively [corresponding to 0.78, 32.2, or 133 ppm]). Levels of 8-OHdG in exposed workers rose in a concentration-related manner although the increases were significant only in the medium and high exposure groups. Toluene, also detected in the workplace air, did not alter levels of 8-OHdG. Formation of micronuclei, a measure of DNA damage, increased in a concentration-related manner to levels that were significantly higher than those in controls. The levels of urinary *trans,trans*-

muconic acid, a biomarker of benzene exposure, were well correlated with levels of 8-OHdG which, in turn, were well correlated with levels of lymphocyte micronuclei. The findings of Liu et al. (1996) are supported by the results of a study by Nilsson et al. (1996) in which concentration-related increased urinary levels of 8-OHdG were reported in male workers (n=30) at a gasoline station. Breathing zone benzene concentrations ranged from 0.003 to 0.6 ppm (mean 0.13 ppm). Significant concentration-related increases in DNA single-strand breaks were also noted in these workers. Collectively, the results of Liu et al. (1996) and Nilsson et al. (1996) provide suggestive evidence that benzene metabolites may induce reactive oxygen species, which could result in oxidative DNA damage and formation of hydroxylated bases such as 8-OHdG (Liu et al. 1996).

Rothman et al. (1995) used the glycophorin A (GPA) gene loss mutation assay to assess the nature of DNA damage in workers heavily exposed to benzene. The GPA assay measures the frequency of variant cells that have lost the expression of the M form of the GPA gene in the peripheral blood of heterozygous (MN) subjects. The variant cells possess the NN phenotype (double-copy expression of the N allele and no expression of M) or the NØ phenotype (single-copy expression of the N allele and no expression of M). The NN variants are thought to arise from mitotic recombination, chromosome loss, and reduplication, or gene conversion. The NØ variants appear to be associated with point mutations, deletions, or gene inactivation. Rothman et al. (1995) demonstrated a significant increase in the frequency of NN cells in benzene-exposed workers (compared with unexposed control subjects) in the absence of a significant effect on the frequency of NØ cells. These results suggest that benzene induces gene-duplicating, but not gene-inactivating, mutations at the GPA locus in benzene-exposed humans.

Sister chromatid exchange was not found to be a significant effect of benzene exposure in humans (Kašuba et al. 2000; Seiji et al. 1990; Yardley-Jones et al. 1988); however, the selection of control subjects in the studies by Seiji et al. (1990) and Yardley-Jones et al. (1988) was poor. Refer to Table 3-4 for a further summary of these results.

In vivo animal studies provide convincing evidence of benzene's genotoxicity (Table 3-4). Furthermore, the finding that male mice are more sensitive than females to benzene-induced chromosomal damage is consistent among reports (Armstrong and Galloway 1993; Barale et al. 1985; Choy et al. 1985; Ciranni et al. 1988; Hatakeyama et al. 1992; Meyne and Legator 1980; Siou et al. 1981). Consistently positive findings for chromosomal aberrations in bone marrow and lymphocytes in animals support the human case reports and epidemiological studies in which chromosomal damage was linked to benzene exposure. Micronucleus assays are popular methods for crudely analyzing DNA damage in animals. Positive results

were observed in all studies testing for increased micronuclei frequencies. One of these micronucleus assays (Luke et al. 1988a) investigated the effects of different inhalation exposure durations on polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) in peripheral blood. PCEs are newly formed erythrocytes that contain mRNA and as a result, exhibit staining when RNA staining reagents are used. NCEs are mature erythrocytes that lack mRNA and are not stained under the same conditions. The researchers found that PCEs are good indicators of recent and acute exposure, while NCEs are good indicators of accumulated long-term exposure (Luke et al. 1988a). Although no human studies were located that reported increased sister chromatid exchange in exposed individuals, increases in sister chromatid exchange were reported in mice and rats (Erexson et al. 1986; Sharma et al. 1985; Tice et al. 1980, 1982). *In vivo*, *trans,trans*-muconaldehyde (a metabolite of benzene) has also been shown to induce highly significant increases in sister chromatid exchanges in mice (Witz et al. 1990a). In addition to oral and inhalation routes, many researchers tested subcutaneous and intraperitoneal routes as well; the results for these alternate routes of exposure were largely positive for chromosomal aberrations in bone marrow (Anderson and Richardson 1981; Kissling and Speck 1972, 1973; Kolachana et al. 1993; Meyne and Legator 1980; Philip and Jensen 1970), micronuclei in bone marrow (Diaz et al. 1980), and sister chromatid exchange in mouse fetus liver cells (Sharma et al. 1985). Binding of benzene and/or its metabolites to DNA, RNA, and proteins has been consistently observed in rats and mice (Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Mani et al. 1999; Mazullo et al. 1989; Turteltaub and Mani 2003). Arfellini et al. (1985) noted that binding to RNA and proteins was more prevalent than binding to DNA. Lévay et al. (1996) observed dose- and time-dependent formation of two DNA adducts in white blood cells and bone marrow cells of mice administered benzene for 7 days via intraperitoneal injection. Turteltaub and Mani (2003) consistently observed DNA and protein binding in mice at intraperitoneal doses as low as 5 µg/kg body weight. One study using intraperitoneal injections reported a dose-dependent increase in sperm head abnormalities in mice exposed to 0.5 or 0.6 mL benzene/kg/day (Topham 1980). The author views sperm head abnormality as a possible indication of heritable mutations. However, from this study alone, it cannot be determined if benzene causes such transmissible genetic mutations. For these and other results from animal *in vivo* studies, see Table 3-4.

Molecular mechanisms of benzene-induced genotoxicity have been studied to some extent in laboratory animals. Schiestl et al. (1997) noted benzene-induced reversion of the mouse pink-eye unstable mutation in premelanocytes of mouse embryos following intraperitoneal injection of the chemical into pregnant dams; the reversions resulted from genomic DNA deletions. Results of Chen et al. (1994) and Eastmond et al. (2001) indicate that benzene-induced micronuclei in mouse bone marrow erythrocytes are formed predominantly from chromosome breakage, but also from aneuploidy.

In vitro studies strongly imply that benzene's genotoxicity is derived primarily from its metabolites. Positive results were obtained for gene mutation in *Salmonella typhimurium* (Glatt et al. 1989; Kaden et al. 1979; Seixas et al. 1982) and sister chromatid exchange in human lymphocyte cell culture (Morimoto 1983) only when exogenous metabolic activators of benzene were used. Similarly, endogenous metabolic activation was required for effects to be seen on DNA synthesis in rat hepatocyte culture (Glauert et al. 1985), DNA adduct formation in rat liver mitoplasts (Rushmore et al. 1984), and RNA synthesis in rat liver mitoplasts and in rabbit and cat bone marrow mitoplasts (Kalf et al. 1982). Endogenous activation occurs naturally by enzymes already within the cells. Exogenous activation requires the addition of enzymes to cellular preparations. In one study using benzene and 13 possible metabolites, Glatt et al. (1989) found that *trans*-1,2-dihydrodiol (with metabolic activators) and the diol epoxides (with or without metabolic activators) produced histidine reversion in *S. typhimurium*. The same researchers also investigated the genotoxicity of these 13 proposed metabolites in V79 Chinese hamster cells; *anti*-diol epoxide, *syn*-diol epoxide, 1,2,3-trihydroxybenzene, 1,2,4-trihydroxybenzene, quinone, hydroquinone, catechol, phenol, and 1,2-dihydrodiol were found to produce genotoxic effects ranging from sister chromatid exchange and micronuclei increases to gene mutations (Glatt et al. 1989). Similar studies with *trans,trans*-muconaldehyde showed that this metabolite is strongly mutagenic in V79 cells and weakly mutagenic in bacteria (Glatt and Witz 1990). Chang et al. (1994) showed that muconaldehyde and its aldehyde metabolites 6-hydroxy-*trans,trans*-2,4-hexadienal and 6-oxo-*trans,trans*-hexadienoic acid were mutagenic in V79 cells.

Several studies revealed a possible connection between certain benzene metabolites and DNA damage via the formation of oxygen radicals. Lewis et al. (1988) found that both 1,2,4-benzenetriol and hydroquinone produce DNA strand breaks, 1,2,4-benzenetriol to a greater degree than hydroquinone. Consistent with these findings was the observation that 1,2,4-benzenetriol generates a greater concentration of oxygen radicals than hydroquinone. The study concluded that 1,2,4-benzenetriol damages DNA by producing oxygen radicals, while hydroquinone probably exerts its genotoxic effects by some other mechanism (Lewis et al. 1988). However, results of Li et al. (1995b) indicate that much of the benzene-mediated DNA damage may result from the generation of reactive oxygen species via a copper-redox cycling mechanism involved in the oxidation of the benzene metabolite, hydroquinone. Increased recombination, which can lead to adverse genetic changes, was observed in Chinese hamster ovary cells exposed to phenol, catechol, or benzoquinone (Winn 2003). Benzene had no effect on recombination. The observed increases in recombination were abolished by the addition of catalase to the cells, suggesting that the effect of the benzene metabolites was elicited by oxidative stress (Winn 2003).

Oxidative damage (manifested as DNA strand breaks) was observed in HL60 cells treated with 1,4-benzoquinone or 1,4-hydroquinone (Hiraku and Kawanishi 1996). 1,4-Benzoquinone and hydroquinone induced DNA strand breaks in Chinese hamster ovary cells while phenol, catechol, 1,2,4-benzenetriol, *trans,trans*-muconic acid, and *S*-phenyl mercapturic acid did not (Sze et al. 1996). No synergism between hydroquinone and the other metabolites was observed.

Phenol, catechol, hydroquinone, and benzene induced morphological transformation and gene mutations in Syrian hamster embryo cells (Tsutsui et al. 1997). Chromosomal aberrations, sister chromatid exchange, and unscheduled DNA synthesis were increased by the benzene metabolites while aneuploidy was observed in cells treated with benzene or catechol (Tsutsui et al. 1997). There was no significant increase ($p>0.005$) in DNA fragmentation in human respiratory epithelial cells exposed to an atmosphere of 5 mg benzene/m^3 for 8 hours, although there was evidence of an inflammatory response (Gosepath et al. 2003). Chromosomal breaks and hyperdiploidy were observed in human lymphocytes after exposure to hydroquinone *in vitro* (Eastmond et al. 1994). Aneusomy of chromosomes 7 and 8 were observed in human umbilical cord blood cells treated with hydroquinone (2, 10, or 50 μM) for 72 hours (Smith et al. 2000). Monosomy 7 and trisomy 8 are two common clonal aberrations observed in myeloid leukemias (Smith et al. 2000). Hydroquinone (26–49 μM) also induced monosomy of chromosomes 5, 7, and 8 in a human lymphoblast cell line (Stillman et al. 1997). Hydroquinone (10–100 μM) and 1,2,4-benzenetriol (10–50 μM) significantly increased monosomy 5 and 7 in human lymphocytes and long-arm deletions in chromosomes 5 and 7 (Zhang et al. 1998b). Benzene metabolites have been shown to form DNA adducts in human bone marrow and HL-60 cells (Bodell et al. 1993; Lévay and Bodell 1992). Zhang et al. (1993) showed that 1,2,4-benzenetriol increased the frequency of micronuclei formation in human lymphocytes in culture, and in HL60 cells in a dose-related manner. An increase in the level of oxidative damage to DNA was also noted in HL60 cells in culture. Extracts from human cells have been shown to have repair activity toward benzoquinone-DNA adducts *in vitro* (Chenna et al. 1995). Benzene (1–5 mM) did not elicit micronuclei formation in whole blood cells treated for 48 hours with or without metabolic activation with 10% S9 rat liver fraction for 2 hours (Zarani et al. 1999). The assay was conducted with blood collected from four subjects. Chen and Eastmond (1995) showed that benzene metabolites can adversely affect human topoisomerases, enzymes involved in DNA replication and repair. No effect of any metabolite was seen on human topoisomerase I or for topoisomerase II for hydroquinone, phenol, 2,2'-biphenol, 4,4'-biphenol, and catechol at concentrations as high as 500 μM. 1,4-Benzoquinone and 1,2,4-benzenetriol inhibited human topoisomerase II *in vitro*, at 500 and 250 μM without bioactivation. However, following bioactivation, phenol and 2,2'-biphenol showed inhibitory effects at doses as low as 50 μM, whereas 4,4'-biphenol inhibited topoisomerase II at concentrations of 10 μM. More recently,

Eastmond et al. (2001) demonstrated decreased activity of topoisomerase II activity in nucleated bone marrow cells of mice administered benzene by oral gavage for subchronic durations.

Available *in vitro* data suggest that benzene itself is genotoxic. Two studies reported that benzene produced DNA breaks in Chinese hamster ovary cells independent of metabolic activators (Douglas et al. 1985; Lakhanisky and Hendrickx 1985). In a study by Aubrecht et al. (1995), benzene was shown to induce intrachromosomal recombination in human lymphoblastoid cell culture. Therefore, benzene appears to have some genotoxic capabilities of its own, but its metabolites seem to be the primary genotoxins in systems in which normal metabolism is occurring. Refer to Table 3-5 for the results of these and other *in vitro* studies.

In summary, chromosome aberrations have been found consistently in bone marrow cells of persons occupationally exposed to benzene. The conclusion, based on human epidemiological studies, that benzene is a human clastogen is well supported by *in vivo* animal studies and *in vitro* cell cultures and subcellular studies. Virtually all studies that looked for effects at the chromosomal level were positive when the ability to metabolize benzene was present. These experimental results are consistent with the chromosomal damage seen in exposed humans. The leukemia observed in some benzene-exposed persons may result from the appearance of a clone of chromosomally abnormal cells in the bone marrow. With respect to genetic effects, no safe human exposure level can be determined from available epidemiological data. Significant increases in sister chromatid exchanges were produced in bone marrow cells and lymphocytes of animals. The significance of sister chromatid exchanges is unknown, but their production by a chemical is generally considered to indicate a genotoxic potential. Exposures generally occur via inhalation, and based on animal studies, effects following oral exposure may be greater than effects following inhalation exposure to comparable levels of benzene. Data presented in this section and elsewhere in this profile (Section 3.4) show that benzene metabolites are the genotoxic entities. It is possible that each metabolite causes a different genotoxic effect. Differences in metabolic capability are probably responsible for some of the variations in response to benzene seen in different test systems.

3.4   TOXICOKINETICS

The toxicokinetics of benzene has been extensively studied. Inhalation exposure is probably the major route of human exposure to benzene, although oral and dermal exposure are also important. Benzene is readily absorbed following inhalation or oral exposure. Although benzene is also readily absorbed from the skin, a significant amount of a dermal application evaporates from the skin surface. Absorbed

benzene is rapidly distributed throughout the body and tends to accumulate in fatty tissues. The liver serves an important function in benzene metabolism, which results in the production of several reactive metabolites. Although it is widely accepted that benzene toxicity is dependent upon metabolism, no single benzene metabolite has been found to be the major source of benzene hematopoietic and leukemogenic effects. At low exposure levels, benzene is rapidly metabolized and excreted predominantly as conjugated urinary metabolites. At higher exposure levels, metabolic pathways appear to become saturated and a large portion of an absorbed dose of benzene is excreted as parent compound in exhaled air. Benzene metabolism appears to be qualitatively similar among humans and various laboratory animal species. However, there are quantitative differences in the relative amounts of benzene metabolites.

3.4.1   Absorption

3.4.1.1   Inhalation Exposure

Inhalation exposure is probably the major route of human exposure to benzene, and numerous studies of absorption of benzene after inhalation exposure in different settings have been conducted (Ashley et al. 1994; Avis and Hutton 1993; Boogaard and van Sittert 1995; Brunnemann et al. 1989; Byrd et al. 1990; Etzel and Ashley 1994; Fustinoni et al. 1995; Ghittori et al. 1995; Gordian and Guay 1995; Hajimiragha et al. 1989; Hanzlick 1995; Karacic et al. 1995; Kok and Ong 1994; Lagorio et al. 1994a; Laitinen et al. 1994; Lauwerys et al. 1994; Lindstrom et al. 1994; Mannino et al. 1995; Nomiyama and Nomiyama 1974a; Ong et al. 1994, 1995; Pekari et al. 1992; Popp et al. 1994; Rauscher et al. 1994; Rothman et al. 1995; Ruppert et al. 1995; Scherer et al. 1995; Shamy et al. 1994; Srbova et al. 1950; Yu and Weisel 1996). Existing evidence indicates that benzene is rapidly absorbed by humans following inhalation exposure. Results from a study of 23 subjects who inhaled 47–110 ppm benzene for 2–3 hours showed that absorption was highest in the first few minutes of exposure, but decreased rapidly thereafter (Srbova et al. 1950). In the first 5 minutes of exposure, absorption was 70–80%, but by 1 hour, it was reduced to approximately 50% (range, 20–60%). Respiratory uptake (the amount of benzene absorbed from the lungs following inhalation of the vapors) in six volunteers including males and females exposed to 52–62 ppm benzene for 4 hours was determined to be approximately 47%(Nomiyama and Nomiyama 1974a). In a similar study, three healthy nonsmoking volunteers were exposed to benzene at levels of 1.6 or 9.4 ppm for 4 hours (Pekari et al. 1992). The amount of benzene absorbed was estimated from the difference between the concentration inhaled and the concentration exhaled. Estimates were 48% for the high dose and 52% for the low dose, supporting the evidence of Nomiyama and Nomiyama (1974a). Yu and Weisel (1996) measured the uptake of benzene by three female subjects exposed to benzene in smoke

generated by burning cigarettes, which resulted in airborne benzene concentrations in the range of 32–69 ppm. Average absorption for exposure periods of 30 or 120 minutes was 64% and did not appear to be influenced by exposure duration.

Studies of occupational exposure to benzene suggest that absorption occurs both by inhalation and dermally in many workplace settings. In a study conducted in 1992 in Finland, car mechanics' exposure to benzene was evaluated (Laitinen et al. 1994). Different work phases were measured at five Finnish garages. Blood samples from car mechanics (eight nonsmokers) were taken 3–9 hours after exposure to benzene. The results were approximated to the time point of 16 hours after exposure. Fourteen air samples were taken from the breathing zone and five stationary samples were collected from the middle of the garage for background concentration levels. The average background concentration (stationary samples) of gasoline vapors was 6 ± 7 cm^3/m^3 (2 ± 2 ppm) and the concentration of benzene was under the detection limit of 0.2 cm^3/m^3 (0.1 ppm). The concentrations of benzene in the breathing zone varied from the detection limit of 0.2 cm^3/m^3 to 1.3 cm^3/m^3 (0.1–0.4 ppm) for unleaded gasoline and from the detection limit to 3.7 cm^3/m^3 (1.2 ppm) for leaded gasoline. The highest benzene exposure level (2.4–3.7 cm^3/m^3 or 0.8–1.2 ppm) was measured when changing the filter to the fuel pump. The mechanics worked without protective gloves, and the risk of contamination and penetration through the skin was significant. During carburetor renewal and gathering, benzene concentrations were 0.5–1.1 cm^3/m^3 (0.2–0.3 ppm). During changing of the fuel filter to electronic fuel-injection system, benzene concentration ranged from 0.9 to 3.4 cm^3/m^3 (0.3–1.1 ppm). The approximated benzene concentrations in blood corresponding to the time point of 16 hours after the exposure showed much higher levels of exposure than could be expected according to the corresponding air measurements (8-hour TWA). The comparison of expected benzene concentrations in blood, if no dermal exposure were present, to the levels at the time point of 16 hours after the exposure showed that the dermal route must be the source of about 68% of exposure (range 1.1–88.2%). Two of eight workers had minimal exposure through the skin (0–1.1%). The other six workers showed high dermal exposure (79.4%).

Exposure to benzene-contaminated water can also provide an opportunity for both inhalation and dermal absorption. In a series of experiments conducted in a single-family residence from June 11 to 13, 1991, exposure to benzene through contaminated residential water was monitored (Lindstrom et al. 1994). The residential water was contaminated with benzene and other hydrocarbons in 1986. Periodic testing conducted from 1986 to 1991 showed benzene concentrations ranging from 33 to 673 µg/L (ppb). The experiment involved an individual taking a 20-minute shower with the bathroom door closed, followed by 5 minutes for drying and dressing; then the bathroom door was opened and this individual was allowed to

leave the house. Integrated 60- and 240-minute whole-air samples were collected from the bathroom, an adjacent bedroom, living room, and in ambient air. Glass, gas-tight syringe grab samples were simultaneously collected from the shower, bathroom, bedroom, and living room at 0, 10, 18, 20, 25, 25.5, and 30 minutes. Two members of the monitoring team were measured for 6 hours using personal Tenax gas GC monitors. For the first 30 minutes of each experiment, one member was based in the bathroom and the other in the living room. Benzene concentrations in the shower head ranged from 185 to 367 µg/L (ppb), while drain level samples ranged from below the detectable limit (0.6 µg/L or ppb) to 198 µg/L (ppb). Analysis of the syringe samples suggested a pulse of benzene moving from the shower stall to the rest of the house over approximately 60 minutes. Peak levels of benzene measured 758– 1,670 µg/m^3 (235–518 ppb) in the shower stall at 18–20 minutes, 366–498 µg/m^3 (113–154 ppb) in the bathroom at 10–25 minutes, 81–146 µg/m^3 (25–45 ppb) in the bedroom at 25.5–30 minutes, and 40– 62 µg/m^3 (12–19 ppb) in the living room at 36–70 minutes. The individual who took the 20-minute shower had estimated inhalation doses of 79.6, 105, and 103 µg (mean=95.9 µg) for the 3 consecutive sampling days. These doses were estimated by taking the products of the concentration of benzene in water, the minute ventilation rate, the duration of exposure, and a 70% benzene absorption factor. This was 2.1–4.9 times higher than corresponding 20-minute bathroom exposures. Adding the average dose absorbed in the bathroom during the 5.5 minutes following the shower (using the overall 20–25 minutes mean syringe level of 318 µg/m^3 [99 ppb]) gave a total average shower-related inhalation dose of 113 µg. An average dermal dose of 168 µg was estimated for the 20-minute shower by multiplying the average concentration of benzene in water by the surface area of the male volunteer, an exposure factor of 75% body surface area exposed, a dermal permeability constant for benzene of 0.11 cm/hour, an exposure duration of 0.33 hours, and a unit conversion factor of 11/1,000 cm^2. The total benzene dose resulting from the shower was estimated to be approximately 281 µg (40% via inhalation and 60% via dermal), suggesting a higher potential exposure to benzene via dermal contact from the water than through vaporization and inhalation. This exposure was 2–3.5 times higher than the mean 6-hour inhalation dose received by the sampling members. The estimated inhalation and dermal doses reported by Lindstrom et al. (1994) have not been validated by others and are therefore of questionable value for quantitative analysis.

Additional evidence of benzene absorption following inhalation exposure comes from data on cigarette smokers. Benzene levels were significantly higher in the venous blood of 14 smokers (median level of 493 ng/L) than in a control group of 13 nonsmokers (median level of 190 ng/L) (Hajimiragha et al. 1989). Cigarette smoke is known to contain benzene (Brunnemann et al. 1989; Byrd et al. 1990), and the subjects had no known exposure to other sources of benzene (Hajimiragha et al. 1989). Kok and Ong (1994)

report blood and urine levels of benzene as 110.9 and 116.4 ng/L, respectively for nonsmokers, and 328.8 and 405.4 ng/L, respectively for smokers. The National Association of Medical Examiners Pediatric Toxicology (PedTox) Registry reported blood benzene concentrations ranging from 0.2 to 4.9 mg/L in eight children who died in fires and were dead at the scene, indicating absorption of benzene from burning materials (Hanzlick 1995). Blood benzene levels taken from U.S. engineers (Group I) and firefighters (Group II) working at burning oil wells in Kuwait were compared to blood benzene levels from non-exposed U.S. citizens (Etzel and Ashley 1994). The median concentrations of benzene in whole blood from Groups I, II, and U.S. reference group were 0.035 μg/L (range ND–0.055 μg/L), 0.18 μg/L (range 0.063–1.1 μg/L), and 0.066 μg/L (range ND–0.54 μg/L), respectively. The median concentration in group II was generally higher than the median concentrations in group I or the reference group. Statistically significant higher concentrations of benzene ($p<0.0001$) were found in group II smokers than in Group II nonsmokers.

Animal data confirm that benzene is rapidly absorbed through the lungs. Inhalation studies with laboratory dogs indicate that distribution of benzene throughout the animal's body is rapid, with tissue values dependent on blood supply. A linear relationship existed between the concentration of benzene in air (200–1,300 ppm) and the equilibrium concentration in blood (Schrenk et al. 1941). At these exposures, the concentrations of benzene in the blood of dogs exposed to benzene reached a steady state within 30 minutes.

In rodents, the extent of uptake increased linearly with concentration for exposures up to 200 ppm. At concentrations of >200 ppm, zero-order kinetics were observed (i.e., uptake became nonlinear, indicating saturation of the metabolic capacity). The percentage of inhaled benzene that was absorbed and retained during a 6-hour exposure period decreased from 33 to 15% in rats and from 50 to 10% in mice as the exposure concentration was increased from about 10 to 1,000 ppm (Sabourin et al. 1987). When rats and mice were exposed to approximately 300 ppm, mice had greater uptake than rats. Mice and rats had different absorption characteristics; the cumulative inhaled dose in mice was greater than that in rats (Eutermoser et al. 1986; Sabourin et al. 1987). Purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours/day, 5 days/week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

3.4.1.2 Oral Exposure

Although definitive scientific data are not available on oral absorption of benzene in humans, case studies of accidental or intentional poisoning indicate that benzene is absorbed by the oral route (Thienes and Haley 1972).

Benzene appears to be efficiently absorbed following oral dosing in animals. Oral absorption of benzene was first demonstrated by Parke and Williams (1953a). After radiolabeled (^{14}C) benzene was administered orally to rabbits (340–500 mg/kg), the total radioactivity eliminated in exhaled air and urine accounted for approximately 90% of the administered dose, indicating that at least this much of the administered dose was absorbed. Studies in rats and mice showed that gastrointestinal absorption was greater than 97% in both species when the animals were administered benzene by gavage (in corn oil) at doses of 0.5–150 mg/kg/day (Sabourin et al. 1987). In many animal studies, benzene is administered orally in oil to insure predictable solubility and dose concentration control. This is unlike the predicted human oral exposure, which is likely to be in drinking water. There are a number of studies in which benzene has been administered to animals in the drinking water, which more closely resembles predicted human oral exposure (Lindstrom et al. 1994). Although no information was located regarding the extent of oral absorption of benzene in aqueous solutions, it is reasonable to assume that oral absorption from water solutions would be nearly 100%.

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats (Turkall et al. 1988). Animals were gavaged with an aqueous suspension of benzene alone, or adsorbed to clay or sandy soil. Plasma concentration, half-life, tissue distribution, respiratory excretion, and urinary excretion were monitored. Peak plasma concentration of radioactivity was increased in the presence of either soil as opposed to benzene alone, while sandy soil also decreased the time to peak plasma concentration as opposed to benzene alone. Soil increased the area under the plasma radioactivity-time curve as opposed to benzene alone, a difference that was significant with clay soil. The half-life in plasma was not affected by soil.

3.4.1.3 Dermal Exposure

Studies conducted *in vivo* in humans and *in vitro* using human skin indicates that benzene can be absorbed dermally. The movement of a substance through the skin to the blood occurs by passive diffusion and has been described mathematically by Fick's law. However, this is an oversimplification of

the process of skin absorption; various factors (e.g., interaction of benzene with molecules within the skin) affect the transport of the solvent through the skin (Lodén 1986).

In vivo experiments on four volunteers, to whom 0.0026 mg/cm^2 of ^{14}C-benzene was applied to forearm skin, indicated that approximately 0.05% of the applied dose was absorbed (Franz 1984). Absorption was rapid, with more than 80% of the total excretion of the absorbed dose occurring in the first 8 hours after application. Calculations were based on urinary excretion data and no correction was made for the amount of benzene that evaporated from the applied site before absorption occurred. In addition, the percentage of absorbed dose excreted in urine that was used in the calculation was based only on data from rhesus monkeys and may not be accurate for humans. In another study, 35–43 cm^2 of the forearm was exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25–2 hours (Hanke et al. 1961). The absorption was estimated from the amount of phenol eliminated in the urine. The absorption rate of liquid benzene by the skin (under the conditions of complete saturation) was calculated to be low, approximately 0.4 mg/cm^2/hour. The absorption due to vapors in the same experiment was negligible. The results indicate that dermal absorption of liquid benzene is of concern, while dermal absorption from vapor exposure may not be of concern because of the low concentration of benzene in vapor form at the point of contact with the skin. No signs of acute intoxication due to liquid benzene dermally absorbed were noted. These results confirm that benzene can be absorbed through skin. However, non-benzene-derived phenol in the urine was not accounted for.

Studies of occupational exposure to benzene suggest that absorption occurs both by inhalation and dermally in many workplace settings. In a study conducted in 1992 in Finland, car mechanics' exposure to benzene was evaluated (Laitinen et al. 1994). Different work phases were measured at five Finnish garages. Blood samples from car mechanics (eight nonsmokers) were taken 3–9 hours after exposure to benzene. The results were approximated to the time point of 16 hours after exposure. Fourteen air samples were taken from the breathing zone and five stationary samples were collected from the middle of the garage for background concentration levels. The mechanics worked without protective gloves, and the risk of contamination and penetration through the skin was significant. The approximated benzene concentrations in blood corresponding to the time point of 16 hours after the exposure showed much higher levels of exposure than could be expected according to the corresponding air measurements (8 hour TWA). The comparison of expected benzene concentrations in blood, if no dermal exposure was present, to the levels at the time point of 16 hours after the exposure showed that the dermal route must be the source of about 68% of exposure (range 1.1–88.2%). Two of eight workers had minimal exposure through the skin (0–1.1%). The other six workers showed high dermal exposure (79.4%).

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In vitro experiments using human skin support the fact that benzene can be absorbed dermally. An experiment on the permeability of excised human skin with regard to benzene (specific activity 99.8 mCi/mmol; total volume of applied benzene not reported) resulted in the absorption of 0.17 mg/cm^2 after 0.5 hours and 1.92 mg/cm^2 after 13.5 hours (Lodén 1986). Following application of 5, 120, 270, and 520 μL/cm^2 of benzene to human skin, total absorption was found to be 0.01, 0.24, 0.56, and 0.9 μL/cm^2, respectively. Thus, the total amount absorbed appears to increase linearly with dose. The study author indicated that evaporation of benzene did not exceed 5%. When exposure time (i.e., the time to complete evaporation) at each dose was measured and plotted as the ordinate of absorption, total absorption was found to increase linearly with exposure time. The percentage of the applied dose absorbed at each concentration was constant at about 0.2% (Franz 1984).

Using results from an *in vitro* study, it was estimated that an adult working in ambient air containing 10 ppm benzene would absorb 7.5 μL/hour from inhalation and 1.5 μL/hour from whole-body (2 m^2) dermal exposure (Blank and McAuliffe 1985). It was also estimated that 100 cm^2 of smooth and bare skin in contact with gasoline containing 5% benzene would absorb 7.0 μL/hour. Diffusion through the stratum corneum was considered the most likely rate-limiting step for dermal absorption because of benzene's low water solubility (Blank and McAuliffe 1985).

Based on an observational study of workers in a tire factory, it was estimated that a worker exposed to benzene as a result of direct skin contact with petroleum naphtha containing 0.5% benzene could absorb 4–8 mg of benzene per day through intact skin (Susten et al. 1985). This amount absorbed was compared with an estimated 14 mg of benzene absorbed as a result of inhalation of 1 ppm for an 8-hour day. The estimate for dermal absorption is theoretical since in many facilities the concentration of benzene in rubber solvents such as petroleum naphtha is less than 0.5% and may be as low as 0.09%.

Benzene is also absorbed dermally by animals. In Rhesus monkeys, minipigs, and hairless mice, dermal absorption was <1% following a single direct (unoccluded) application of liquid benzene (Franz 1984; Maibach and Anjo 1981; Susten et al. 1985). As with humans, absorption appeared to be rapid, with the highest urinary excretion of the absorbed dose observed in the first 8 hours following exposure (Franz 1984). Multiple applications, as well as application to stripped skin, resulted in greater skin penetration (Maibach and Anjo 1981). The percentage of absorption of the applied dose of benzene in each of these animals was approximately 2–3-fold higher than that of humans.

Data indicate that soil adsorption decreases the dermal bioavailability of benzene. A study in which male rats were treated dermally with 0.004 mg/cm^2 ^{14}C-benzene, with or without 1 g of clay or sandy soil, reported benzene absorption half-lives of 3.1, 3.6, and 4.4 hours for pure benzene, sandy soil, and clay soil, respectively (Skowronski et al. 1988).

Benzene in air was rapidly absorbed through the skin of hairless mice that were attached to respirators to avoid pulmonary uptake of the benzene vapors (Tsuruta 1989). The rate of absorption of benzene through the skin increased linearly with dose. The skin absorption rate for 200 ppm was 4.11 nmol/cm^2/hour (0.31 μg/cm^2/hour); at 1,000 ppm, the rate was 24.2 nmol/cm^2/hour (1.89 μg/cm^2/hour), and at 3,000 ppm, the rate was 75.5 nmol/cm^2/hour (5.90 μg/cm^2/hour). The skin absorption coefficient was 0.619 cm/hour.

McDougal et al. (1990) estimated permeability constants of 0.15 and 0.08 cm/hour for rat and human skin, respectively, based on the appearance of benzene in the blood of rats dermally exposed to benzene vapors at a concentration of 40,000 ppm for 4 hours. A physiologically based pharmacodynamic (PBPK) model was used to estimate the permeability of the vapor in rat and human skin. These results indicate that dermal absorption of benzene may be greater in rats than humans. Therefore, results in rats may provide a conservative estimate of dermal absorption of benzene in humans.

In an *in vitro* experiment using Fischer 344 rat skin, the partition coefficient for skin:air was determined for benzene at 203 ppm (Mattie et al. 1994). The partition coefficient of a chemical in skin is an indicator of the capacity of the skin to retain the chemical, and may reflect the rate at which a chemical is absorbed through the skin and enters the circulation. Results indicated a partition coefficient of 35, with an equilibration time of 4 hours. The skin:air partition coefficient is necessary for developing the dermal compartment of a PBPK model.

Based on data for skin absorption of benzene vapors in mice and occupational exposure data, Tsuruta (1989) estimated the ratio of skin absorption rate to pulmonary uptake for humans exposed to benzene to be 0.037. Dermal absorption could account for a relatively higher percentage of total benzene uptake in occupational settings where personnel, using respirators but not protective clothing, are exposed to high concentrations of benzene vapor.

3.4.2   Distribution

3.4.2.1   Inhalation Exposure

Information on the distribution of benzene in humans comes primarily from case studies. The data suggest that benzene is distributed throughout the body following absorption into blood. Since benzene is lipophilic, a high distribution to fatty tissue might be expected. Following inhalation exposure to benzene, the chemical has been detected in the biological fluids and tissues of the subjects (Pekari et al. 1992; Tauber 1970; Winek and Collom 1971; Winek et al. 1967). Fluid and tissue levels of benzene have been reported in cases of both accidental and intentional lethal exposures. Levels of 0.38 mg% in blood (mg% = mg per 100 mL of blood or mg per 100 g of tissue), 1.38 mg% in the brain, and 0.26 mg% in the liver were reported in a worker who died from exposure to very high air concentrations of the chemical (Tauber 1970). An autopsy (time after death not indicated) performed on a youth who died while sniffing reagent-grade benzene revealed benzene concentrations of 2.0 mg% in blood, 3.9 mg% in brain, 1.6 mg% in liver, 1.9 mg% in kidney, 1 mg% in stomach, 1.1 mg% in bile, 2.23 mg% in abdominal fat, and 0.06 mg% in urine (Winek and Collom 1971). Benzene crosses the human placenta and is present in the cord blood in amounts equal to or greater than those in maternal blood (Dowty et al. 1976). Benzene is expected to readily bind to plasma proteins (Travis and Bowers 1989). Furthermore, benzene metabolites have been found to form covalent adducts with proteins from blood in humans (Bechtold et al. 1992b; Rappaport et al. 2002a, 2002b; Yeowell-O'Connell et al. 1998) and mice (McDonald et al. 1994). The relatively widespread distribution of benzene and its metabolites to other tissues and organs indicates that

protein adduct formation in the blood does not adversely affect distribution, although no confirming studies were located.

Results from animal studies indicate that absorbed benzene is distributed among several compartments. The parent compound is preferentially stored in the fat, although the relative uptake in tissues also appears to be dependent on the perfusion rate of tissues by blood.

Following a 10-minute inhalation exposure of pregnant mice to 2,000 ppm benzene, parent compound and its metabolites were found to be present in lipid-rich tissues, such as brain and fat, and in well-perfused tissues, such as liver and kidney. Benzene was also found in the placenta and fetuses immediately following inhalation of benzene (Ghantous and Danielsson 1986). During inhalation exposure of rats to 500 ppm, benzene levels reached a steady-state concentration within 4 hours in blood (11.5 μg/mL), 6 hours in fat (164.4 μg/g), and less than 2 hours in bone marrow (37.0 μg/g) (Rickert et al. 1979). Benzene was also distributed to the kidney, lung, liver, brain, and spleen. The benzene metabolites phenol, catechol, and hydroquinone were detected in blood and bone marrow following 6 hours of exposure to benzene, with levels in bone marrow exceeding the respective levels in blood. The levels of phenol in blood and bone marrow decreased much more rapidly after exposure ceased than did those of catechol or hydroquinone, suggesting the possibility of accumulation of the latter two compounds.

Benzene was rapidly distributed throughout the bodies of dogs exposed via inhalation to concentrations of 800 ppm for up to 8 hours/day for 8–22 days (Schrenk et al. 1941). Fat, bone marrow, and urine contained about 20 times the concentration of benzene in blood; benzene levels in muscles and organs were 1–3 times that in blood; and erythrocytes contained about twice the amount of benzene found in plasma. During inhalation exposure of rats to 1,000 ppm (2 hours/day, for 12 weeks), benzene was stored longer (and eliminated more slowly) in female and male rats with higher body fat content than in leaner animals (Sato et al. 1975).

Benzene was detected in the liver, lung, and blood of rats and mice examined immediately following a 6-hour exposure to benzene vapors at a concentration of 50 ppm (Sabourin et al. 1988). Sabourin and coworkers (Sabourin et al. 1987, 1988) also examined effects exposure concentration, exposure rate, and route of administration on the comparative metabolism of benzene in rats and mice. Results of these studies are summarized in Section 3.4.3 (Metabolism).

3.4.2.2 Oral Exposure

No studies were located regarding distribution in humans after oral exposure to benzene.

In Sprague-Dawley rats administered a single dose of 0.15, 1.5, 15, 150, or 500 mg/kg of ^{14}C-benzene by gavage, benzene was rapidly absorbed and distributed to various organs and tissues within 1 hour of administration (Low et al. 1989). One hour after rats were dosed with 0.15 or 1.5 mg/kg of benzene, tissue distribution of benzene was highest in liver and kidney, intermediate in blood, and lowest in the Zymbal gland, nasal cavity tissue, and mammary gland. At higher doses, beginning with 15 mg/kg, benzene disproportionately increased in the mammary glands and bone marrow. Bone marrow and adipose tissue proved to be depots of benzene at the higher dose levels. The highest tissue concentrations of benzene's metabolite hydroquinone 1 hour after administration of 15 mg/kg of benzene were in the liver, kidney, and blood, while the highest concentrations of the metabolite phenol were in the oral cavity, nasal cavity, and kidney. The major tissue sites of benzene's conjugated metabolites were blood, bone marrow, oral cavity, kidney, and liver for phenyl sulfate and hydroquinone glucuronide; muconic acid was also found in these sites. Additionally, the Zymbal gland and nasal cavity were depots for phenyl glucuronide, another conjugated metabolite of benzene. The Zymbal gland is a specialized sebaceous gland and a site for benzene-induced tumors. Therefore, it is reasonable to expect that lipophilic chemicals like benzene would partition readily into this gland. However, benzene did not accumulate in the Zymbal gland; within 24 hours after administration, radiolabel derived from ^{14}C-benzene in the Zymbal gland constituted less than 0.0001% of the administered dose.

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats (Turkall et al. 1988). Animals were gavaged with an aqueous suspension of ^{14}C-benzene alone, or adsorbed to clay or sandy soil. Two hours after exposure, stomach tissue contained the highest amount of radioactivity, followed by fat in all treatment groups. No differences in tissue distribution patterns were detected for the three treatments.

3.4.2.3 Dermal Exposure

No studies were located regarding distribution in humans after dermal exposure to benzene.

A study of male rats treated dermally with 0.004 mg/cm^2 of ^{14}C-benzene, with and without 1 g of clay or sandy soil, revealed soil-related differences in tissue distribution following treatment. The ^{14}C activity (expressed as a percentage of initial dose per g of tissue) 48 hours after treatment with soil-adsorbed

benzene was greatest in the treated skin (0.059–0.119%), followed by the kidney (0.024%) and liver
(0.013–0.015%), in both soil groups. In the pure benzene group, the kidney contained the largest amount
of radioactivity (0.026%), followed by the liver (0.013%) and treated skin (0.11%) (Skowronski et al.
1988). In all three groups, <0.01% of the radioactivity was found in the following tissues: duodenum,
fat, bone marrow, esophagus, pancreas, lung, heart, spleen, blood, brain, thymus, thyroid, adrenal, testes,
untreated skin, and remaining carcass.

3.4.3   Metabolism

Although the metabolism of benzene has been studied extensively, the steps leading to benzene toxicity
are not yet fully understood. It is generally understood that both cancer and noncancer effects are caused
by one or more reactive metabolites of benzene. Available data indicate that metabolites produced in the
liver are carried to the bone marrow where benzene toxicity is expressed. Benzene metabolism may
occur, at least in part, in the bone marrow. Benzene metabolism has been demonstrated in isolated
perfused rabbit lung preparations (Powley and Carlson 2002). As discussed in detail in Section 3.5,
available evidence suggests that multiple benzene metabolites may collectively be responsible for the
expression of benzene toxicity.

Data regarding metabolism of benzene in humans are derived primarily from studies using inhalation
exposures. Benzene is excreted both unchanged via the lungs and as metabolites (but also as parent
compound in small amounts) in the urine. The rate and percentage of excretion via the lungs are
dependent on exposure dose and route. Qualitatively, the metabolism and elimination of benzene appear
to be similar in humans and laboratory animals, but no directly comparable studies are available
(Henderson et al. 1989; Sabourin et al. 1988).

The metabolic scheme shown in Figure 3-3 is based on results of numerous mechanistic studies of
benzene metabolism (see Henderson et al. 1989; Huff et al. 1989; and Ross 1996, 2000 for
comprehensive reviews of benzene metabolism). The first step is the cytochrome P-450 2E1 (CYP2E1)
catalyzed oxidation of benzene to form benzene oxide (Lindstrom et al. 1997), which is in equilibrium
with its oxepin (Vogel and Günther 1967). Several pathways are involved in the metabolism of benzene
oxide. The predominant pathway involves nonenzymatic rearrangement to form phenol (Jerina et al.
1968), the major initial product of benzene metabolism (Parke and Williams 1953a). Phenol is oxidized
in the presence of CYP2E1 to catechol or hydroquinone, which are oxidized via myeloperoxidase (MPO)
to the reactive metabolites 1,2- and 1,4-benzoquinone, respectively (Nebert et al. 2002). The reverse

Figure 3-3. Metabolic Pathways for Benzene
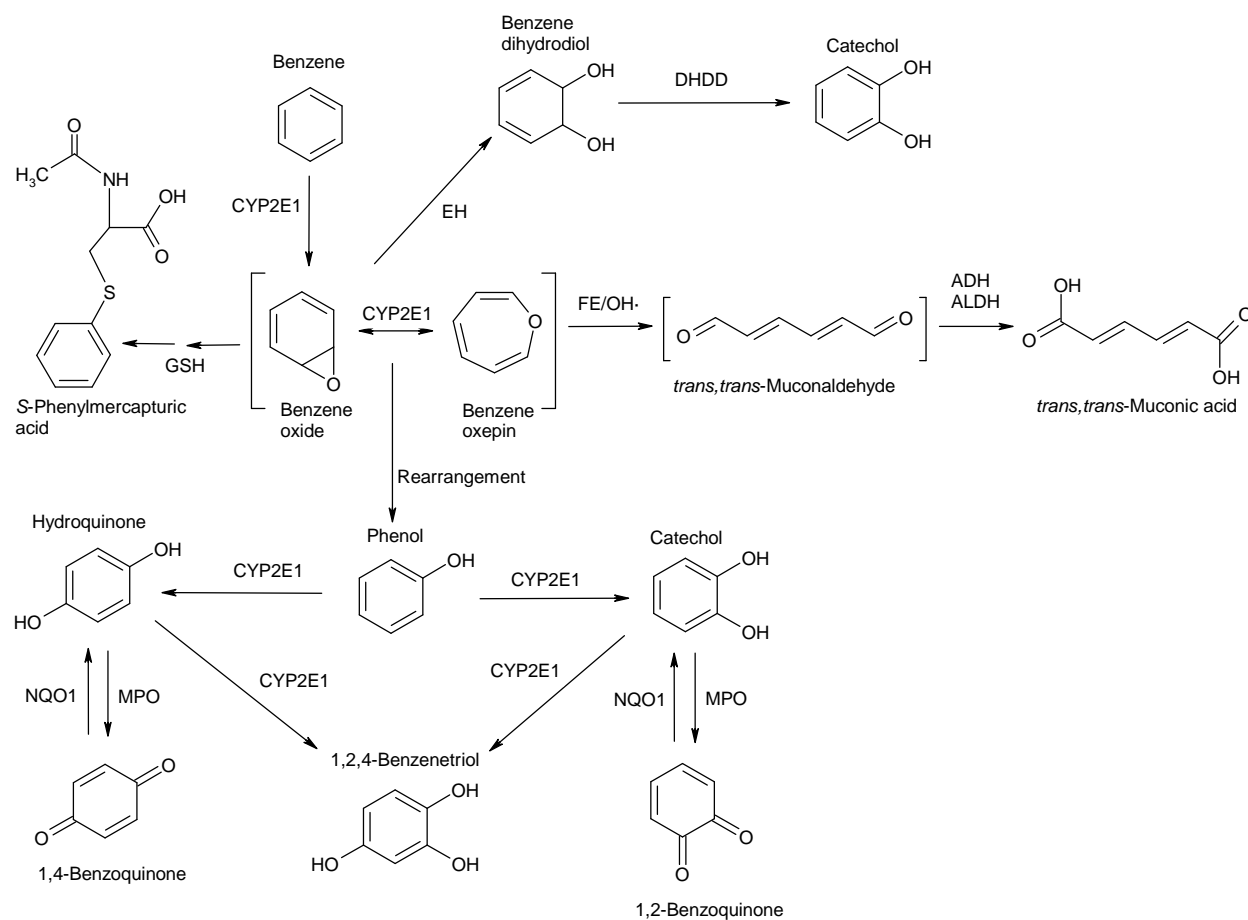
ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase; CYP2E1 = cytochrome P-450 2E1;
DHDD = dihydrodiol dehydrogenase; EH = epoxide hydrolase; GSH = glutathione; MPO = myeloperoxidase;
NQ01 = NAD(P)H:quinone oxidoreductase

Source: adapted from Nebert et al. 2002; Ross 2000

reaction (reduction of 1,2- and 1,4-benzoquinone to catechol and hydroquinone, respectively) is catalyzed by NAD(P)H:quinone oxidoreductase (NQ01) (Nebert et al. 2002). Both catechol and hydroquinone may be converted to the reactive metabolite 1,2,4-benzenetriol via CYP2E1 catalysis. Alternatively, benzene oxide may undergo epoxide hydrolase-catalyzed conversion to benzene dihydrodiol and subsequent dihydrodiol dehydrogenase-catalyzed conversion to catechol (Nebert et al. 2002; Snyder et al. 1993a, 1993b). Each of the phenolic metabolites of benzene (phenol, catechol, hydroquinone, and 1,2,4-benzenetriol) can undergo sulfonic or glucuronic conjugation (Nebert et al. 2002; Schrenk and Bock 1990); the conjugates of phenol and hydroquinone are major urinary metabolites of benzene (Sabourin et al. 1989a; Wells and Nerland 1991). Other pathways of benzene oxide metabolism include: (1) reaction with glutathione (GSH) to form S-phenylmercapturic acid (Nebert et al. 2002; Sabourin et al. 1988; Schafer et al. 1993; Schlosser et al. 1993; Schrenk et al. 1992; van Sittert et al. 1993), and (2) iron-catalyzed ring-opening conversion to *trans,trans*-muconic acid, presumably via the reactive *trans,trans*-muconaldehyde intermediate (Bleasdale et al. 1996; Nebert et al. 2002; Ross 2000; Witz et al. 1990b, 1990c, 1996).

Results of several studies provide strong evidence for the involvement of CYP2E1 in the oxidation of benzene. For example, no signs of benzene-induced toxicity were observed in transgenic CYP2E1 knockout mice (that do not express hepatic CYP2E1 activity) following exposure to benzene vapors (200 ppm, 6 hours/day for 5 days) that caused severe genotoxicity and cytotoxicity in wild-type mice (Valentine et al. 1996a, 1996b). Pretreatment of mice with CYP inhibitors (toluene, propylene glycol, β-diethyl amino ethyl diphenyl propyl acetate hydrogen chloride [SKF-525A]) has been demonstrated to reduce both benzene metabolite formation (Andrews et al. 1977; Gill et al. 1979; Ikeda et al. 1972; Tuo et al. 1996) and resulting genotoxicity (DNA damage as assessed by the alkaline comet assay) in mice (Tuo et al. 1996). Pretreatment with CYP inducers (3-methylcholanthrene and β-naphthoflavone) increased both benzene metabolism and benzene clastogenicity (Gad-El-Karim et al. 1986). Immunoinhibition studies in rat and rabbit hepatic microsomes provide additional support to the major role of CYP2E in benzene metabolism (Johansson and Ingelman-Sundberg 1988; Koop and Laethem 1992). Occupationally exposed workers with a phenotype corresponding to rapid CYP2E1 metabolism were more susceptible to benzene hematotoxicity than workers not expressing this phenotype (Rothman et al. 1997). *In vitro* studies using human liver microsomes demonstrate a positive correlation between benzene metabolism and CYP2E1 activity (Nedelcheva et al. 1999; Seaton et al. 1994). Although CYP2E1 appears to be the major catalyzing agent in initial benzene metabolism, other CYPs, such as CYP2B1 and CYP2F2, may also be involved (Gut et al. 1996a, 1996b; Powley and Carlson 2000, 2001; Sheets and Carlson 2004; Sheets et al. 2004; Snyder et al. 1993a, 1993b).

CYPs involved in benzene metabolism are found in all tissues. However, the predominant repository is the liver, which is considered to be the primary site of benzene metabolism. By demonstrating that partial hepatectomy diminished both the rate of metabolism of benzene and its toxicity in rats exposed to benzene via subcutaneous injection, Sammett et al. (1979) provided suggestive evidence that one or more benzene metabolites formed in the liver are necessary for toxicity. *In vitro* studies have demonstrated that pulmonary microsomes of humans and laboratory animals are capable of metabolizing benzene, which appears to be catalyzed by both CYP2E1 and CYP2F2 (Powley and Carlson 1999, 2000; Sheets et al. 2004). There is some indication that CYP2E1-catalyzed benzene metabolism may also occur in bone marrow, a major target tissue of benzene toxicity. Andrews et al. (1979) demonstrated that rabbit bone marrow is capable of metabolizing benzene. Schnier et al. (1989) subsequently found that rabbit bone marrow contains CYP2E1. Irons et al. (1980) demonstrated that benzene metabolism by rat bone marrow (*in situ*) was complete and independent of metabolism by the liver, with concentrations of phenol greater than catechol and hydroquinone. Although the total metabolism by bone marrow was limited (total metabolites present were 25% of those in blood), the concentration of metabolites in the bone marrow exceeded that in the blood. Similar studies have been conducted in mice (Ganousis et al. 1992). Fibroblasts had elevated levels of glutathione-S-transferase activity relative to macrophages, whereas macrophages had higher levels of UDP-glucuronyltransferase and peroxidase activity. These data suggest that cell-specific metabolism of benzene in the marrow may contribute to the toxicity of benzene in this tissue compartment. In addition, comparison of the detoxifying activities of rat and mouse bone marrow stromal cells indicates that rats have higher levels of glutathione and quinone reductase, which are known to play critical roles in modulating hydroquinone-induced toxicity; this suggests a metabolic basis for the observed increased susceptibility of mice to benzene-induced hematotoxicity (Zhu et al. 1995). Bernauer et al. (1999, 2000) recently noted the presence of CYP2E1 in bone marrow samples of mice (several strains), rats, rabbits, and humans. However, although Irons et al. (1980) demonstrated that the isolated perfused rat femur was capable of metabolizing a very small amount of benzene (approximately 0.0002% of ^{14}C-benzene was recovered as metabolites), neither benzene oxide nor phenol were detected in a test of benzene metabolism using microsomal preparations of bone marrow from rats (Lindstrom et al. 1999), indicating that bone marrow is not a likely source of initial metabolic oxidation for benzene. No studies were located the potential for human bone marrow tissue to metabolize benzene.

Mouse liver microsomes and cytosol have been shown to catalyze ring opening in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) *in vitro*, producing *trans,trans*-muconaldehyde, a six-carbon diene dialdehyde also referred to as muconic dialdehyde (Goon et al. 1993; Latriano et al.

1986), a known hematotoxin (Witz et al. 1985) and toxic metabolite of benzene (Henderson et al. 1989).

Metabolism of benzene and *trans,trans*-muconaldehyde in the isolated perfused rat liver indicated that

benzene was metabolized to muconic acid, a ring-opened metabolite of benzene (Grotz et al. 1994).

Trans,trans-muconaldehyde was metabolized to muconic acid and three other metabolites. These studies

indicate that ring-opening of benzene occurs in the liver. Other recent literature identifies the following

metabolites after incubation of benzene with mouse liver microsomes: phenol, hydroquinone,

trans,trans-muconaldehyde, *6*-oxo-*trans,trans*-2,4-hexadienoic acid, 6-hydroxy-*trans,trans*-2,4-hexa-

dienal, and 6-hydroxy-*trans,trans*-2,4-hexadienoic acid (Zhang et al. 1995a). β-Hydroxymuconaldehyde,

a new metabolite, was also identified. Additional work by Zhang et al. (1995b) suggests that *cis,cis*-

muconaldehyde is formed first, followed by *cis,trans*-muconaldehyde, and finally converted to

trans,trans-muconaldehyde. Muconic dialdehyde has been shown to be metabolized *in vivo* in mice to

muconic acid (Witz et al. 1990c). These data suggest that muconic dialdehyde is the precursor of

muconic acid in animals exposed to benzene. Small amounts of muconic acid were found in the urine of

rabbits and mice that received oral doses of ^{14}C-benzene (Gad-El-Karim et al. 1985; Parke and Williams

1953a). The percentage of this metabolite formed varied with the administered benzene dose and was

quite high at low doses (17.6% of 0.5 mg/kg benzene administered to C57BL/6 mice) (Witz et al. 1990c).

Other studies in animals support these results (Brondeau et al. 1992; Ducos et al. 1990; McMahon and

Birnbaum 1991; Sabourin et al. 1989a; Schad et al. 1992). This pathway also appears to be active in

humans (Bechtold and Henderson 1993; Ducos et al. 1990, 1992; Lee et al. 1993; Melikian et al. 1993,

1994). For instance, urine samples from male and female smokers and nonsmokers were obtained from

subjects who applied for life insurance (Melikian et al. 1994). Samples from pregnant women were

obtained during 7–35 weeks of pregnancy. Questionnaires were filled out on smoking history and

occupation. The levels of muconic acid and cotinine (a biomarker for cigarette smoking) in the urine for

the groups of pregnant and nonpregnant smokers and nonsmokers were compared with previously

reported data in male smokers. Results showed the mean levels of muconic acid in the groups of male,

female-nonpregnant, and female-pregnant smokers were 3.6-, 4.8-, and 4.5-fold higher than the mean

concentration of this acid in the nonsmoking groups. The differences in the mean muconic acid

concentrations between smoking and nonsmoking groups were significant in male, and nonpregnant and

pregnant female smokers. Mean concentrations of muconic acid levels in nonpregnant female smokers

are similar to that of male smokers. Mean concentrations of muconic acid in groups of 42 male smokers

and 53 female smokers were 0.22±0.03 and 0.24±0.02 mg/g creatinine, or 0.13±0.06 and

0.13±0.07 mg/mg cotinine, respectively. Mean concentrations of muconic acid in groups of 63 pregnant

and 53 nonpregnant female smokers were 0.27±0.04 and 0.24±0.02 mg/g creatinine, or 0.24±0.06 and

0.13±0.07 mg/mg cotinine, respectively. Because of its relative importance in benzene toxicity,

additional modeling studies, including molecular orbital studies, have been conducted to further describe
how *trans,trans*-muconaldehyde is transformed to muconic acid (Bock et al. 1994).

Kenyon et al. (1995) compared their urinary profile of metabolites in B6C3F$_1$ mice after oral dosing with
phenol with the results of Sabourin et al. (1989a) who administered a comparable oral dose of benzene to
B6C3F$_1$ mice. The analysis of Kenyon et al. (1995) indicated that phenol administration resulted in lower
urinary levels of hydroquinone glucuronide, and higher levels of phenol sulfate and phenol glucuronide
compared to benzene administration. Kenyon et al. (1995) hypothesized that the differences in the
urinary metabolite profiles between phenol and benzene after oral dosing were due to zonal differences in
the distribution of metabolizing enzymes within the liver. Conjugating enzymes are more concentrated in
the periportal area of the liver, the first region to absorb the compound, whereas oxidizing enzymes are
more concentrated in the pericentral region of the liver. Based on this hypothesis, during an initial pass
through the liver after oral administration, phenol would have a greater opportunity to be conjugated as it
was absorbed from the gastrointestinal tract into the periportal region of the liver, thus resulting in less
free phenol being delivered into the pericentral region of the liver to be oxidized. With less free phenol
available for oxidation, less hydroquinone would be produced, relative to conjugated phenol metabolites.
In contrast, benzene must be oxidized before it can be conjugated. Therefore, metabolism of benzene
would be minimal in the periportal region of the liver, with most of the benzene reaching the pericentral
region to be oxidized to hydroquinone. Based on this scheme, the authors suggest that benzene
administration would result in more free phenol being delivered to oxidizing enzymes in the pericentral
region of the liver than administration of phenol itself (Kenyon et al. 1995).

Benzene has been found to stimulate its own metabolism, thereby increasing the rate of toxic metabolite
formation. Pretreatment of mice, rats, and rabbits subcutaneously with benzene increased benzene
metabolism *in vitro* without increasing CYP2E1 concentrations (Arinc et al. 1991; Gonasun et al. 1973;
Saito et al. 1973). In contrast, there was no significant effect on the metabolism of benzene when
Fischer 344 rats and B6C3F$_1$ mice, pretreated with repeated inhalation exposure to 600 ppm of benzene,
were again exposed to 600 ppm benzene (Sabourin et al. 1990). The rate of benzene metabolism can be
altered by pretreatment with various compounds. Benzene is a preferential substrate of CYP2E1, which
also metabolizes alcohol and aniline. CYP2E1 can be induced by these substrates and is associated with
the generation of hydroxyl radicals, probably via futile cycling of the cytochrome (Chepiga et al. 1991;
Parke 1989; Snyder et al. 1993a, 1993b). It is possible that hydroxy radical formation by CYP2E1 may
play a role in the benzene ring-opening pathway, leading to the formation of *trans,trans*-muconaldehyde.
Phenol, hydroquinone, benzoquinone, and catechol have also been shown to induce CYPs in human

hematopoietic stem cells (Henschler and Glatt 1995). Therefore, exposure to chemicals that stimulate the activity of this enzyme system prior to exposure to benzene could increase the rate of benzene metabolism.

Both NADPH-linked and ascorbate-induced lipid peroxidation activities induced *in vitro* were lowered 5.5 and 26%, respectively, in rats following oral administration of 1,400 mg/kg/day of benzene for 3 days, followed by intraperitoneal injection of phenobarbital. These results suggest that benzene alters hepatic drug metabolism and lipid peroxidation. The decrease in lipid peroxidation could be due to the antioxidant property of the metabolites (Pawar and Mungikar 1975).

The ultimate disposition and metabolic fate of benzene depends on animal species, dose, and route of exposure. The dose of benzene affects both the total metabolism and the concentrations of individual metabolites formed. In mice, the percentage of hydroquinone glucuronide decreased as the dose increased. In both rats and mice, the percentage of muconic acid decreased as the dose increased. The shift in metabolism may affect the dose-response relationship for toxicity, and has been observed in all animal species studies thus far (Sabourin et al. 1989a, 1992; Witz et al. 1990b, 1990c). The effect of species differences in metabolism of inhaled benzene was evidenced by the fact that mice have a higher minute volume per kg body weight than rats (1.5 times higher). This caused the blood concentration of benzene to reach equilibrium more quickly in mice than in rats, but the steady-state level in blood was not influenced (Sabourin et al. 1987). Species differences in benzene metabolism following oral exposure were elucidated in rats and mice administered benzene by gavage at doses of 0.5–150 mg/kg/day (Sabourin et al. 1987). At doses below 15 mg/kg, >90% of the benzene was metabolized, while at doses above 15 mg/kg, an increasing percentage of orally administered benzene was exhaled unmetabolized. Total metabolites per unit body weight were equal in rats and mice at doses up to 50 mg/kg/day. However, total metabolites in mice did not increase at higher doses, suggesting saturation of metabolic pathways (Sabourin et al. 1987).

The integrated dose to a tissue over a 14-hour period (6-hour exposure, 8 hours following exposure) was calculated for benzene metabolites in rats and mice that were exposed to 50 ppm of radiolabeled (^{3}H) benzene (Sabourin et al. 1988). The major metabolic products in rats were detoxification products that were marked by phenyl conjugates. In contrast, mice had substantial quantities of the markers for toxification pathways (muconic acid, hydroquinone glucuronide, and hydroquinone sulfate) in their tissues. Muconic acid and hydroquinone glucuronide were also detected in mouse bone marrow. These results may explain why mice are more susceptible to benzene-induced toxicity than rats.

In a study by Orzechowski et al. (1995), hepatocytes from adult male Wistar rats and NMRI mice were incubated for 1 hour with 0.5 mM ^{14}C-benzene, and the supernatant analyzed for metabolites. Formation of sulfate conjugates of benzene, hydroquinone, and 1,2,4-benzenetriol was also studied in a separate experiment. Mouse hepatocytes produced two metabolites (1,2,4-trihydroxybenzene sulfate and hydroquinone sulfate) that were not found in rat hepatocyte incubations. These sulfate metabolites were found in incubations including benzene, or the metabolites themselves, hydroquinone and 1,2,4-benzenetriol. Mouse hepatocytes were almost three times more effective in metabolizing benzene, compared to rat hepatocytes. This difference was accounted for in the formation of hydroquinone, hydroquinone sulfate, and 1,2,4-trihydroxybenzene sulfate. These *in vitro* experiments indicate there are both quantitative and qualitative differences in rodent metabolism of benzene.

Data produced *in vitro* by mouse and rat liver microsomes also indicate species differences in benzene metabolism (Schlosser et al. 1993). Quantitation of metabolites from the microsomal metabolism of benzene indicated that after 45 minutes, mouse liver microsomes from male B6C3F$_1$ mice had converted 20% of the benzene to phenol, 31% to hydroquinone, and 2% to catechol. In contrast, rat liver microsomes from male Fischer 344 rats converted 23% to phenol, 8% to hydroquinone, and 0.5% to catechol. Mouse liver microsomes continued to produce hydroquinone and catechol for 90 minutes, whereas rat liver microsomes had ceased production of these metabolites by 90 minutes. Muconic acid production by mouse liver microsomes was <0.04 and <0.2% from phenol and benzene, respectively, after 90 minutes.

There are quantitative differences in the benzene metabolites produced by different species (Sabourin et al. 1988). Fischer 344 rats exposed to 50 ppm benzene had undetectable amounts of phenol, catechol, and hydroquinone in the liver, lungs, and blood. The major water-soluble metabolites were muconic acid, phenyl sulfate, prephenyl mercapturic acid, and an unknown. The unknown was present in amounts equal to the amounts of phenyl sulfate in the liver; phenyl sulfate and the unknown were the major metabolites in the liver. B6C3F$_1$ mice exposed to 50 ppm benzene had detectable levels of phenol and hydroquinone in the liver, lungs, and blood; catechol was detectable only in the liver and not in the lungs or blood. As in the rat, the unknown was present in amounts equal to the amounts of phenyl sulfate in the liver. Mice had more muconic acid in the liver, which indicates a greater risk for them from *trans,trans*-muconaldehyde (Sabourin et al. 1988).

The effect of dose rate on benzene metabolism was studied in Fischer 344 rats and B6C3F$_1$ mice that had either long inhalation exposures to low concentrations or short exposures to high concentrations of benzene (Sabourin et al. 1989a, 1989b). Inhalation occurred at 1 of 3 exposure regimens, all having the same integral amount of benzene: 600 ppm benzene for 0.5 hour, 150 ppm for 2 hours, or 50 ppm for 6 hours. Results indicated no dose-rate effect in rats. In mice, however, the fast exposure rate (0.5 hour times 600 ppm) produced less muconic acid in the blood, liver, and lungs. In the blood and lungs, less hydroquinone glucuronide and more prephenyl mercapturic acid were produced at the higher exposure rates. At the highest benzene exposure concentrations or fastest benzene exposure rate in mice, there was a reduction in the ratios of muconic acid and hydroquinone glucuronide to the metabolite phenylsulfate. Furthermore, with increased dose rate or increased exposure concentration, mice tended to shift a greater portion of their benzene metabolism toward detoxification pathways. Likewise, the detoxification pathways for benzene appear to be low-affinity, high-capacity pathways, whereas pathways leading to the putative toxic metabolites appear to be high-affinity, low-capacity systems (Henderson et al. 1989). Accordingly, if the exposure dose regimen, via inhalation, extends beyond the range of linear metabolism rates of benzene (200 ppm by inhalation) (Sabourin et al. 1989b), then the fraction of toxic metabolites formed relative to the amount administered will be reduced. Bois and Paxman (1992) used a PBPK model to assess effects of dose rate on the disposition of benzene metabolites. Simulations were performed for rats exposed either for 15 minutes to 32 ppm or for 8 hours to 1 ppm (equivalent 8-hour TWAs). The amount of metabolites (hydroquinone, catechol, and muconaldehyde) formed was 20% higher after the 15-minute exposure at the higher level than after the 8-hour exposure at the lower level. Differences between the model predictions (Bois and Paxman 1992) and the empirical data of Sabourin et al. (1989a, 1989b) may be related, at least in part, to the higher benzene exposure levels (50, 150, and 600 ppm) used by Sabourin and coworkers.

A number of investigators have suggested that covalent binding of benzene metabolites to cellular macromolecules is related to benzene's mechanism of toxicity, although the relationship between adduct formation and toxicity is not clear. Benzene metabolites have been found to form covalent adducts with proteins from blood in humans (Bechtold et al. 1992b). Benzene metabolites form covalent adducts with nucleic acids and proteins in rats and mice (Norpoth et al. 1988; Rappaport et al. 1996); covalently bind to proteins in mouse or rat liver, bone marrow, kidney, spleen, blood, and muscle *in vivo* (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Creek et al. 1997; Longacre et al. 1981a, 1981b; Sun et al. 1990); bind to proteins in perfused bone marrow preparations (Irons et al. 1980) and in rat and mouse liver DNA *in vivo* (Creek et al. 1997; Lutz and Schlatter 1977); and bind to DNA in rabbit and rat bone marrow mitochondria *in vitro* (Rushmore et al. 1984). Exposure-related increases in blood levels of

albumin adducts of benzene oxide and 1,4-benzoquinone were noted among workers occupationally
exposed to benzene air concentrations ranging from 0.07 to 46.6 ppm (Rappaport et al. 2002a, 2002b).
Several reactive metabolites of benzene have been proposed as agents of benzene hematotoxic and
leukemogenic effects. These metabolites include benzene oxide, reactive products of the phenol pathway
(catechol, hydroquinone, and 1,4-benzoquinone), and *trans*,*trans*-muconaldehyde. See Section 3.5.2 for a
discussion of mechanisms of benzene toxicity.

3.4.4   Elimination and Excretion

3.4.4.1 Inhalation Exposure

Available human data indicate that following inhalation exposure to benzene, the major route for
elimination of unmetabolized benzene is via exhalation. Absorbed benzene is also excreted in humans via
metabolism to phenol and muconic acid followed by urinary excretion of conjugated derivatives (sulfates
and glucuronides). In six male and female volunteers exposed to 52–62 ppm benzene for 4 hours,
respiratory excretion (the amount of absorbed benzene excreted via the lungs) was approximately 17%;
no gender-related differences were observed (Nomiyama and Nomiyama 1974a, 1974b). Results from a
study of 23 subjects who inhaled 47–110 ppm benzene for 2–3 hours showed that 16.4–41.6% of the
retained benzene was excreted by the lungs within 5–7 hours (Srbova et al. 1950). The rate of excretion
of benzene was the greatest during the first hour. The study also showed that only 0.07–0.2% of the
retained benzene was excreted in the urine. Other studies suggest that benzene in the urine may be a
useful biomarker of occupational exposure (Ghittori et al. 1993). Results of a study involving a single
human experimental subject exposed to concentrations of benzene of 6.4 and 99 ppm for 8 hours and
1 hour, respectively, suggested that excretion of benzene in breath has three phases and could possibly
have four phases. The initial phase is rapid and is followed by two (or three) slower phases (Sherwood
1988). The initial phase with a high exposure concentration (99 ppm) and a short-term exposure duration
(1 hour) had a more rapid excretion rate (half-life=42 minutes) and a greater percentage of the total dose
excreted (17%) than did the initial phase with a low exposure concentration (6.4 ppm) and longer
exposure duration (8 hours) (half-life=1.2 hours, percentage of total dose excreted=9.3%). Subsequent
phases showed an increase in the half-lives. These results also showed that urinary excretion of phenol
conjugate was biphasic, with an initial rapid excretion phase, followed by a slower excretion phase. A
greater proportion of the total dose was excreted in urine than in breath (Sherwood 1988). The urinary
excretion of phenol in workers was measured following a 7-hour workshift exposure to 1–200 ppm
benzene. A correlation of 0.881 between exposure level and urinary phenol excretion was found (Inoue et
al. 1986). Urine samples were collected from randomly chosen subjects not exposed to known sources of

benzene, from subjects exposed to sidestream cigarette smoke, or from supermarket workers presumed exposed to benzene from polyvinyl chloride (PVC) meat packing wrap (Bartczak et al. 1994). Samples were analyzed for identification of muconic acid. Muconic acid concentrations of 8–550 ng/mL were found in all urine samples. Kok and Ong (1994) report blood and urine levels of benzene as 110.9 and 116.4 ng/L, respectively, in nonsmokers, and 328.8 and 405.4 ng/L, respectively in smokers. A significant correlation was found between benzene levels in blood and benzene levels in urine. Similar results were found for filling station attendants in Italy (Lagorio et al. 1994b).

Popp et al. (1994) reported a mean blood benzene level in car mechanics of 3.3 µg/L. Urinary muconic acid and *S*-phenylmercapturic acid levels increased during the work shift, and were well correlated with the blood levels and the benzene air levels, which reached a maximum of 13 mg/m^3.

As discussed in Section 3.4.3, the mean urinary levels of muconic in groups of male, female-nonpregnant, and female-pregnant smokers were 3.6-, 4.8-, and 4.5-fold higher than the mean concentration of this acid in the nonsmoking groups (Melikian et al. 1994). The differences in the mean muconic acid concentrations between smoking and nonsmoking groups were significant in male (p=0.001), and nonpregnant (p=0.001) and pregnant female smokers (p=0.002). Mean concentrations of muconic acid levels in nonpregnant female smokers are similar to that of male smokers. Mean concentrations of muconic acid in groups of 42 male smokers and 53 female smokers were 0.22±0.03 and 0.24±0.02 mg/g creatinine, or 0.13±0.06 and 0.13±0.07 mg/mg cotinine, respectively. Mean concentrations of muconic acid in groups of 63 pregnant and 53 nonpregnant female smokers were 0.27±0.04 and 0.24±0.02 mg/g creatinine, or 0.24±0.06 and 0.13±0.07 mg/mg cotinine, respectively. Mean concentrations of urinary cotinine in pregnant smokers were significantly lower than in the group of nonpregnant female smokers (1.13±0.12 mg/g creatinine compared to 1.82±0.14 mg/g creatinine). Benzene levels ranging from 0.01 to 0.18 µg/kg have been detected in samples of human breast milk (Fabietti et al. 2004).

Animal data show that exhalation is the main route for excretion of unmetabolized benzene and that metabolized benzene is excreted primarily in urine. Only a small amount of an absorbed dose is eliminated in feces. A biphasic pattern of excretion of unmetabolized benzene in expired air was observed in rats exposed to 500 ppm for 6 hours, with half-times for expiration of 0.7 hour for the rapid phase and 13.1 hours for the slow phase (Rickert et al. 1979). The half-life for the slow phase of benzene elimination suggests the accumulation of benzene. The major route of excretion following a 6-hour nose-only inhalation exposure of rats and mice to various concentrations of ^{14}C-benzene appeared to be dependent on the inhaled concentration (Sabourin et al. 1987). At similar exposures to vapor

concentrations of 10–1,000 ppm, the mice received 150–200% of the equivalent dose in rats on a per kg body weight basis. At all concentrations, fecal excretion accounted for <3.5% of the radioactivity for rats and <9% for mice. At lower exposure concentrations (i.e., 13–130 ppm in rats and 11–130 ppm in mice), <6% of the radioactivity was excreted in expired air. At the highest exposure concentrations (rats, 870 ppm; mice, 990 ppm), both rats and mice exhaled a significant amount of unmetabolized benzene (48 and 14%, respectively) following termination of the exposure. The majority of the benzene-associated radioactivity that was not exhaled was found in the urine and in the carcass 56 hours after the end of exposure to these high concentrations. The radioactivity in the carcass was associated with the pelt of the animals. The authors assumed that this was due to contamination of the pelt with urine, since the inhalation exposure had been nose-only. Further investigation confirmed that the radioactivity was associated with the fur of the animals. Accordingly, the percentage of the total radioactivity excreted by these animals (urine and urine-contaminated pelt) that was not exhaled or associated with feces was 47–92% for rats and 80–94% for mice. At exposures of 260 ppm in rats, 85–92% of the radioactivity was excreted as urinary metabolites, while at exposures of 130 ppm in mice, 88–94% of the radioactivity was excreted as urinary metabolites. The total urinary metabolite formation was 5–37% higher in mice than in rats at all doses. This may be explained by the greater amount of benzene inhaled by mice per kg of body weight (Sabourin et al. 1987). Purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours/day, 5 days/week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

3.4.4.2 Oral Exposure

No studies were located regarding excretion in humans after oral exposure to benzene. Data on excretion of benzene or its metabolites in human breast milk after oral exposure were not found.

Radiolabeled benzene (340 mg/kg) was administered by oral intubation to rabbits; 43% of the label was recovered as exhaled unmetabolized benzene and 1.5% was recovered as carbon dioxide (Parke and Williams 1953a). Urinary excretion accounted for about 33% of the dose. The isolated urinary metabolites were mainly in the form of conjugated phenols. Phenol was the major metabolite accounting for about 23% of the dose or about 70% of the benzene metabolized and excreted in the urine. The other phenols excreted (percentage of dose) were hydroquinone (4.8%), catechol (2.2%), and trihydroxy-benzene (0.3%). L-Phenyl-N-acetyl cysteine accounted for 0.5% of the dose. Muconic acid accounted for 1.3%; the rest of the radioactivity (5–10%) remained in the tissues or was excreted in the feces (Parke and Williams 1953a).

Mice received a single oral dose of either 10 or 200 mg/kg radiolabeled benzene (McMahon and
Birnbaum 1991). Radioactivity was monitored in urine, feces, and breath. At the low dose, urinary
excretion was the major route of elimination. Hydroquinone glucuronide, phenylsulfate, and muconic
acid were the major metabolites at this dose, accounting for 40, 28, and 15% of the dose, respectively. At
200 mg/kg, urinary excretion decreased to account for 42–47% of the administered dose, while
respiratory excretion of volatile components increased to 46–56% of the administered dose. Fecal
elimination was minor and relatively constant over both doses, accounting for 0.5–3% of the dose.

The effect of dose on the excretion of radioactivity, including benzene and metabolites, following oral
administration of ^{14}C-benzene (0.5–300 mg/kg) has been studied in rats and mice (Sabourin et al. 1987).
At doses of <15 mg/kg for 1 day, 90% of the administered dose was excreted in the urine of both species.
There was a linear relationship for the excretion of urinary metabolites up to 15 mg/kg; above that level,
there was an increased amount of ^{14}C eliminated in the expired air. Mice and rats excreted equal amounts
up to 50 mg/kg; above this level, metabolism apparently became saturated in mice. In rats, 50% of the
150 mg/kg dose of ^{14}C was eliminated in the expired air; in mice, 69% of the 150 mg/kg dose of ^{14}C was
eliminated in expired air (Sabourin et al. 1987). The label recovered during exhalation was largely in the
form of unmetabolized benzene, suggesting that saturation of the metabolic pathways had occurred. Dose
also affected the metabolite profile in the urine. At low doses, a greater fraction of the benzene was
converted to putative toxic metabolites than at high doses, as reflected in urinary metabolites.

Mathews et al. (1998) reported similar results following oral (gavage) administration of ^{14}C-benzene to
rats, mice, and hamsters in single doses from as low as 0.2 mg/kg and up to 100 mg/kg. For example,
>95% of a 0.5 mg/kg dose was recovered in the urine of rats; a small amount (3%) was recovered in
expired air. At benzene doses of 10 and 100 mg/kg, elimination in the breath rose to 9 and 50%,
respectively, indicating the likely saturation of benzene metabolism. Excretion in the feces was minimal
at all dose levels. Similar results were noted for mice and hamsters. Both dose and species differences
were noted in the composition of urinary metabolites. Phenyl sulfate was the major metabolite in rat
urine at all dose levels, accounting for 64–73% of urinary radioactivity. Phenyl sulfate (24–32%) and
hydroquinone glucuronide (27–29%) were the predominant urinary metabolites in mice. At a dose of
0.1 mg/kg, mice produced a considerably higher proportion of muconic acid than rats (15 versus 7%). In
hamsters, hydroquinone glucuronide (24–29%) and muconic acid (19–31%) were the primary urinary
metabolites. Two additional metabolites (1,2,4-trihydroxybenzene and catechol sulfate) were recovered
from the urine of hamsters, but not rats or mice.

3.4.4.3 Dermal Exposure

Limited data on excretion of benzene after dermal exposure in humans were found. Four human male subjects were given a dermal application of 0.0024 mg/cm^2 ^{14}C benzene (Franz 1984). A mean of 0.023% (range, 0.006–0.054%) of the applied radiolabel was recovered in the urine over a 36-hour period. Urinary excretion of the radiolabel was greatest in the first two hours following skin application. More than 80% of the total excretion occurred in the first 8 hours. In another study, 35–43 cm^2 of the forearm were exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25–2 hours (Hanke et al. 1961). The absorption was estimated from the amount of phenol eliminated in the urine. The absorption rate of liquid benzene by the skin (under the conditions of complete saturation) was calculated to be low, approximately 0.4 mg/cm^2/hour. The absorption due to vapors in the same experiment was negligible. Although there was a large variability in the physiological values, the amount of excreted phenol was 8.0–14.7 mg during the 24-hour period after exposure. It is estimated that approximately 30% of dermally absorbed benzene is eliminated in the form of phenol in the urine.

Data on excretion of benzene or its metabolites in human breast milk after dermal exposure were not found.

Monkeys and minipigs were exposed dermally to 0.0026–0.0036 mg/cm^2 of ^{14}C-benzene (Franz 1984). After application, the urine samples were collected over the next 2–4 days at 5-hour intervals. The rate of excretion was highest in the first two collection periods. The total urinary excretion of radioactivity was found to be higher in monkeys than in minipigs with the same exposure. Mean excretion in monkeys was 0.065% (range, 0.033–0.135%) of the applied dose compared to 0.042% (range, 0.030–0.054%) in minipigs.

Results of a study in which male rats were dermally treated with 0.004 mg/cm^2 of ^{14}C-benzene, with or without 1g of clay or sandy soil, showed that for all treatment groups, the major routes of excretion were the urine and, to a lesser extent, the expired air (Skowronski et al. 1988). The highest amount of radioactivity in urine appeared in the first 12–24 hours after treatment (58.8, 31.3, and 25.1% of the absorbed dose, respectively, for pure benzene, sandy soil–adsorbed benzene, and clay soil-adsorbed benzene). In the group treated with pure benzene, 86.2% of the absorbed dose was excreted in the urine. Sandy soil and clay soil significantly decreased urinary excretion to 64.0 and 45.4%, respectively, of the absorbed dose during the same time period. Rats receiving pure benzene excreted 12.8% of the absorbed

dose in expired air within 48 hours. Only 5.9% of the radioactivity was collected in expired air 48 hours after treatment with sandy soil–adsorbed benzene, while experiments with clay soil–adsorbed benzene revealed that 10.1% of the radioactivity was located in expired air. Less than 1% of the absorbed dose was expired as $^{14}CO_2$ in all groups. The ^{14}C activity in the feces was small (<0.5% of the applied radioactivity) in all groups 48 hours after treatment. Phenol was the major urinary metabolite detected in the 0–12-hour urine samples of all treatment groups. The percentage of total urinary radioactivity associated with phenol was 37.7% for benzene alone, 44.2% for benzene adsorbed to sandy soil, and 45.5% for benzene adsorbed to clay soil. Smaller quantities of hydroquinone, catechol, and benzenetriol were also detected (Skowronski et al. 1988).

3.4.4.4 Other Routes of Exposure

The metabolic fate of benzene can be altered in fasted animals. In nonfasted rats that received an intraperitoneal injection of 88 mg of benzene, the major metabolites present in urine were total conjugated phenols (14–19% of dose), glucuronides (3–4% of dose), and free phenol (2–3% of dose). However, in rats fasted for 24 hours preceding the same exposure, glucuronide conjugation increased markedly (18–21% of dose) (Cornish and Ryan 1965). Free phenol excretion (8–10% of dose) was also increased in fasted, benzene-treated rats. There was no apparent increase in total conjugated phenol excretion in fasted rats given benzene.

When ^{14}C-benzene (0.5 and 150 mg/kg) was injected intraperitoneally into rats and mice, most of the ^{14}C-benzene and ^{14}C-metabolites were excreted in the urine and in the expired air. A smaller amount of ^{14}C-benzene was found in the feces due to biliary excretion (Sabourin et al. 1987). Monkeys were dosed intraperitoneally with 5–500 mg/kg radiolabeled benzene, and urinary metabolites were examined (Sabourin et al. 1992). The proportion of radioactivity excreted in the urine decreased with increasing dose, whereas as the dose increased, more benzene was exhaled unchanged. This indicated saturation of benzene metabolism at higher doses. Phenyl sulfate was the major urinary metabolite. Hydroquinone conjugates and muconic acid in the urine decreased as the dose increased. When C57BL/6 mice and DBA/2 mice were given benzene subcutaneously in single doses (440, 880, or 2,200 mg/kg) for 1 day, or multiple doses (880 mg/kg) 2 times daily for 3 days, no strain differences were observed in the total amount of urinary ring-hydroxylated metabolites (Longacre et al. 1981a). Although each strain excreted phenol, catechol, and hydroquinone, differences in the relative amounts of these metabolites were noted. The more sensitive DBA/2 mice excreted more phenol but less hydroquinone than the more resistant C57BL/6 mice, while both strains excreted similar amounts of catechol. DBA/2 mice excreted more

phenyl glucuronide but less sulfate conjugate. Both strains excreted similar amounts of phenyl

mercapturic acid (Longacre et al. 1981a).

3.4.5   Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and

disposition of chemical substances to quantitatively describe the relationships among critical biological

processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry

models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of

potentially toxic moieties of a chemical that will be delivered to any given target tissue following various

combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based

pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to

quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to

delineate and characterize the relationships between: (1) the external/exposure concentration and target

tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and

Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can

be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from

route to route, between species, and between subpopulations within a species. The biological basis of

PBPK models results in more meaningful extrapolations than those generated with the more conventional

use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model

representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and

Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of

toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen

1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-

specific physicochemical parameters, and species-specific physiological and biological parameters. The

numerical estimates of these model parameters are incorporated within a set of differential and algebraic

equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations

provides the predictions of tissue dose. Computers then provide process simulations based on these

solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

If PBPK models for benzene exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

Several PBPK models have been developed that simulate the disposition of benzene in humans (Bois et al. 1996; Brown et al. 1998; Fisher et al. 1997; Medinsky et al. 1989c; Sinclair et al. 1999; Travis et al. 1990), mice (Cole et al. 2001; Medinsky et al. 1989a, 1989b; Sun et al. 1990; Travis et al. 1990), and rats (Bois et al. 1991a; Medinsky et al. 1989a, 1989b; Sun et al. 1990; Travis et al. 1990). A comparative summary of the models is provided in Table 3-6. All of the models have the same general structure (Figure 3-5). Most of the models simulate inhalation and oral exposures; one model provides a simulation of dermal absorption (Sinclair et al. 1999). Physiological parameters and partition coefficients for simulating benzene biokinetics of human females were reported for the Brown et al. (1998) and Fisher et al. (1997) models. Flow-limited exchange of benzene between blood and tissues is assumed in all models, with excretion of benzene in exhaled air and, in one case, to breast milk (Fisher et al. 1997). All models include simulations of blood, fat, liver, lung, and lumped compartments representing other slowly-perfused tissues (e.g., skeletal muscle) and rapidly-perfused tissues (e.g., kidneys, other viscera). Simulation of bone marrow, the primary target for benzene toxicity, is included in the models reported by Bois et al. (1991a, 1996), Sinclair et al. (1999), and Travis et al. (1990).

Simulations of metabolism in the various models vary in complexity. In the simplest representation, metabolic elimination of benzene is simulated as a single capacity-limited process, represented with

Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance
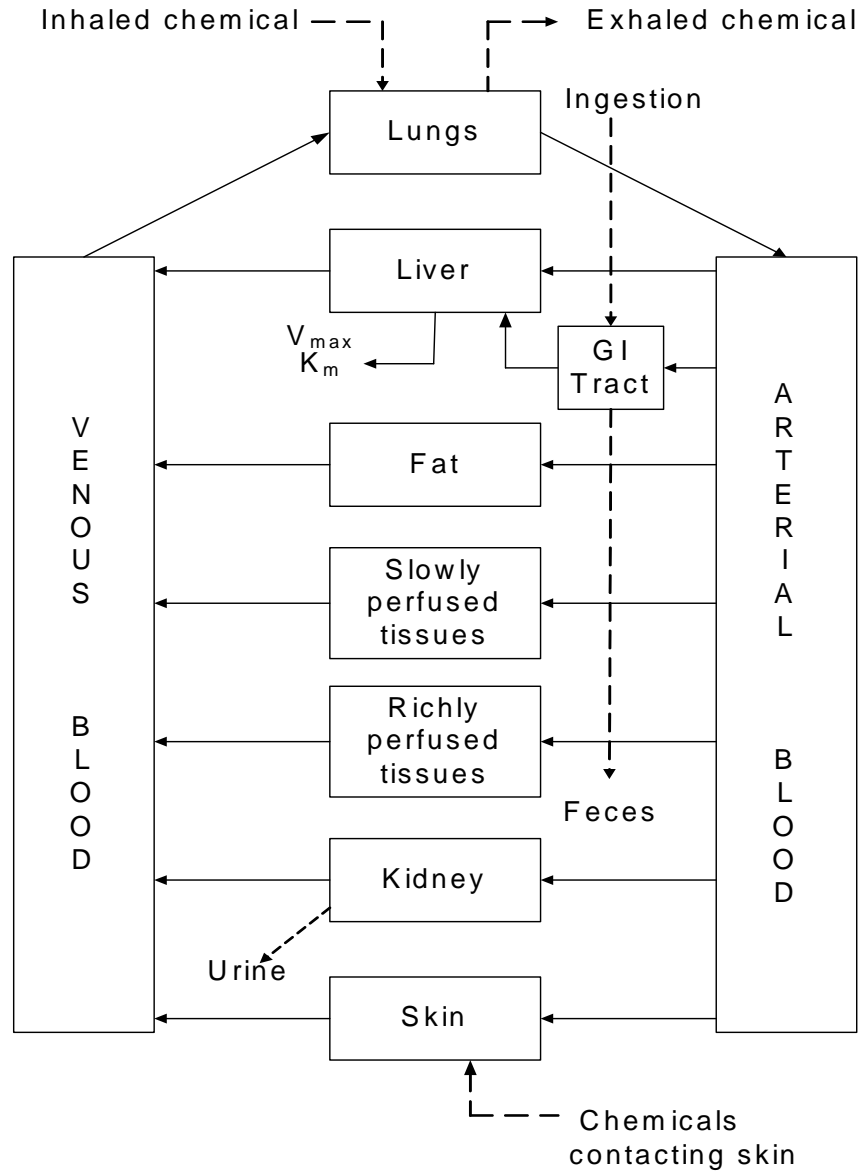
Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: adapted from Krishnan and Andersen 1994

Figure 3-5. General Structure of Physiologically Based Pharmacokinetic Models of Benzene*
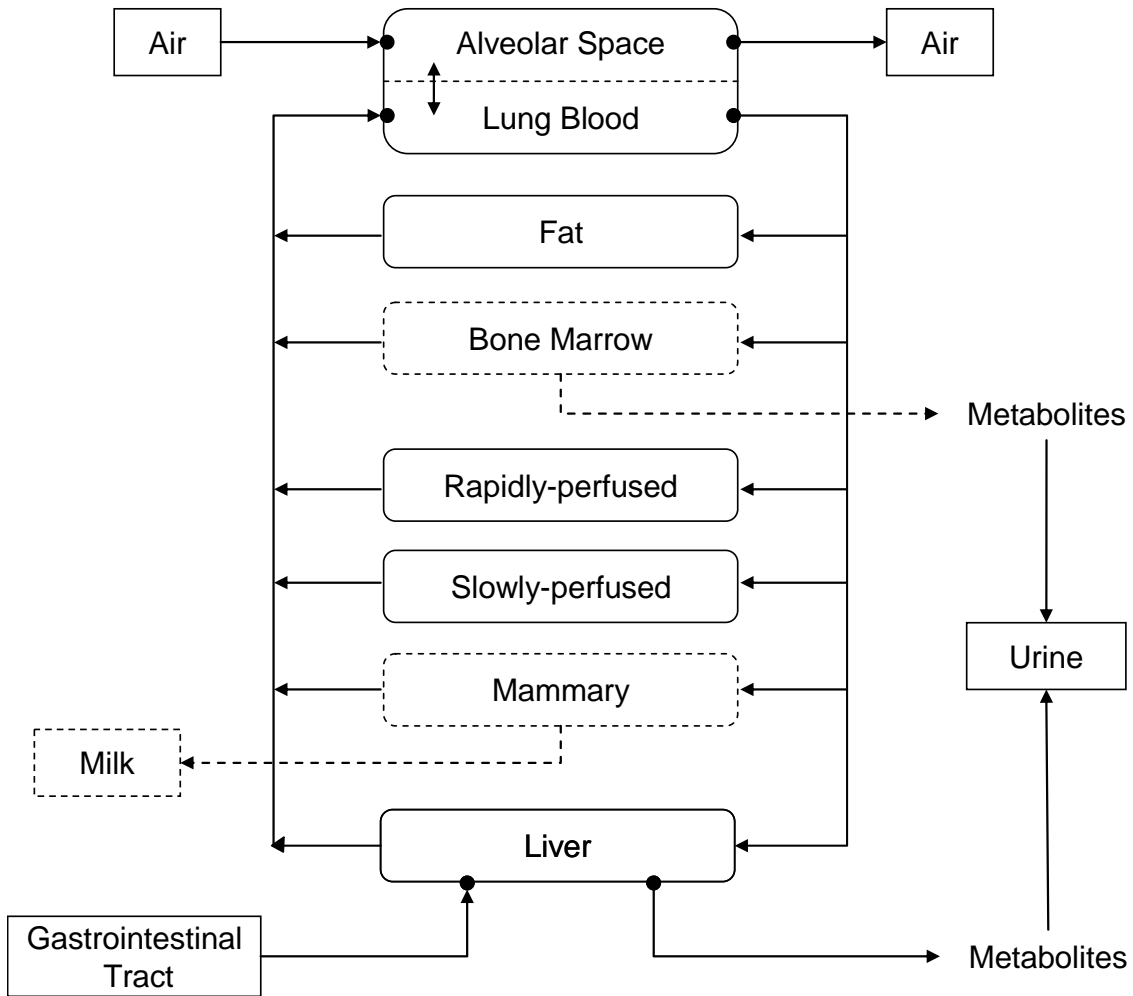
*Tissues shown with dashed lines are not simulated in all models. Flow-limited exchange of benzene between blood and tissues is assumed. Metabolism is simulated to varying degrees of complexity (see Table 3-6 for model comparison).

3. HEALTH EFFECTS

Table 3-6. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

Refer-ence	Species[a]	Absorption pathways[b]	Tissues[c]	Metabolic pathways[d]		Excretion pathways[e]		Comment
Bois et al. 1991a	R	IH, OR	BL, BM, FA, LI, LU, RP, SP	BM, LI:	BZ→BO(c) BO→BG(c) BO→PH(f) BO→GSH(c) BG→DI(c) PH→HQ(c) PH→CA(c)	EH: BZ UR: PH		Simulates metabolic pathways in bone marrow, and phenol conjugation in lung and gastrointestinal tract
				BM, LU, LU, GI:	PH→PHCO(c)			
Bois et al. 1996	H	IH	BL, BM, FA, LU, LI, RP, SP	BM, LI: LI:	BZ→M$_{tot}$(c) PHX$_{end}$→PH(z)	EH: BZ UR: M$_{tot}$ PH		Simulates metabolic pathways in bone marrow, and endo-genous production of phenolic meta-bolites
Brown et al. 1998	H (m,f)	IH	BL, FA, LI, LU, RP, SP	LI:	BZ→M$_{tot}$(f)	EH: BZ		Simulates males or females
Cole et al. 2001	M	IH, OR	BL, FA, LI, LU, RP, SP	LI:	BZ→BO(c) BO→PH(f) BO→PMA(f) BO→MA(f) PH→HQ(c) PH→PHCO(c) PH→CA(c) CA→THB(c) HQ→HQCO(c)	EH: BZ UR: CA MA PHCO PMA HQCO THB		All metabolism is assigned to the liver
Fisher et al. 1997	H	IH	FA, LU, LI, RP, SP, MI	LI:	BZ→M$_{tot}$(c)	EH: BZ MI: BZ		Simulates transfer of benzene to breast milk
Medinsky et al. 1989a, 1989b, 1989c	H, M, R	IH, OR	FA, LI, LU, RP, SP	LI:	BZ→BO(c) BO→PHCO(c) BO→PMA(c) BO→HQCO(c) BO→MA(c)	EH: BZ		All metabolism is assigned to the liver

Table 3-6. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

Refer-ence	Species[a]	Absorption pathways[b]	Tissues[c]	Metabolic pathways[d]		Excretion pathways[e]		Comment
Sinclair et al. 1999	H	IH, OR, DE	BL, BM, LI, LU, MU, RP	BM, LI:	$BZ \rightarrow M_{tot}(c)$	EH: UR:	BZ M_{tot} PH	Simulates dermal exposure and absorption
Sun et al. 1990	M, R	IH, OR	BL, FA, LI, LU, RBC, RP, SP	LI: RBC:	$BZ \rightarrow BO(c)$ $BO \rightarrow PHCO(c)$ $BO \rightarrow PMA(c)$ $BO \rightarrow HQCO(c)$ $BO \rightarrow MA(c)$ $BO \rightarrow HBA(c,f)$	EH:	BZ	Simulates formation of hemoglobin adducts in red blood cells derived from benzene oxide
Travis et al. 1990	H, M, R	IH, OR	BL, BM, FA, LI, LU, MU, RP	BM, LI:	$BZ \rightarrow M_{tot}(c)$	EH:	BZ	Total metabolism of benzene in the bone marrow and liver

[a]Species simulated: H=human; M=mouse; R=rat; m=male; f=female
[b]Absorption pathways simulated: IH=inhalation; OR=oral; DE=dermal
[c]Tissues simulated: BL=blood; BM=bone marrow; FA=fat; LI=liver; LU=lung; MU=muscle; RBC=red blood cells; RP=other rapidly-perfused tissues; SP=other slowly-perfused tissues
[d]Metabolic pathways simulated: BZ=benzene; BD=benzene diols; BO=benzene oxide; BG=Benzene glycol; CA=catechol; HBA=hemoglobin adduct; HQ=hydroquinone; HQCO=ydroqinione conjugates; MA=muconic acid; M_{tot}=total metabolites; PH=phenol; PHCO=phenol conjugates; PMA=phenylmercapturic acid; THB=trihydroxybenzene; PHX_{end}=endogenous phenolic metabolites; (c)=capacity-limited; (f)=first-order; (z)= zero-order
[e]Excretion pathways simulated: EH=exhalation; MI=breast milk; UR=urine

Michaelis-Menten function of benzene concentration in tissue (Bois et al. 1996; Brown et al. 1998; Fisher et al. 1997; Sinclair et al. 1999; Travis et al. 1990). In the more complex representations, the major pathways of metabolism of benzene, including conjugation reactions, are simulated as capacity-limited or first-order processes (Bois et al. 1991a; Cole et al. 2001; Medinsky et al. 1989a, 1989b, 1989c; Sun et al. 1990). In most of the models, all metabolic pathways are attributed to the liver; however, four of the models include simulations of metabolism in bone marrow (Bois et al. 1991a, 1996; Sinclair et al. 1999; Travis et al. 1990), and one model includes simulations of the formation of sulfate and glucuronide conjugates of phenol in the gastrointestinal and respiratory tracts (Bois et al. 1991a). The Sun et al. (1990) model includes a simulation of the formation of hemoglobin adducts derived from benzene oxide. In models that simulate the disposition of the metabolites, metabolites are assumed to be excreted in urine either at a rate equal to their formation (Cole et al. 2001), or in accordance with a first-order excretion rate constant (Bois et al. 1991a, 1996; Sinclair et al. 1999); the difference being, in the latter, the mass balance for formation and excretion of metabolites is simulated, allowing predictions of metabolite levels in tissues. All of the models use typical parameters and values for species-specific blood flows and tissue volumes.

Brief summaries of the models presented in Table 3-6 are provided below, with emphasis on unique features that are applicable to risk assessment.

Medinsky et al. 1989a, 1989b, 1989c

Description of the Model.   The Medinsky et al. (1989a, 1989b, 1989c) model simulates absorption and disposition of benzene in the human, mouse, and rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process; absorption and excretion of benzene in the lung are assumed to be flow-limited. Exchange of benzene between blood and tissues is assumed to be flow-limited. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolism of benzene to benzene oxide as a function of the concentration of benzene in liver. Conversion of benzene oxide to phenol conjugates, phenylmercapturic acid, hydroquinone conjugates, and muconic acid are simulated as parallel, capacity-limited reactions in liver. The model simulates rates of formation of metabolites, but not the disposition (e.g., excretion) of metabolites. Metabolism parameter values (V_{max}, K_m) for the mouse and rat models were estimated by optimization of the model to observations of total metabolites formed in mice and rats exposed by inhalation or oral routes to benzene (Medinsky et al. 1989b; Sabourin

et al. 1987). Human metabolism parameter values were derived from allometric scaling of the values for mice (Medinsky et al. 1989c).

Risk Assessment.   The model has been used to predict the amounts of benzene metabolites formed in rats and mice after inhalation or oral exposures (Medinsky et al. 1989a, 1989b). For inhalation concentrations up to 1,000 ppm, mice were predicted to metabolize at least 2–3 times more benzene than rats. For oral doses >50 mg/kg, rats were predicted to metabolize more benzene on a kg-body weight basis than mice. The model also predicts different metabolite profiles in the two species: mice were predicted to produce primarily hydroquinone glucuronide and muconic acid, metabolites linked to toxic effects, whereas rats were predicted to produce primarily phenyl sulfate, a detoxification product. These predictions agree with experimental data and provide a framework for understanding the greater sensitivity of the mouse to benzene toxicity.

Validation of the Model.   The model was calibrated with data from Sabourin et al. (1987). Bois et al. (1991b) compared predictions made to observations of benzene exhaled by rats following exposures to 490 ppm benzene, reported by Rickert et al. (1979), as well as the data from which the model was calibrated (Sabourin et al. 1987). In general, the model tended to overestimate observations to which it was not specifically fitted.

Target Tissues.   The model simulates amounts and concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as amounts of metabolites formed. It does not simulate concentrations of metabolites in these tissues. It does not simulate bone marrow, a target of benzene metabolites.

Species Extrapolation.   The model has been applied to simulations of mice, rats, and humans (Medinsky et al. 1989a, 1989b, 1989c).

High-low Dose Extrapolation.   The model has been evaluated for simulating inhalation exposures in rodents ranging from 1 to 1,000 ppm and oral gavage doses ranging from 0.1 to 300 mg/kg (Bois et al. 1991b; Medinsky et al. 1989a, 1989b, 1989c).

Interroute Extrapolation.   The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes (Medinsky et al. 1989a, 1989b, 1989c).

Strengths and Limitations.   Strengths of the model are that it simulates disposition of inhaled and ingested (single dose) benzene, including rates and amounts of major metabolites formed in mice, rats, and humans. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) the model attributes all metabolism to the liver; (3) the model does not simulate the fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites (e.g., muconaldehyde) in tissues; and (4) the model does not simulate bone marrow, a major target tissue for benzene metabolites.

Sun et al. 1990

Description of the Model.   The Sun et al. (1990) model is an extension of the mouse and rat models developed by Medinsky et al. (1989a, 1989b, 1989c). The Sun et al. (1990) model includes a simulation of the formation of hemoglobin adducts derived from benzene oxide. Adduct formation is represented as the sum of capacity-limited and first-order functions of the concentration of benzene oxide in the liver. Parameter values were estimated by optimization to measurements of hemoglobin adduct formations in rats and mice exposed to single oral gavage doses of benzene (Sun et al. 1990).

Risk Assessment.   The model has been applied to predicting the levels of hemoglobin adducts in mice and rats following inhalation or oral exposures to benzene. This approach could be potentially useful for predicting exposure levels that correspond to measured hemoglobin adduct levels, for use of adducts as an exposure biomarker.

Validation of the Model.   The model was calibrated against measurements of hemoglobin adduct formation in mice and rats that received single oral gavage doses of benzene ranging from 0.008 to 800 mg/kg (Sun et al. 1990). The model was evaluated by comparing predictions to observations of amounts of hemoglobin adducts formed in mice and rats exposed to benzene vapor concentrations of 5, 50, or 600 ppm for 6 hours (Sabourin et al. 1989a).

Target Tissues.   The model predicts hemoglobin adduct formation after oral and inhalation exposure (Sun et al. 1990).

Species Extrapolation.   The model has been applied to simulations for mice and rats.

High-low Dose Extrapolation.   The model was calibrated with observations made in mice and rats exposed to single gavage doses ranging from 0.1 to 10,000 μmol/kg (0.008–800 mg/kg), and evaluated for predicting observations in mice and rats exposed by inhalation to 600 ppm benzene.

Interroute Extrapolation.   The Sun model examined two routes of exposure, oral and inhalation. The model was found to be useful in predicting the concentrations of hemoglobin adducts in blood in rodents after oral and inhalation exposure.

Strengths and Limitations.   Strengths of the model are that it extends the Medinsky et al. (1989a, 1989b, 1989c) models to simulate hemoglobin adduct formation secondary to formation of benzene oxide. A limitation of the adduct model is that it simulates production of adducts as a function of benzene oxide concentration in liver and does not consider other potential pathways of adduct formation through hydroquinone, phenol, or muconaldehyde.

Travis et al. 1990

Description of the Model.   The Travis et al. (1990) model simulates the absorption and disposition of benzene in the human, mouse, and rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process. Absorption and excretion of benzene in the lung are assumed to be flow-limited, as are exchanges of benzene between blood and tissues. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolic elimination of benzene as a function of the concentration of benzene in bone marrow and liver. The model simulates rates of metabolic elimination of benzene, but not the rates of formation of specific metabolites or their disposition (e.g., excretion). For the purpose of comparing model predictions to observations, 80% of the total metabolite formed in 24 hours (and excreted in urine) was assumed to be phenol. Metabolism parameter values (V_{max}, K_m) were estimated by optimization of the model to observations of total metabolites formed (i.e., excreted in urine) in humans, mice, and rats exposed to benzene by inhalation or oral routes to benzene. The V_{max} for metabolism in bone marrow in humans was assumed to be 4% of that of liver, consistent with optimized values for rodents.

Risk Assessment.   This model has been used to predict the amounts of benzene in expired air, concentrations of benzene in blood, and total amount of benzene metabolized following inhalation exposures to humans and inhalation, intraperitoneal, oral gavage, or subcutaneous exposures in mice or

rats (Travis et al. 1990). Cox (1996) applied the model to derive internal dose-response relationships for benzene in humans.

Validation of the Model.   The model was evaluated by comparing predictions with observations made in mouse and rat inhalation studies (Rickert et al. 1979; Sabourin et al. 1987; Sato et al. 1975; Snyder et al. 1981); mouse oral gavage studies (Sabourin et al. 1987); mouse subcutaneous injection studies (Andrews et al. 1977); and rat intraperitoneal injection studies (Sato and Nakajima 1979). Predictions of benzene in expired air and/or blood concentrations were also compared to observations made in humans who inhaled concentrations ranging from 5 to 100 ppm benzene (5 ppm: Berlin et al. 1980; Sherwood 1972; 25–57 ppm: Sato et al. 1975; Sherwood 1972; Nomiyama and Nomiyama 1974a, 1974b; 99–100 ppm: Sherwood 1972; Teisinger and Fiserova-Bergerova 1955). Further evaluations of predictions of benzene in workers are reported in Sinclair et al. (1999) and Sherwood and Sinclair (1999), who compared model predictions with observations of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations ranging from 1 to 1,100 ppm.

Target Tissues.   The model simulates amounts and concentrations of benzene in blood, bone marrow (a target tissue), liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues; and amounts of metabolites formed in liver and bone marrow. It does not simulate concentrations of metabolites in these tissues.

Species Extrapolation.   The model has been applied to simulations for mice, rats, and humans (Sherwood and Sinclair 1999; Sinclair et al. 1999; Travis et al. 1990).

High-low Dose Extrapolation.   The model has been evaluated for simulating inhalation exposures in humans ranging from 1 to 1,110 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999; Travis et al. 1990). Evaluations of predictions in rodents included observations made during inhalation exposures that ranged from 11 to 1,000 ppm and oral gavage doses that ranged from 0.5 to 300 mg/kg.

Interroute Extrapolation.   The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., benzene concentration in blood, amount benzene metabolized) resulting from exposures by these routes (Travis et al. 1990).

Strengths and Limitations.   Strengths of the model are that it simulates (1) disposition of inhaled and ingested (single dose) benzene in mice, rats, and humans; and (2) concentrations of benzene, and

rates and amount of benzene metabolized in bone marrow, a target tissue for benzene metabolites. Limitations of the model include: (1) the model simulates metabolic elimination of benzene, but not the rates of formation of major metabolites; and (2) the model does not simulate fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites in tissues.

Fisher et al. 1997

Description of the Model.   The Fisher et al. (1997) model extends the model reported by Travis et al. (1990) to include a simulation of lactational transfer of benzene to breast milk in humans. Other tissues simulated include blood, fat, liver, lung, other slowly-perfused tissues, and rapidly-perfused tissues. Absorption and excretion of benzene in the lung and exchange of benzene between blood and tissues are assumed to be flow-limited, as is excretion of benzene in breast milk. The lactational transfer model includes simulations of breast milk production and loss from nursing; the latter is represented as a first-order process. Estimates of blood:air and blood:milk partition coefficients during lactation (from which the milk:blood partition coefficient could be calculated) were measured in nine lactating subjects (Fisher et al. 1997). The model simulates capacity-limited (i.e., Michaelis-Menten) metabolism of metabolic elimination of benzene as a function of the concentration of benzene in liver. Rates of formation of specific metabolites and their disposition (e.g., excretion) are not simulated. Metabolism parameter values (K_m, V_{max}) and tissue:blood partition coefficients were derived from Travis et al. (1990).

Risk Assessment.   This model has been used to predict benzene concentrations in breast milk and lactational transfers to breast feeding infants (Fisher et al. 1997). Exposures to the threshold limit value (TLV) (10 ppm, 8 hours/day, 5 days/week) were predicted to yield 0.053 mg of benzene in breast milk per 24 hours. This approach has potential applicability to assessing lactational exposures to infants resulting from maternal exposures.

Validation of the Model.   The lactation model was evaluated (Fisher et al. 1997) by comparing predictions for perchloroethylene (not benzene) with those predicted by a perchloroethylene model developed by Schreiber (1993). Other components of the biokinetics model were derived from the Travis et al. (1990) model, which has undergone evaluations against data obtained from studies in humans.

Target Tissues.   The model simulates concentrations of benzene in blood, breast milk, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as rates of metabolic

elimination of benzene. It does not simulate concentrations of metabolites in these tissues and does not simulate metabolism in bone marrow, a major target of benzene metabolites.

Species Extrapolation.   The model has been applied to simulations for humans (Fisher et al. 1997).

High-low Dose Extrapolation.   The lactational model has not been evaluated for simulating inhalation exposures to benzene in humans; therefore, applicability to high-low dose extrapolations cannot be assessed.

Interroute Extrapolation.   The model was developed to simulate inhalation exposures. Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations.   Strengths of the model are that it simulates the disposition of inhaled benzene in females during lactation, including transfers of benzene to breast milk and nursing infants; concentrations of benzene in blood and tissues; and rates of metabolic elimination of benzene metabolized. Limitations of the model include that the model does not simulate rates of formation of major metabolites and that the model does not simulate kinetics of uptake or metabolism of benzene in bone marrow, a major target of benzene toxicity.

Sinclair et al. 1999

Description of the Model.   The Sinclair et al. (1999) model is an extension of the human model developed by Travis et al. (1990) to include a simulation of first-order urinary excretion of total metabolites and phenol, and dermal absorption of benzene.

Risk Assessment.   The model has been applied to predicting the levels of benzene in exhaled air and phenol in urine in workers exposed to benzene (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Validation of the Model.   The model was evaluated against measurements of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations ranging from 1 to 1,100 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Target Tissues.   The model simulates amounts and concentrations of benzene in blood, bone marrow (a target tissue), liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues; rates of metabolic elimination of benzene in liver and bone marrow; and excretion of total metabolites formed and phenol.

Species Extrapolation.   The model has been applied to simulations for humans (Sinclair et al. 1999).

High-low Dose Extrapolation.   The model was evaluated against observations of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations ranging from 1 to 1,100 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Interroute Extrapolation.   The model simulates inhalation, oral, and dermal exposures.

Strengths and Limitations.   Strengths of the model are that it extends the Travis et al. (1990) model to include simulation of dermal absorption of benzene.

Bois et al. 1991a

Description of the Model.   The Bois et al. (1991a) model simulates absorption and disposition of benzene and the benzene metabolite, phenol, in the rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene and phenol are simulated as a first-order function for dose. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene and phenol between blood and tissues. Excretion of phenol is simulated as a first-order transfer to urine. The model simulates capacity-limited (i.e., Michaelis-Menten) and first-order metabolism of benzene and metabolites in bone marrow, liver, gastrointestinal tract, and respiratory tract (see Table 3-6). All pathways are assumed to be capacity-limited reactions, except for the spontaneous hydrolysis of benzene oxide to form phenol, which is simulated as a first-order process. The model simulates rates of formation of metabolites and first-order excretion of phenol; however, disposition (e.g., excretion) of other metabolites is not simulated. Parameter values, including metabolism parameter values, were optimized to a reference set of observations of metabolites formed in rats exposed by inhalation or to single gavage doses of benzene (see below).

Risk Assessment.   This model has been used to predict amounts of benzene and phenol metabolites formed in rats during oral gavage exposures to benzene equivalent to those administered in NTP (1986) and to inhalation exposures equivalent to the OSHA PEL (Bois and Paxman 1992; Bois et al. 1991a). Model simulations indicate that dose rate may be an important factor in benzene toxicity. For example, when the model was applied to simulations for rats exposed either for 15 minutes to a benzene vapor concentration of 32 ppm or for 8 hours to 1 ppm (equivalent 8-hour TWAs), the amount of metabolites (hydroquinone, catechol, and muconaldehyde) formed was 20% higher after the 15-minute exposure at the higher level than after the 8-hour exposure at the lower level (Bois and Paxman 1992). These metabolites have been identified as being important in the genesis of bone marrow toxicity after benzene exposure (Eastmond et al. 1987). These types of analyses, if extended to humans, would be applicable to evaluations of the adequacy of short-term exposure limits.

Validation of the Model.   The model was calibrated (Bois and Paxman 1992; Bois et al. 1991a) with observations made in rats exposed to single oral gavage doses of benzene, or to inhalation exposures of 13–870 ppm (Sabourin et al. 1987, 1989b), in rats administered single parenteral doses of phenol (Cassidy and Houston 1984), and in *in vitro* metabolism studies (Sawahata and Neal 1983). Further evaluations against data not used in the calibration were not reported.

Target Tissues.   The model simulates amounts and concentrations of benzene and phenol in bone marrow, a target tissue for benzene metabolites, as well as in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues. The model also simulates amounts of specific metabolites formed and urinary excretion of the major urinary metabolite, phenol. It does not simulate concentrations of metabolites, other than phenol, in these tissues.

Species Extrapolation.   The model has been applied to simulations for rats. A human model has been developed that implements a scaled-down version of the rat metabolism model (see Bois et al. 1996).

High-low Dose Extrapolation.   The model has been evaluated for simulating inhalation exposures in rats ranging from 13 to 870 ppm and oral gavage doses ranging from 15 to 300 mg/kg.

Interroute Extrapolation.   The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes.

Strengths and Limitations.   Strengths of the model are that it simulates disposition of inhaled and ingested benzene (and phenol), including rates and amounts of most of the major metabolites formed in rats. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) although the model simulates the fate of benzene and phenol, it does not simulate the fate of other metabolites formed and, therefore, cannot be used to predict concentrations of these metabolites in tissues; and (3) the model, as configured in Bois et al. (1991a), does not simulate benzene disposition in humans.

Bois et al. 1996

Description of the Model.   The Bois et al. (1996) model simulates inhalation absorption and disposition of benzene in humans. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene between blood and tissues. The model simulates metabolic elimination of benzene as a single capacity-limited (i.e., Michaelis-Menten) reaction, occurring in bone marrow and liver. Endogenous formation of phenolic metabolites is also simulated as a zero-order process occurring in liver. The model simulates first-order excretion of total metabolites and the phenol fraction (approximately 80% of total). Parameter values (physiological and chemical) were estimated by Bayesian optimization techniques (Markov Chain Monte Carlo analysis) using reference observations of benzene concentration in blood and urinary excretion of phenol in human subjects who were exposed to benzene in air (Pekari et al. 1992).

Risk Assessment.   The model has been used to predict rates and amounts of benzene metabolized in human populations (Bois et al. 1996). The population model (population geometric means and standard deviations of parameter values) was derived using Markov Chain Monte Carlo analysis with observations from three human subjects serving as the reference data for inter-individual variability (from Pekari et al. 1992). The population model predicts probability distributions of model outputs (for example, rates or amounts of benzene metabolized for a given exposure). This approach could be used to evaluate uncertainty factors in risk assessments intended to account for uncertainties in our understanding of benzene pharmacokinetics variability.

Validation of the Model.   The model was calibrated with observations of benzene concentrations in blood and urinary phenol levels, made in three human subjects who were exposed to 1.7 or 10 ppm

benzene for 4 hours (Pekari et al. 1992). Further evaluations against data not used in the calibration have not been reported.

Target Tissues.   The model simulates amounts and concentrations of benzene in bone marrow, a target of benzene toxicity, as well as blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues. Amounts of total metabolites formed and excreted are simulated; however, the model does not simulate concentrations of metabolites in these tissues.

Species Extrapolation.   The model has been applied to simulations for humans (Bois et al. 1996).

High-low Dose Extrapolation.   The model has been evaluated for simulating inhalation exposures in human subjects ranging from 1.7 to 10 ppm (Bois et al. 1996).

Interroute Extrapolation.   The model simulates inhalation exposures. Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations.   Strengths of the model are that it simulates disposition of inhaled benzene and rates of total metabolism in humans. Limitations include that the model has not been evaluated for multiple exposures and that the model simulates total metabolism of benzene, and not the rates of formation of the major metabolites of benzene of toxicological interest.

Brown et al. 1998

Description of the Model.   The Brown et al. (1998) model simulates inhalation absorption and disposition of benzene in humans. Tissues simulated include the blood, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Absorption and excretion of benzene in the lung and exchange of benzene between blood and tissues are assumed to be flow-limited. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolic elimination of benzene as a function of the concentration of benzene in liver. Rates of formation of specific metabolites, or their disposition (e.g., excretion), are not simulated. For the purpose of comparing model predictions to observations, 80% of the total metabolites formed and excreted in urine (i.e., amount of benzene eliminated by metabolism) in 24 hours was assumed to be phenol. The K_m parameter for metabolism was derived from Travis et al. 1990; the V_{max} was estimated by optimization of the model to observations of blood concentrations of

benzene and benzene in exhaled breath of female and male subjects who were exposed to 25 ppm benzene for 2 hours (Sato et al. 1975). Partition coefficients for males and females were derived from vial equilibrium studies conducted on blood and/or tissues from males and females (Fisher et al. 1997; Paterson and Mackay 1989).

Risk Assessment.   This model has been used to predict the benzene concentrations in blood and amounts of benzene metabolized in females and males who experience the same inhalation exposure scenarios. Females were predicted to metabolize 23–26% more benzene than similarly-exposed males. This difference was attributed, in part, to a higher blood:air partition coefficient for benzene in females.

Validation of the Model.   The model was calibrated by comparing predictions of blood concentrations of benzene and benzene in exhaled breath of female and male subjects who were exposed to 25 ppm benzene for 2 hours (Brown et al. 1998; Sato et al. 1975). Further evaluations against data not used in the calibration were not been reported.

Target Tissues.   The model simulates concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as rates of metabolic elimination of benzene. It does not simulate concentrations of metabolites in these tissues and does not simulate metabolism in bone marrow, a major target of benzene metabolites.

Species Extrapolation.   The model has been applied to simulations for humans.

High-low Dose Extrapolation.   The model has been evaluated for simulating inhalation exposures in humans. Evaluations of predictions included observations made during inhalation exposures to 25 ppm (Brown et al. 1998; Sato et al. 1975).

Interroute Extrapolation.   The model simulates inhalation and has been applied to predicting internal dose metrics (e.g., benzene concentration in blood, amount benzene metabolized) resulting from exposures by this route (Brown et al. 1998). Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations.   Strengths of the model are that it simulates disposition of inhaled benzene in female and male humans as well as the concentrations of benzene and rates of metabolic elimination of benzene metabolized. Limitations of the model include: (1) the model does not simulate

rates of formation of major benzene metabolites; (2) the model does not simulate fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites in tissues; and (3) the model does not simulate kinetics of uptake or metabolism of benzene in bone marrow, a major target of benzene toxicity.

Cole et al. 2001

Description of the Model.   The Cole et al. (2001) model simulates absorption and disposition of benzene in the mouse. Tissues simulated include the blood, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene between blood and tissues. The model simulates capacity-limited (i.e., Michaelis-Menten) and first-order metabolism of benzene and metabolites in liver (see Table 3-6). Capacity-limited reactions in bone marrow and liver include benzene to benzene oxide, phenol to hydroquinone, phenol to catechol, catechol to trihydroxybenzene, and conjugation of phenol and hydroquinone. First-order reactions in liver include conversion of benzene oxide to phenol, muconic acid, and phenylmercapturic acid. The model simulates rates of formation of metabolites, tissue distribution of benzene oxide, phenol, and hydroquinone; and first-order excretion of metabolites in urine. Capacity-limited metabolism parameter values were estimated from *in vitro* studies of mouse liver (Lovern et al. 1999; Nedelcheva et al. 1999; Seaton et al. 1995); first-order parameters were estimated by optimization of model output to observations of metabolites formed in mice exposed by inhalation or to single gavage doses (Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988). Blood:tissue partition coefficients for benzene and metabolites were derived from Medinsky et al. (1989a) or estimated based on the n-octanol-water partition coefficient (Poulin and Krishnan 1995).

Risk Assessment.   This model has been used to predict amounts of benzene exhaled and amounts of benzene metabolites produced in mice during inhalation exposures or following oral gavage exposures to benzene (Cole et al. 2001).

Validation of the Model.   The model was calibrated with observations made in mice exposed to single oral gavage doses of benzene, or to inhalation exposures (Cole et al. 2001; Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988). Further evaluations against data not used in the calibration have not been reported.

Target Tissues.   The model simulates amounts and concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly perfused and slowly perfused tissues; rates of formation of metabolites; tissue distribution of benzene oxide, phenol, and hydroquinone; and first-order excretion of metabolites in urine. It does not simulate concentrations of metabolites in bone marrow, a target tissue for benzene metabolites.

Species Extrapolation.   The model has been applied to simulations for mice (Cole et al. 2001).

High-low Dose Extrapolation.   The model has been evaluated for simulating inhalation exposures in mice (50 ppm) and oral gavage doses ranging from 0.1 to 100 mg/kg (Cole et al. 2001; Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988).

Interroute Extrapolation.   The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes (Cole et al. 2001).

Strengths and Limitations.   Strengths of the model are that it simulates disposition of inhaled and ingested benzene, including rates and amounts of major metabolites. Most of the metabolism parameter values were derived empirically from *in vitro* studies, rather than by model optimization. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) the model does not simulate the metabolism of benzene in bone marrow, a major target of benzene toxicity; (3) the model, as configured in Cole et al. (2001), does not simulate benzene disposition in humans.

3.5   MECHANISMS OF ACTION

3.5.1   Pharmacokinetic Mechanisms

Benzene is readily absorbed via all natural routes of exposure (inhalation, oral, and dermal) and distributed throughout the body via the blood. Based on physical properties such as slight water solubility, high lipid solubility, and nonpolarity, benzene is expected to enter the blood via passive diffusion from gut, lungs, and skin. Benzene is expected to readily bind to plasma proteins (Travis and Bowers 1989). Being moderately lipophilic, benzene tends to accumulate in fatty tissues. However, benzene metabolism is relatively rapid and required for hematopoietic and leukemogenic effects to be expressed. Multiple reactive metabolites appear to be involved in benzene toxicity. As discussed in detail in Section 3.5.2, potential candidates include benzene oxide, phenolic metabolites (phenol,

catechol, hydroquinone, 1,2,4-benzenetriol, and 1,2- and 1,4-benzoquinone), and *trans,trans*-muconalde-hyde. Both human and animal data demonstrate the importance of CYP2E1 in benzene metabolism (see Section 3.4.3 for a detailed discussion). Metabolism is assumed to take place primarily in the liver, with some secondary metabolism in the bone marrow, the site of characteristic benzene toxicity. Processes involved in transport of hepatic metabolites of benzene to the critical toxicity target (bone marrow) are not known, although some degree of covalent binding of reactive benzene metabolites to blood proteins is expected. At relatively low exposure levels, urinary excretion of conjugated benzene derivatives represents the major excretory pathway for benzene. Biliary excretion represents a minor excretory pathway.

3.5.2   Mechanisms of Toxicity

Numerous mechanistic studies have been conducted in an effort to elucidate mechanisms of benzene-induced hematotoxic and leukemogenic effects, widely recognized as the most critical effects of benzene exposure. Conversely, benzene-induced effects on reproduction, development, and the nervous system have not been studied in sufficient detail to assess mechanisms of toxicity for these end points. The database of information for benzene-induced hematotoxic and leukemogenic effects has been reviewed extensively (e.g., Bird et al. 2005; Irons 2000; Morgan and Alvares 2005; Ross 1996, 2000, 2005; Schnatter et al. 2005; Smith 1996a, 1996b; Snyder 2000a, 2000b, 2002; Snyder and Hedli 1996; Snyder and Kalf 1994). It is generally believed that reactive hepatic metabolites of benzene are transported to the major toxicity target (bone marrow). Additional metabolism likely occurs in bone marrow. Phenolic metabolites (phenol, hydroquinone, catechol, 1,2,4-benzenetriol, and 1,2- and 1,4-benzoquinone) appear to play a major role in benzene toxicity. Smith (1996a, 1999b) noted that the phenolic metabolites can be metabolized by bone marrow peroxidases, such as myeloperoxidase (MPO), to highly reactive semiquinone radicals and quinones that stimulate the production of reactive oxygen species. These steps lead to damage to tubulin, histone proteins, topoisomerase II, other DNA associated proteins, and DNA itself (clastogenic effects such as strand breakage, mitotic recombination, chromosome translocations, and aneuploidy). Damage to stem or early progenitor cells would be expressed as hematopoietic and leukemogenic effects.

Results of several mechanistic studies demonstrate that benzene hematotoxicity is dependent upon metabolism (see Section 3.4.3 for a detailed discussion). Inhibition of benzene metabolism reduced its toxicity (Andrews et al. 1977). Partial hepatectomy decreased both benzene metabolism and toxicity (Sammett et al. 1979). Pretreatment with inducers of metabolism increased both benzene metabolism and

toxicity (Gad-El-Karim et al. 1985, 1986). Inhibition of CYPs (enzymes that catalyze oxidation pathways in benzene metabolism) reduced benzene-induced genotoxicity (Tuo et al. 1996). Mice lacking CYP2E1 or microsomal epoxide hydrolase expression were not susceptible to benzene levels known to cause myelotoxicity and cytotoxicity in wild type mice (Bauer et al. 2003; Valentine et al. 1996a, 1996b). Occupationally-exposed workers with a phenotype corresponding to rapid CYP2E1 metabolism were more susceptible to benzene toxicity than those expressing slow CYP2E1 metabolism (Rothman et al. 1997). The enzyme NAD(P)H:quinone oxidoreductase (NQ01), which maintains quinones in reduced form where they are more readily conjugated and excreted (Nebert et al. 2002), is another example of the importance of metabolism in benzene hematotoxicity. Between 22% (Caucasian) and 45% (Asian) of the population is homozygous for an NQ01 allele whereby NQ01 production is negligible. Rothman et al. (1997) found that workers homozygous for an NQ01 allele whereby NQ01 production is negligible (wild type) exhibited a 2.4-fold increased risk for benzene hematotoxicity than workers with the normal genotype. Greater than 7-fold increased risk of benzene hematotoxicity was noted in workers who expressed both rapid CYP2E1 metabolism and the NQ01 wild type (Rothman et al. 1997).

Benzene metabolism involves the production of reactive metabolites that may act directly on cellular macromolecules (proteins and DNA). No single metabolite has been implicated; effects are probably due to many metabolites, which include benzene oxide, reactive products of the phenol pathway (catechol, hydroquinone, and 1,4-benzoquinone), and *trans,trans*-muconaldehyde. Evidence that benzene oxide may play a role in benzene toxicity includes findings that benzene oxide is a product of oxidative benzene metabolism in mouse, rat, and human liver microsomes (Lovern et al. 1997), benzene oxide can be released from the liver into the blood (Lindstrom et al. 1997), benzene oxide-protein adducts have been found in the blood and bone marrow of mice exposed to benzene (McDonald et al. 1994), and benzene oxide hemoglobin and albumin adducts have been detected in the blood of workers exposed to benzene (Rappaport et al. 2002a, 2002b; Yeowell-O'Connell et al. 1998).

Urinary *trans,trans*-muconic acid has been detected in humans and animals following benzene exposure, although its purported reactive precursor (*trans,trans*-muconaldehyde; see Figure 3-3) has not been detected *in vivo*. The highly reactive *trans,trans*-muconaldehyde, which has been found in mouse hepatic microsomes (Latriano et al. 1986), can undergo reductive and oxidative metabolism (Goon et al. 1992) and has been shown to be hematotoxic (Witz et al. 1985). A small amount (<0.05%) of parenterally-administered *trans,trans*-muconaldehyde to mice reached the bone marrow (Zhang et al. 1997). Rivedal and Witz (2005) found *trans,trans*-muconaldehyde to be a strong inhibitor of gap junction intercellular communication in rat liver epithelial cells.

Phenolics (phenol, catechol, hydroquinone; 1,2,4-benzenetriol; 1,2- and 1,4-benzoquinone) are major metabolites of benzene that have been shown to persist in bone marrow following inhalation exposure (Rickert et al. 1979; Sabourin et al. 1988). Hydroquinone induces chromosomal damage in lymphocytes *in vitro* in a manner similar to that observed in lymphocytes of benzene-exposed workers (Eastmond et al. 1994; Stillman et al. 1997; Zhang et al. 1998b). Kolachana et al. (1993) demonstrated that both hydroquinone and 1,2,4-benzenetriol cause oxidative damage to DNA in mouse bone marrow (*in vivo*) and human myeloid cells (*in vitro*). Glutathione adducts of 1,4-benzoquinone have been shown to be hematotoxic in bone marrow of mice exposed to benzene (Bratton et al. 1997). Additional evidence that phenolic metabolites may play an important role in benzene toxicity includes the finding that MPO, an enzyme found in high concentration in bone marrow (Bainton et al. 1971), catalyzes the oxidation of polyphenols to reactive quinones, semiquinones, and oxygen radicals (Nebert et al. 2002). This can lead to strand breaks and inhibition of topoisomerases and microtubule assembly, which could result in chromosome damage (Chen and Eastmond 1995; Eastmond et al. 2001; Irons and Neptune 1980; Smith 1996a, 1996b). Eastmond et al. (2005) demonstrated that hydroquinone can be readily activated to a potent topoisomerase II inhibitor in the presence of human MPO and H_2O_2 and that partial inhibition occurs at hydroquinone concentrations as low as 50 nM. Irons and Neptune (1980) suggested that benzene-derived hydroquinone may inhibit cell replication by covalently binding to tubulin, a protein essential for spindle formation in mitosis.

Benzene-induced effects on DNA have been studied in some detail. Schwartz et al. (1985) demonstrated that benzene metabolites inhibit mitochondrial DNA polymerase. Hydroquinone has been shown to inhibit ribonucleotide reductase, a key step in DNA synthesis (Li et al. 1997, 1998). Rushmore et al. (1984) found that benzene metabolites covalently bind to mitochondrial DNA and inhibit RNA synthesis. Benzene metabolites bound to hepatic DNA have been observed in animals following inhalation exposure to radiolabeled benzene (Lutz and Schlatter 1977). However, the reported levels of DNA adduct formation appear to be low. For example, Creek et al. (1997) observed adducts to both protein and DNA in the range of nanograms per kilogram in mice given radiolabeled benzene.

Benzene-induced DNA damage may result from the oxidation of DNA by reactive oxygen species that are produced during benzene metabolism. Both 1,4-benzoquinone and hydroquinone are known to increase superoxide, nitric oxide, and hydrogen peroxide in HL-60 cells (Rao and Snyder 1995). Chen et al. (2004) observed nitric oxide-derived benzene metabolites, namely nitrobenzene, nitrophenyl, and nitrophenol isomers in the bone marrow of mice 1 hour following intraperitoneal injection of a 400 mg/kg

dose of benzene. These nitro metabolites were either not detected in other tissues or were present in much smaller concentrations, indicating that they were most likely produced in bone marrow. Brunmark and Cadenas (1988) described a metabolic pathway from hydroquinone leading to the production of glutathionyl-benzenetriol, which can undergo autoxidation leading to superoxide formation. Rao (1996) suggested that benzene-induced DNA damage is mediated by the release of free iron in the bone marrow (probably by polyphenolic metabolites), followed by the chelation of iron by hydroquinone or benzenetriol to yield a reactive oxygen-generating species such as superoxide, which causes oxidative damage to DNA.

The expression of benzene toxicity may involve multiple benzene metabolites. All of the known unconjugated metabolites of benzene, with the exception of phenol and 1,2,4-benzenetriol, have been shown to decrease erythropoiesis (Snyder and Hedli 1996). In mice, the combination of phenol and hydroquinone resulted in exacerbated loss of bone marrow cellularity (Eastmond et al. 1987), increased peroxidatic activation of hydroquinone (Subrahmanyam et al. 1989, 1990), and increased DNA damage (Lévay and Bodell 1992; Marrazzini et al. 1994). Combinations of either phenol and hydroquinone, or phenol and catechol, were more hematotoxic than any of the metabolites given alone (Guy et al. 1991). The combination of hydroquinone and muconaldehyde was the most potent in inhibiting erythropoiesis (Snyder et al. 1989). Catechol was found to stimulate the peroxidase-mediated activation of hydroquinone and produced a synergistic genotoxic effect in lymphocytes (Robertson et al. 1991).

3.5.3   Animal-to-Human Extrapolations

Pathways of benzene metabolism are generally similar among various rodent and nonhuman primate species. However, species differences exist regarding capacity to metabolize benzene and relative proportions of various benzene metabolites formed.

Species differences exist in absorption and retention of benzene. For example, following 6-hour exposures to low concentrations (7–10 ppm) of benzene vapors, mice retained 20% of the inhaled benzene, whereas rats and monkeys retained only 3–4% (Sabourin et al. 1987, 1992). Mice exhibit a greater overall capacity to metabolize benzene, compared to rats. An Inhalation exposure to 925 ppm resulted in an internal dose of 152 mg/kg in mice, approximately 15% of which was excreted as parent compound, and an internal dose of 116 mg/kg in rats, approximately 50% of which was excreted unchanged (Henderson et al. 1992; Sabourin et al. 1987).

The proportions of benzene metabolites produced depend on both species and exposure concentration. Hydroquinones and muconic acid (potential sources of benzene toxicity) were detected in much higher concentrations in the blood, liver, lung, and bone marrow of mice than rats, following a 6-hour inhalation exposure to benzene at a concentration of 50 ppm (Sabourin et al. 1988). It is generally understood that metabolic profiles of benzene in mice and humans are more similar than those of humans and rats. Sabourin et al. (1989a) noted increased production of detoxification metabolites (phenylglucuronide and prephenylmercapturic acid) and decreased production of potentially toxic metabolites (hydroquinones and muconic acid) in both mice and rats exposed to benzene at much higher concentrations (600 ppm in air or 200 mg/kg orally), which indicates that extrapolation of toxicological results from studies using high exposure concentrations to low exposure scenarios may result in an underestimation of risk.

Recent PBPK models have tried to address benzene metabolism in an effort to derive animal-to-human extrapolations (Bois et al. 1991a, 1996; Cole et al. 2001; Medinsky 1995; Medinsky et al. 1989a, 1989b, 1989c; Travis et al. 1990). Each model described a multicompartmental model that attempted to relate the generation of metabolites to end points of benzene toxicity. The generation of hydroquinone and muconaldehyde in the liver, with further metabolism in the bone marrow, has been addressed as well as the available data allow. However, the models are not sufficiently refined to allow them to accurately predict human metabolism.

3.6   TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to

the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active

chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist

in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens

(Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are

similar in structure and action to endogenous estrogen. Although the public health significance and

descriptive terminology of substances capable of affecting the endocrine system remains controversial,

scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or

elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction,

development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that

are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering,

for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought

to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994;

Giwercman et al. 1993; Hoel et al. 1992).

No information was located to indicate that benzene may adversely affect the endocrine system.

3.7   CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to

maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential

effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect

effects on the fetus and neonate resulting from maternal exposure during gestation and lactation.

Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their

susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the

extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is

a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less

susceptible than adults to health effects, and the relationship may change with developmental age

(Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are

critical periods of structural and functional development during both prenatal and postnatal life, and a

particular structure or function will be most sensitive to disruption during its critical period(s). Damage

may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

No clear evidence of age-related differences in susceptibility to benzene toxicity was located. Benzene crosses the placenta and can be found in cord blood at concentrations that equal or exceed those of maternal blood (Dowty et al. 1976). Nursing infants can be exposed to benzene in the breast milk (Fabietti et al. 2004). Limited animal studies indicate that *in utero* exposure to benzene results in hematological changes similar to those observed in animals exposed only as adults (Corti and Snyder 1996; Keller and Snyder 1986, 1988). There is some indication that parental occupational exposure to benzene may play a role in childhood leukemia (Buckley et al. 1989; McKinney et al. 1991; Shaw et al.

1984; Shu et al. 1988). However, none of these studies indicate whether children may be at greater risk
than adults for benzene toxicity. Results of Infante-Rivard et al. (2005) indicate that maternal exposure to
benzene during pregnancy or from 2 years before pregnancy up to birth does not result in increased risk of
childhood acute lymphoblastic leukemia (ALL), a frequent form of childhood cancer (Infante-Rivard et
al. 2005). However, no information was located regarding the risk of childhood AML, the form of
leukemia most frequently associated with exposure to benzene.

Children could potentially be at increased risk for significant benzene exposure via the inhalation route
based on higher activity levels and ventilation rates than adults. However, no information was located to
indicate that children are at increased risk for benzene toxicity. Age-related differences in benzene
metabolism could potentially affect susceptibility. Results of one human study indicate that CYP2E1, a
major enzyme involved in benzene metabolism, is not present in the fetus, but appears in rapidly
increasing concentrations during early postnatal development (Vieira et al. 1996). This suggests that
fetuses and neonates may be at decreased risk of benzene toxicity due to a reduced metabolic capacity.
No information was located regarding potential age-related differences in pharmacodymanic processes
such as benzene-target interactions in the hematopoietic system.

3.8   BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have
been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC
1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers
as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic
substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target
molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The
preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in
readily obtainable body fluid(s), or excreta. However, several factors can confound the use and
interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures
from more than one source. The substance being measured may be a metabolite of another xenobiotic
substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic
compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental
conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the

body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to benzene are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by benzene are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

3.8.1   Biomarkers Used to Identify or Quantify Exposure to Benzene

Several biomarkers of exposure to benzene have been reported in the literature. Unmetabolized benzene can be detected in the expired air and urine of humans exposed to benzene vapors (Farmer et al. 2005; Fustinoni et al. 2005; Ghittori et al. 1993; Nomiyama and Nomiyama 1974a, 1974b; Sherwood 1988; Srbova et al. 1950; Waidyanatha et al. 2001). Urinary phenol measurements have routinely been used for monitoring occupational exposure to benzene (OSHA 1987), and urinary phenol levels appear to be correlated with exposure levels (Astier 1992; Inoue et al. 1986, 1988b; Karacic et al. 1987; Pagnotto et al. 1961; Pekari et al. 1992).

Urinary *trans,trans*-muconic acid has been widely studied as a biomarker of exposure to benzene (Boogaard and van Sittert 1995, 1996; Ducos et al. 1990, 1992; Inoue et al. 1989b, 2000; Lee et al. 1993; Melikian et al. 1993, 1994; Pezzagno et al. 1999; Popp et al. 1994; Qu et al. 2005; Rothman et al. 1998; Ruppert et al. 1997; Sanguinetti et al. 2001; van Sittert et al. 1993; Weaver et al. 2000). Urinary *S*-phenylmercapturic acid levels have also been correlated with occupational exposure to benzene

(Boogaard and van Sittert 1995, 1996; Farmer et al. 2005; Inoue et al. 2000; Jongeneelen et al. 1987; Popp et al. 1994; Qu et al. 2005). Significant exposure-response trends for urinary *trans*,*trans*-muconic acid and *S*-phenylmercapturic acid levels have been demonstrated in occupationally-exposed subjects at exposure levels of ≤1 ppm (Qu et al. 2005). The American Conference of Governmental Industrial Hygienists (ACGIH) has established 25 μg *S*-phenylmercapturic acid/g creatinine in the urine and 500 μg *trans*,*trans*-muconic acid/g creatinine in the urine as Biological Exposure Indices (BEIs) for benzene exposure in the workplace (ACGIH 2006). The BEI is primarily an index of exposure and not a level at which health effects might occur from exposure to benzene. Positive correlations have been made between benzene workplace air levels and urinary catechol and hydroquinone in exposed workers (Inoue et al. 1988a, 1988b; Rothman et al. 1998).

Hemoglobin and albumin adducts of the benzene metabolites, benzene oxide and 1,4-benzoquinone, have been used as biomarkers of exposure to benzene (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Smith and Rothman 2000; Yeowell-O'Connell et al. 1998, 2001). Furthermore, DNA adducts with benzene metabolites have been found after benzene exposure (Hedli et al. 1991; Lutz and Schlatter 1977; Reddy et al. 1989).

Ong et al. (1995) evaluated various biomarkers of benzene exposure for their relationship with environmental benzene levels. Muconic acid in the urine correlated best with environmental benzene concentrations. Urinary hydroquinone levels were the most accurate biomarker of exposure for the phenolic metabolites of benzene, followed by phenol and catechol. No correlation was found between environmental benzene levels and unmetabolized benzene in the urine, although other studies suggest that benzene in the urine may be a useful biomarker of occupational exposure (Ghittori et al. 1993).

The biomarkers discussed in the preceding paragraphs appear to be adequate indicators of exposure to benzene at relatively high occupational exposure levels, and may serve as biomarkers in acute exposure scenarios involving relatively high levels of benzene. However, some of these biomarkers do not appear to be reliable indicators of environmental exposure to benzene (concentrations below the common industrial standard of 1 ppm TWA). For example, results from data collected on 152 chemical workers showed a linear relationship between the concentration of benzene in breathing zone air (when greater than 10 ppm) and urinary concentrations of catechol and hydroquinone (Inoue et al. 1988a). Workers who had an average work-site exposure of 10 ppm benzene showed no significant differences in the concentration of urinary catechol or hydroquinone when compared to a group of unexposed subjects. In a study of pharmacy workers exposed to benzene levels measured in the parts per billion (ppb) range, there

was no significant difference in urinary levels of *trans,trans*-muconic acid between subjects exposed to 1.5 ppb and those exposed to 2.5 ppb (Sanguinetti et al. 2001). Recent reports indicate that urinary benzene may serve as the most sensitive biomarker of exposure to benzene concentrations well below 1 ppm (Farmer et al. 2005; Fustinoni et al. 2005).

Several additional factors must be taken into account when assessing the reliability of biomarkers of exposure to benzene. High and variable background levels of phenol and its metabolites result from ingestion of vegetables, exposure to other aromatic compounds, ingestion of ethanol, and inhalation of cigarette smoke (Nakajima et al. 1987). Relatively high urinary phenol levels (5–42 mg/L) have been found in persons with no known exposure to benzene (NIOSH 1974). Although muconic acid is used as a marker for benzene exposure, muconic acid in the urine can also result from ingestion of sorbic acid, a common food preservative (Ducos et al. 1990). Inoue et al. (1989b) suggested that individual urinary *trans,trans*-muconic acid content was not a useful index of benzene exposure due to large variations in measured individual background urinary *trans,trans*-muconic acid values.

In summary, several benzene metabolites may serve as biomarkers of exposure to benzene. Urinary benzene appears to be the most sensitive biomarker for low-level exposure to benzene. Refer to Tables 7-1 and 7-2 for information regarding analytical methods for determining benzene in biological samples and benzene metabolites in urine.

3.8.2   Biomarkers Used to Characterize Effects Caused by Benzene

In addition to using levels of benzene and benzene metabolites for monitoring purposes, various biological indices might also be helpful in characterizing the effects of exposure to benzene. As with monitoring for benzene exposure, monitoring for effects may best be accomplished through the use of a series of biomarkers with correlation of the results. Decreases in erythrocyte and leukocyte counts have been used as an indicator of high occupational exposures. Monitoring of benzene workers has included monthly blood counts, with workers being removed from areas of high benzene exposure when leukocyte counts fell below 4,000/mm^3 or erythrocyte counts fell below 4,000,000/mm^3 (ITII 1975; OSHA 1987). Hayes (1992) indicates that benzene-related leukopenia, commonly thought of as an intermediate end point in the process of developing benzene-related leukemia, is considered a biomarker of benzene poisoning in China, not necessarily related to leukemia. Leukocyte alkaline phosphatase (LAP) activity was increased in benzene workers exposed to about 31 ppm for a chronic time period (Songnian et al. 1982). Increased LAP activity is an indicator of myelofibrosis and is associated with both decreased

white blood cell counts and changes in bone marrow activity. The change in LAP activity could be used in the diagnosis of benzene poisoning since it was more sensitive than the change in the leukocyte count, although it is not a biomarker that is specific for benzene. Additionally, it seems reasonable that chromosomal aberrations in bone marrow and peripheral blood lymphocytes and sister chromatid exchanges could be used to monitor for benzene effects (Eastmond et al. 1994; Van Sittert and de Jong 1985). Benzene metabolites have also been found to form adducts with DNA (Chenna et al. 1995; Lutz and Schlatter 1977; Norpoth et al. 1988; Rushmore et al. 1984; Snyder et al. 1987).

Exposure to benzene causes toxic effects in the bone marrow via its metabolites and possibly by benzene via solvent effects (Eastmond et al. 1987; Gad-El-Karim et al. 1985; Hedli et al. 1991; Irons et al. 1980). Therefore, it is possible that hematological tests could be used as markers of hematotoxicity. To date, surveillance and early diagnosis of benzene hematotoxicity rely primarily on the complete blood count, including hemoglobin, hematocrit, erythrocyte count, leukocyte count, and differential and platelet counts. In effect, complete blood counts and marrow exams should be good for early detection of preleukemic lesions. Additionally, cytogenetic tests of marrow cells are being used. Workers exposed to benzene in the air have shown elevated levels of delta-aminolevulinic acid in erythrocytes and elevated coproporphyrin in the urine (Kahn and Muzyka 1973). These may be biomarkers for disruption of porphyrin synthesis and may be early indicators of adverse hematological effects. These effects are not specific for benzene. Hedli et al. (1991) observed that in rats, benzene metabolite-DNA adducts were observed in the bone marrow at doses that did not affect bone marrow cellularity, and suggested that monitoring of the bone marrow DNA adducts might be a sensitive bioassay of genotoxic effects of benzene exposure. Work by other researchers also suggests that monitoring DNA adducts or products of DNA damage might be useful (Bodell et al. 1993; Chen et al. 1994; Lagorio et al. 1994a; Reddy et al. 1989).

3.9   INTERACTIONS WITH OTHER CHEMICALS

Studies have been conducted on the interaction of benzene with other chemicals, both *in vivo* and in the environment. Benzene metabolism is complex, and various xenobiotics can induce or inhibit specific routes of detoxification and/or activation in addition to altering the rate of benzene metabolism and clearance from the body. Toluene, Aroclor 1254, phenobarbital, acetone, and ethanol are known to alter the metabolism and toxicity of benzene. Interactions reported in *in vivo* studies occurred at relatively high benzene exposure levels, which would not likely be encountered near hazardous waste sites.

Ethanol and benzene induce the formation of the hepatic cytochrome P-450 isoenzyme, CYP2E1, in rabbits and rats (Gut et al. 1993; Johansson and Ingelman-Sundberg 1988), although benzene derivatives, such as toluene and xylene, can inhibit the enzymatic activity of the isozyme (Koop and Laethem 1992). Ethanol enhances both the metabolism (*in vitro*) and the toxicity (*in vivo*) of benzene in animals (Baarson et al. 1982; Nakajima et al. 1985). Administration of ethanol (5 or 15% in drinking water, 4 days/week for 13 weeks) to mice exposed to benzene vapors at a concentration of 300 ppm, 6 hours/day, 5 days/week for 13 weeks) resulted in greater severity of benzene-induced hematological effects (anemia, lymphocytopenia, bone marrow aplasia, transient increases in normoblasts and peripheral blood atypia) relative to benzene-exposed mice not given ethanol (Baarson et al. 1982). The modulating effects of benzene were dose dependent. The enhancement of the hematotoxic effects of benzene by ethanol may be of particular concern for benzene-exposed workers who consume alcohol (Nakajima et al. 1985), although the interactions demonstrated in the mice occurred at much higher benzene exposure concentrations than would likely be experienced in workplace air. Benzene can interfere with the disappearance of ethanol from the body. Accordingly, increased central nervous system disturbances (e.g., depression) may occur following concurrent exposure to high levels of benzene and ethanol.

Other chemicals that induce specific isoenzymes of cytochrome P-450 can increase the rate of benzene metabolism and may alter metabolism pathways favoring one over another. Ikeda and Ohtsuji (1971) presented evidence that benzene hydroxylation was stimulated when rats were pretreated with phenobarbital and then exposed to 1,000 ppm of benzene vapor for 8 hours/day for 2 weeks. Additionally, phenobarbital pretreatment increased the rate of metabolism by 40% in rats and 70% in mice (Pawar and Mungikar 1975). In contrast, rats exposed to phenobarbital showed no effects on the metabolism of micromolar amounts (35–112.8 μmol) of benzene *in vitro* (Nakajima et al. 1985).

Co-administration of toluene inhibited the biotransformation of benzene to phenol in rats (Ikeda et al. 1972; Inoue et al. 1988b). This was due to competitive inhibition of the oxidation mechanisms involved in the metabolism of benzene. Phenobarbital pretreatment of the rats alleviated the suppressive effect of toluene on benzene hydroxylation by the induction of oxidative activities in the liver. This effect has been observed in other studies in rats (Purcell et al. 1990).

Mathematical models of benzene and phenol metabolism suggest that the inhibition by benzene of phenol metabolism, and by phenol on benzene metabolism, occurs through competition for a common reaction site, which can also bind catechol and hydroquinone (Schlosser et al. 1993). Flavonoids have been shown

to inhibit phenol hydroxylase or increase phenol hydroxylase activity in a dose-dependent manner, dependent on the oxidation potential of the flavonoid (Hendrickson et al. 1994).

SKF-525A and carbon monoxide are classic inhibitors of cytochrome P-450s. The binding between P-450 and carbon monoxide or SKF-525A is coordinate covalent. Carbon monoxide inhibits all cytochrome P-450 isoenzymes since it binds to the heme component of cytochrome P-450, whereas SKF-525A inhibits specific types. SKF-525A inhibited benzene metabolism in the rat (Ikeda et al. 1972). Injection of 80 mg/kg of SKF-525A in rats resulted in a depression of phenol excretion. It also prolonged phenol excretion and interfered in the conversion of benzene to glucuronides and free phenols. Carbon monoxide, aniline, aminopyrine, cytochrome C, and metyrapone inhibited benzene metabolism *in vitro* by mouse liver microsomes (Gonasun et al. 1973).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to benzene than will most persons exposed to the same level of benzene in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of benzene, or compromised function of organs affected by benzene. Populations who are at greater risk due to their unusually high exposure to benzene are discussed in Section 6.7, Populations with Potentially High Exposures.

Variability in human susceptibility to benzene toxicity may be related, at least in part, to genetic polymorphisms associated with metabolic processes. As discussed in Section 3.4.3, the flavoenzyme, NAD(P)H:quinone oxidoreductase (NQ01), catalyzes the reduction of 1,2- and 1,4-benzoquinone (reactive metabolites of benzene) to catechol and hydroquinone, respectively (Nebert et al. 2002), thus protecting cells from oxidative damage by preventing redox cycling. The NQ01*1 (wild type) allele codes for normal NQ01 enzyme and activity. An NQ01*2 allele encodes a nonsynonymous mutation that has negligible NQ01 activity. Approximately 5% of Caucasians and African Americans, 15% of Mexican-Americans, and 20% of Asians are homozygous for the NQ01*2 allele (Kelsey et al. 1997; Smith and Zhang 1998). Rothman et al. (1997) demonstrated that workers expressing negligible NQ01 activity were at increased risk of benzene poisoning (Rothman et al. 1997). In the same group of workers, those expressing rapid CYP2E1 activity were also at increased risk of benzene poisoning. Those workers with polymorphisms for both negligible NQ01 activity and rapid CYP2E1 activity exhibited greater than 7-fold increased risk of benzene poisoning than workers not expressing these polymorphisms. These

results indicate that individuals expressing rapid CYP2E1 activity may also be at increased risk for benzene toxicity.

Polymorphisms have been described for many of the glutathione genes (Cotton et al. 2000; Hengstler et al. 1998; Strange and Fryer 1999), the myeloperoxidase gene (Williams 2001), and the epoxide hydrolase gene (Omiecinski et al. 2000), which are known to be involved in benzene metabolism. However, the potential involvement of such polymorphisms in benzene toxicity have not been demonstrated in benzene-exposed workers.

Individuals with medical conditions that include reduced bone marrow function or decreased blood factors would be at increased risk for benzene toxicity. Treatments for certain medical conditions might result in decreases in particular blood factors, which could lead to increased susceptibility to benzene poisoning.

Ethanol can increase the severity of benzene-induced anemia, lymphocytopenia, and reduction in bone marrow cellularity, and produce transient increases in normoblasts in the peripheral blood and atypical cellular morphology (Baarson et al. 1982). The enhancement of the hematotoxic effects of benzene by ethanol is of particular concern for benzene-exposed workers who consume alcohol (Nakajima et al. 1985). Accordingly, increased central nervous system disturbances (e.g., depression) may be expected following concurrent exposure to benzene and ethanol.

Gender-related differences in susceptibility to benzene toxicity have been observed in animals. For example, Kenyon et al. (1998) exposed male and female mice to benzene vapor concentrations of 100 or 600 ppm and found increased benzene metabolism and associated genotoxicity in males, relative to females. Brown et al. (1998) used a PBPK modeling approach to assess potential gender-related differences in susceptibility to benzene. Their results suggest that women exhibit a higher blood/air partition coefficient and maximum velocity of benzene metabolism than men, and that women metabolize 23-26% more benzene than men under similar exposure scenarios. However, gender-related differences in susceptibility among benzene-exposed workers were not located in available reports.

At early stages of human development, metabolic pathways may not be fully functional, which might result in a lower level of susceptibility. Young children might experience increased susceptibility to benzene by inhalation due to increased breathing rates and potential for increased absorption, relative to

adults. However, no definitive human or animal data were located regarding age-related differences in susceptibility to benzene.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to benzene. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to benzene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to benzene:

Goldfrank LR, Flomenbaum NE, Lewin NA, et al., eds. 1998. Goldfrank's toxicologic emergencies. 6th ed. Stamford: Appleton & Lange, 1384-1398.

Haddad LM, Shannon MW, Winchester JF. 1998. Clinical management of poisoning and drug overdose. 3rd ed. Philadelphia, PA: W.B Saunders Company, 940-941.

3.11.1 Reducing Peak Absorption Following Exposure

Human exposure to benzene can occur by inhalation, oral, or dermal routes. General recommendations for reducing absorption of benzene following acute high-level inhalation exposure have included moving the patient to fresh air and monitoring for respiratory distress (Bronstein and Currance 1988; Haddad et al. 1998; HSDB 2007; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). The administration of 100% humidified supplemental oxygen with assisted ventilation, when required, has also been suggested (Bronstein and Currance 1988; Haddad et al. 1998; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). In the case of eye exposure, irrigation with copious amounts of water or saline has been recommended (Bronstein and Currance 1988; Haddad et al. 1998; HSDB 2007; Stutz and Janusz 1988). The removal of contaminated clothing and a thorough washing of exposed areas with soap and water have also been recommended (Bronstein and Currance 1988; Ellenhorn and Barceloux 1988; Haddad et al. 1998; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). Some sources suggest the administration of water or milk to the victim after ingestion of benzene (Stutz and Janusz 1988). Emesis may be indicated following recent substantial ingestion of benzene (Ellenhorn and Barceloux 1988); however, other sources do not recommend the use of emetics because of the risk of aspiration pneumonitis, especially once the patient loses consciousness (Bronstein and Currance 1988; Haddad et al. 1998). Some sources recommend gastric lavage if indicated (Haddad et al. 1998; Kunisaki and Augenstein 1994).

While lavage may be useful, induction of emesis should be regarded with great caution because the rapid onset of central nervous system depression may lead to aspiration. Although of unproven value, administration of a charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, has also been suggested as a way to stimulate fecal excretion of the chemical before it is completely absorbed by the body (HSDB 2007; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). Diazepam and phenytoin may be helpful in controlling seizures (Bronstein and Currance 1988; HSDB 2007; Stutz and Janusz 1988). Administration of epinephrine or other catecholamines has not been recommended because of the possibility of myocardial sensitization and subsequent arrhythmia (Haddad et al. 1998; HSDB 2007; Nahum and Hoff 1934).

3.11.2 Reducing Body Burden

Following absorption into the blood, benzene is rapidly distributed throughout the body. Since benzene is lipophilic, it is preferentially distributed to lipid-rich tissues. The initial stage of benzene metabolism is the formation of benzene oxide via P-450 mixed-function oxidases. Detoxification pathways generally involve the formation of glutathione conjugates of benzene oxide and glucuronide or sulfate conjugates of phenol or its subsequent metabolites, catechol, hydroquinone, and trihydroxybenzene. Other metabolites of benzene also have known toxic effects. Exhalation is the main route for excretion of unmetabolized benzene, while metabolized benzene is excreted primarily in the urine. Studies in humans and animals indicate that both exhalation and urinary excretion occur in several phases, with half-lives of minutes to hours. Hence, benzene and its metabolites have relatively short half-lives in the body, and while some of these metabolites are clearly toxic, accumulation of substantial body burdens are not expected.

No methods are currently used for reducing the body burden of benzene. It is possible that methods could be developed to enhance the detoxification and elimination pathways, such as ensuring sufficient glutathione stores in the body by the administration of N-acetyl-L-cysteine.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Administration of indomethacin, a nonsteroidal anti-inflammatory drug, has been shown to prevent benzene-induced myelotoxicity in mice and the accompanying increase in prostaglandin E in the bone marrow (Kalf et al. 1989; Renz and Kalf 1991). Co-administration of indomethacin also prevented an increase in the number of micronucleated polychromatic erythrocytes in peripheral blood. The authors suggest that these results suggest a role for prostaglandin synthetase in benzene-induced myelotoxic and genotoxicity, and a way to interfere with that process with substances such as indomethacin.

Prostaglandins have been shown to inhibit hematopoiesis (Kalf et al. 1989). Additionally, prostaglandin synthetase could be involved in the oxidation of phenol and/or hydroquinone to toxic metabolites (Kalf et al. 1989).

The use of indomethacin to block benzene toxicity has led to data that indicate that myelotoxicity may involve the destruction of stromal macrophages that produce IL-1, a cytokine essential for hematopoiesis (Renz and Kalf 1991). External administration of recombinant IL-1 to mice prior to benzene administration prevents the myelotoxicity, presumably by providing a source of the cytokine (Renz and Kalf 1991). Further indication that IL-1 is affected by benzene exposure comes from the work of Carbonnelle et al. (1995), who showed that exposure of human monocytes to micromolar amounts of hydroquinone for 2 hours resulted in significantly decreased secretion of IL-1α and IL-1β at concentrations of 5 μM and above. RNA and protein synthesis were also inhibited. Additional research in this area indicates that tumor necrosis factor may provide protection against the inhibitory effects of hydroquinone on human hematopoietic progenitor cells (Colinas et al. 1995). The research of Shankar et al. (1993) determined that pretreatment with Protein A, a glycoprotein that acts as a multipotent immunostimulant, modulated the toxicity of benzene. Groups of six female albino rats (Swiss Wistar) were injected intraperitoneally with 1.0 mL/kg/body weight (879 mg/kg) benzene once daily for 3 consecutive days. Another group (six per group) was administered intravenously with 60 μg/kg Protein A twice weekly for 2 weeks and then injected with 879 mg/kg benzene intraperitoneally once daily for 3 consecutive days. Controls were injected with normal saline. All of the animals were killed 24 hours after receiving the last benzene injection. Blood was collected from the jugular vein for enumeration of total leukocyte counts. Routine autopsy was performed on all animals and the liver, thymus, spleen, and kidney organs were collected for organ weights. In benzene-only treated animals, there was a significant decrease in the total leukocyte counts in the peripheral blood as well as a significant decrease in the number of lymphocytes, a decrease in the gross organ weights of thymus and spleen, a significant increase in the iron content and lipid peroxidation of the liver and bone marrow, and an increase in low molecular weight iron in the bone marrow. Pretreatment with Protein A prevented these parameters from changing.

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of benzene is available. Where adequate information is not

available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of benzene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of Benzene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to benzene are summarized in Figure 3-6. The purpose of this figure is to illustrate the existing information concerning the health effects of benzene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Virtually all of the information regarding health effects in humans comes from studies of workers exposed to benzene-containing solvents and/or adhesives. Exposures to benzene occurred at rotogravure printing shops; at shoe, rubber, and raincoat manufacturing plants; and during chemical manufacturing processes. Case reports and cohort studies describe both acute and chronic health effects. The predominant route of exposure in these studies is inhalation. Dermal contact is also suspected as a possible route of exposure in these studies.

As seen in Figure 3-6, inhalation information for humans is available regarding death; acute-, intermediate-, and chronic-duration systemic effects; immunologic, neurologic, reproductive, developmental, and genotoxic effects; and cancer. However, as mentioned above, human exposure to

Figure 3-6. Existing Information on Health Effects of Benzene

Human

Animal
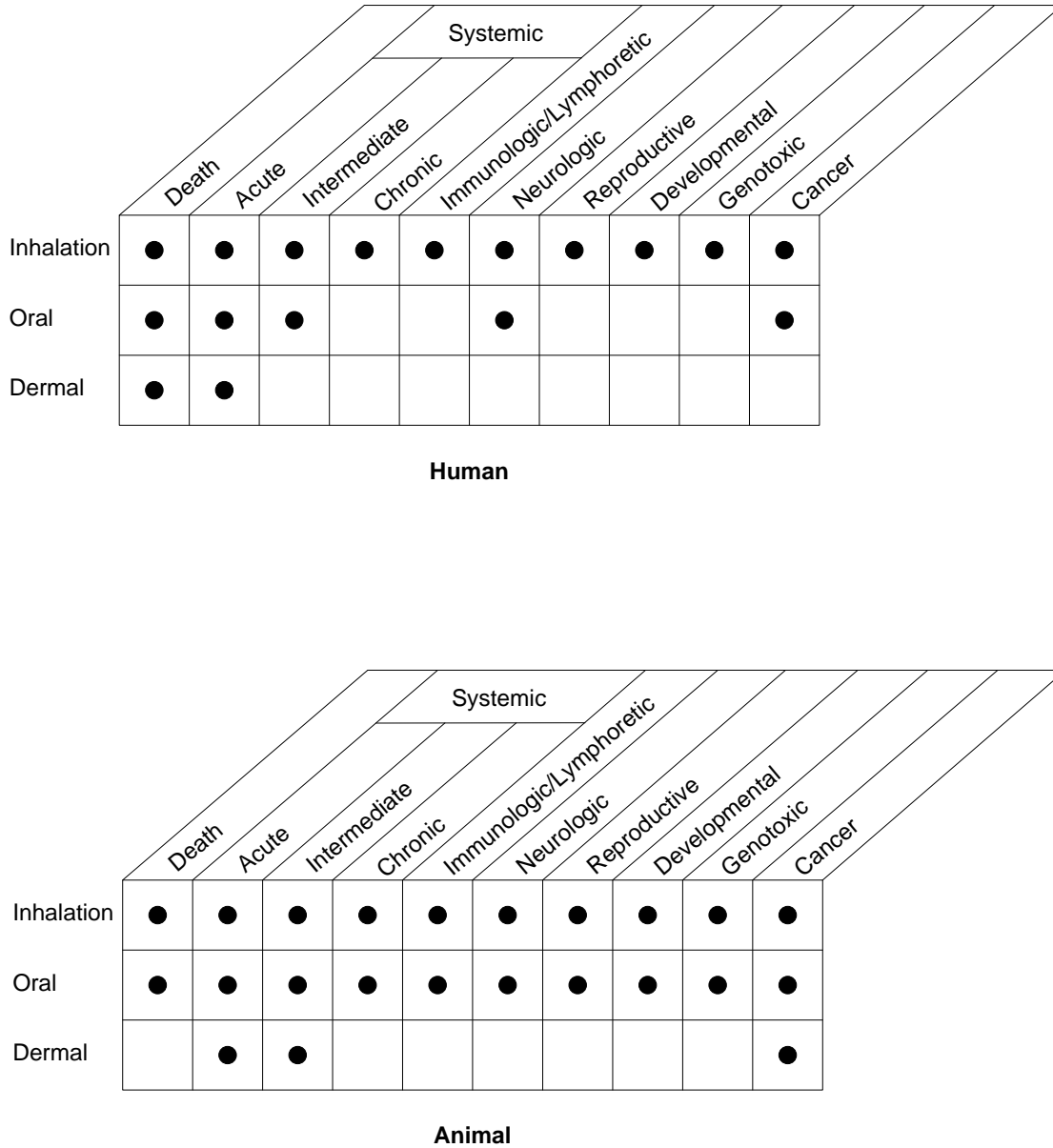
● Existing Studies

benzene in specific work environments probably occurs not only by inhalation, but also by the dermal route. Limited information is available regarding direct skin contact with benzene by humans. Additionally, oral studies in humans are limited to isolated case reports of death and acute-duration systemic effects subsequent to accidental or intentional ingestion of benzene, although one study (Hunting et al. 1995) described effects in vehicle maintenance workers who siphoned gasoline by mouth. There is limited information on effects of dermal exposure of humans to benzene, including death, acute-duration effects, and cancer.

Inhalation and oral studies in animals provide data on death; systemic effects after acute-, intermediate-, and chronic-duration exposure; and immunologic, neurologic, reproductive, developmental, genotoxic, and cancer effects. Furthermore, data exist regarding acute- and intermediate-duration systemic effects and cancer in animals after dermal exposure to benzene.

3.12.2 Identification of Data Needs

Acute-Duration Exposure.   There are reports on the health effects resulting from acute exposure of humans and animals to benzene via the inhalation, oral, and dermal routes. The primary target organs for acute exposure are the hematopoietic system, nervous system, and immune system. Acute effects on the nervous system and immune system are discussed below under Neurotoxicity and Immunotoxicity. Information is also available for levels that cause death in humans (e.g., Cronin 1924; Flury 1928; Greenburg 1926; Tauber 1970; Thienes and Haley 1972) and in animals (e.g., Cornish and Ryan 1965; Drew and Fouts 1974; Smyth et al. 1962) following inhalation and oral exposures.

No acute human or animal data on hematological effects from oral or dermal exposure are available. However, there are acute inhalation data that characterize the effects of benzene on the hematological system in humans and animals. Data regarding effects on the human hematological system following acute inhalation exposure to benzene are scant, but indicate leukopenia, anemia, and thrombocytopenia after more than 2 days of occupational exposure to more than 60 ppm benzene (Midzenski et al. 1992). Data for hematological effects in animals after acute-duration inhalation exposure are more extensive. Changes in peripheral erythrocytes (Chertkov et al. 1992; Cronkite et al. 1985; Rozen et al. 1984; Ward et al. 1985), in peripheral leukocytes (Aoyama et al. 1986; Chertkov et al. 1992; Gill et al. 1980; Green et al. 1981b; Li et al. 1986; Ward et al. 1985; Wells and Nerland 1991), and in bone marrow cells (Chertkov et al. 1992; Corti and Snyder 1996; Cronkite et al. 1989; Dempster and Snyder 1991; Gill et al. 1980; Green et al. 1981b; Neun et al. 1992; Toft et al. 1982) were seen in rats and mice. An acute-duration inhalation

MRL of 0.009 ppm was determined based on the LOAEL for immunologic effects in the mouse (Rozen et al. 1984) (discussed below under Immunotoxicity). No acute-duration oral studies were suitable for deriving MRLs. Additional studies that include dose-response information on hematological effects following acute oral exposure could be designed to provide information that could be useful in deriving an acute-duration oral MRL for benzene. Such studies could be designed to serve as validation for existing PBPK models. Acute dermal exposure at levels that are likely to be found in the environment and at hazardous waste sites is not likely to cause adverse health effects.

Intermediate-Duration Exposure.   There is sufficient information in humans and animals to identify the hematopoietic, nervous, and immunological systems as targets for benzene toxicity. The effects on the nervous system and immune system are discussed in the sections below titled Neurotoxicity and Immunotoxicity. Data on adverse hematological effects in humans are available following intermediate-duration exposures to benzene in the workplace (Aksoy and Erdem 1978; Aksoy et al. 1972). However, the exposure levels and durations were not well defined and, therefore, could not be used to calculate an MRL. Studies in rats and mice following inhalation exposure can be used to define NOAELs and LOAELs for hematological and immunological effects (e.g., Baarson et al. 1984; Cronkite et al. 1982, 1985, 1989; Dow 1992; Farris et al. 1993, 1997a, 1997b; Green et al. 1981a, 1981b; Luke et al. 1988b; Plappert et al. 1994a, 1994b; Rosenthal and Snyder 1987; Seidel et al. 1989; Snyder et al. 1978a, 1980; Toft et al. 1982; Vacha et al. 1990; Ward et al. 1985; Wolf et al. 1956). An intermediate-duration inhalation MRL of 0.006 ppm was derived, based on a LOAEL for immunologic effects in the mouse (Rosenthal and Snyder 1987) (discussed below under Immunotoxicity). Data on hematological effects from oral exposures in animals are also available (Hsieh et al. 1988b, 1990, 1991; NTP 1986; Shell 1992; Wolf et al. 1956), but no intermediate-duration oral MRL could be derived because the threshold for hematological (and immunological) effects could not be identified. Additional studies that include dose-response information on hematological effects following intermediate-duration oral exposure could be designed to provide information that could be useful in deriving an intermediate-duration oral MRL for benzene. Such studies could be designed to serve as validation for existing PBPK models. Intermediate-duration dermal exposure at levels that are likely to be found in the environment and at hazardous waste sites is not likely to cause adverse health effects.

Chronic-Duration Exposure and Cancer.   The primary target for adverse systemic effects of benzene following chronic exposure is the hematological system. Hematotoxicity was reported in studies of humans chronically exposed to benzene in the workplace air (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974, 1987; Cody et al. 1993; Dosemeci et al. 1996; Doskin 1971; Erf and Rhoads 1939;

Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Lan et al. 2004a, 2004b; Li et al. 1994; Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b; Townsend et al. 1978; Ward et al. 1996; Yin et al. 1987c). Several studies of occupational inhalation exposure to benzene did not find clinically-defined hematological effects (Collins et al. 1991, 1997; Tsai et al. 1983, 2004), but they had a high degree of uncertainty regarding estimations of benzene exposure levels. The study of workers of shoe manufacturing industries in Tianjin, China (Lan et al. 2004a, 2004b) identified the lowest LOAEL for hematotoxicity and was selected as the principal study for deriving a chronic-duration inhalation MRL of 0.003 ppm for benzene. Chronic-duration animal studies are available for hematological effects following inhalation exposure and provide support to the human data (Snyder et al. 1978a, 1980, 1982, 1984). No human data are available to evaluate hematological effects following oral exposure. Although chronic duration oral animal studies are available for hematological effects (Huff et al. 1989; Maltoni et al. 1983, 1985; NTP 1986), the most extensive study (NTP 1986) did not conclusively define a NOAEL or less serious LOAEL for end points that could be used to derive an MRL. However, a chronic-duration oral MRL of 0.0005 mg/kg/day was derived for benzene based on route-to-route extrapolation of the same results (Lan et al. 2004a, 2004b) that served as the basis for the chronic-duration inhalation MRL. Additional chronic-duration oral animal data could be designed to provide support to the chronic-duration oral MRL and to assist in defining threshold levels for populations living near hazardous waste sites. Dermal data for humans and animals were not available. However, chronic-duration dermal exposure at levels that are likely to be found in the environment and at hazardous waste sites is not likely to cause adverse health effects.

EPA, IARC, and the Department of Health and Human Services have concluded that benzene is a human carcinogen based on sufficient data in humans supported by animal evidence (IARC 2004; IRIS 2007; NTP 2005). Epidemiological studies and case reports provide clear evidence of a causal relationship between occupational exposure to benzene and the occurrence of acute myelogenous leukemia (AML) (Hayes et al. 1997; IARC 1982, 1987; EPA 1995, 1998; IRIS 2007; Rinsky et al. 1987, 2002; Yin et al. 1996a, 1996b), as well as is suggestive evidence of associations between benzene and non-Hodgkin's lymphoma (NHL) and multiple myeloma (Hayes et al. 1997; Rinsky et al. 1987). Studies of workers in Ohio (the Pliofilm study) (e.g., Rinsky et al. 1981, 1987; 2002) and China (the NCI/CAPM study) (e.g., Hayes et al. 1997; Yin et al. 1996a, 1996b) provide the strongest data on the leukemogenic potential of benzene, including exposure-response information, and data from the Pliofilm study was used as the basis of inhalation and oral cancer risk values for benzene (EPA 1998; IRIS 2007). Additional studies on these and other cohorts could better characterize exposure level and exposure duration relationships for benzene

and leukemia, particularly at low levels of exposure, and clarify the potential of benzene to induce NHL and multiple myeloma.

Benzene is a multiple site carcinogen in rats and mice following inhalation exposure (Cronkite 1986; Cronkite et al. 1984, 1985, 1989; Farris et al. 1993; Maltoni et al. 1982a, 1982b, 1983, 1985, 1989; Snyder et al. 1980) and oral exposure (Huff et al. 1989; Maltoni et al. 1983, 1985, 1989; NTP 1986), inducing lymphomas and other neoplasms in numerous tissues not affected in humans. Although contributing to the weight of evidence for carcinogenicity, the animal studies do not identify a suitable model for leukemia in humans. An appropriate animal model would help to provide a better understanding of how benzene causes cancer, particularly the mechanism of benzene leukemogenesis. The exact mechanism of benzene carcinogenicity is not known, but it has been postulated that some benzene metabolites are capable of forming adducts with DNA and are responsible for reduced immune function which could potentially lead to cancer. The clastogenic properties of benzene may play a role in its carcinogenicity. DNA adduct formation could occur with both inhalation and oral exposures (Ding et al. 1983; Sasiadek et al. 1989). Questions that need to be answered with regard to the mechanism of benzene carcinogenesis include how benzene metabolites produce greater-than-additive effects, determination of the critical target genes, whether aplastic anemia is essential to the development of leukemia, and determination of the role of cytokines and growth factor pathways in benzene toxicity.

Genotoxicity.   Evidence for the genotoxicity of benzene in humans comes from studies of chronically-exposed populations (Andreoli et al. 1997; Bogadi-Šare et al. 1997; Ding et al. 1983; Forni and Moreo 1967, 1969; Forni et al. 1971a; Hallberg et al. 1996; Hartwich et al. 1969; Hedli et al. 1991; Karacic et al. 1995; Kašuba et al. 2000; Liu et al. 1996; Major et al. 1992, 1994; Nilsson et al. 1996; Picciano 1979; Pitarque et al. 1996, 1997; Popp et al. 1992; Qu et al. 2003a, 2003b; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Sellyei and Kelemen 1971; Smith et al. 1998; Sul et al. 2002; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Türkel and Egeli 1994; Van den Berghe et al. 1979; Yardley-Jones et al. 1990; Zhang et al. 1998b, 1999). These exposures have occurred primarily via inhalation, although some dermal exposure cannot be ruled out. In spite of the lack of accurate exposure data, exposure to multiple chemicals, and often inappropriate control groups, the association between benzene exposure and the appearance of structural and numerical chromosome aberrations in human lymphocytes suggests that benzene can be considered a human clastogen. Benzene-induced cytogenetic effects, including chromosome and chromatid aberrations, sister chromatid exchanges, and micronuclei, have been consistently found in *in vivo* animal studies (Anderson and Richardson 1981; Au et al. 1991; Chen et al. 1994; Chang et al. 2005; Eastmond et al. 2001; Erexson et al. 1986; Farris et al. 1996; Fujie et

al. 1992; Healy et al. 2001; Lee et al. 2005; Kolachana et al. 1993; Ranaldi et al. 1998; Siou et al. 1981; Toft et al. 1982; Ward et al. 1992). Binding of benzene and/or its metabolites to DNA, RNA, and proteins has been consistently observed in rats and mice (Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Mani et al. 1999; Mazullo et al. 1989; Turteltaub and Mani 2003). Inhalation exposure of mice has yielded identifiable benzene-derived hemoglobin adducts (Sabourin et al. 1990). Ward et al. (1992) have shown that intermediate-duration exposure of mice to benzene by inhalation at levels below the current PEL may induce gene mutations in the lymphocytes.

Standard short-term *in vitro* assays indicate that benzene's genotoxicity is derived primarily from its metabolites, although benzene has been shown to produce DNA breaks in Chinese hamster ovary cells independent of metabolic activators (Douglas et al. 1985; Eastmond et al. 1994; Lakhanisky and Hendrickx 1985; Zhang et al.1993). There is good evidence that benzene affects cell cycle progression, RNA and DNA synthesis, as well as DNA binding (Forni and Moreo 1967, 1969; Hartwich et al. 1969; Sellyei and Kelemen 1971; Van den Berghe et al. 1979). Chen and Eastmond (1995) showed that benzene metabolites can adversely affect human topoisomerases (enzymes involved in DNA replication and repair); however, evidence exists that some repair of DNA binding with benzene metabolites occurs in human cells (Chenna et al. 1995).

The genotoxicity of benzene has been extensively studied and demonstrated in both humans and laboratory animals. Although some information is available regarding possible mechanisms of benzene genotoxicity and carcinogenicity, additional mechanistic studies would be helpful to more completely characterize mechanisms responsible for these effects.

Reproductive Toxicity.   Reproductive data are available on women occupationally exposed to benzene (Mukhametova and Vozovaya 1972; Vara and Kinnunen 1946). The data suggest spontaneous abortions, menstrual disturbances, and ovarian atrophy. These studies are limited by the difficulty in identifying appropriate controls, problems in controlling for concomitant exposures to other chemicals, and inadequate follow-up. Only one study was found that described the reproductive effects on men exposed to benzene (Stucker et al. 1994). There are some data available from inhalation studies on reproductive effects of benzene in animals. Although there are data on adverse gonadal effects (e.g., atrophy/degeneration, decrease in spermatozoa, moderate increases in abnormal sperm forms) (Ward et al. 1985; Wolf et al. 1956), data on reproductive outcomes are either negative (Coate et al. 1984; Green et al. 1978; Kuna et al. 1992; Murray et al. 1979; Tatrai et al. 1980a) or inconclusive or conflicting (i.e., number of live fetuses, incidences of pregnancies) (Murray et al. 1979; Ungvary and Tatrai 1985).

Gofmekler (1968) showed infertility in female rats after intermediate-duration inhalation exposure to 210 ppm, but the results are poorly reported. Thus, they provide only suggestive evidence that benzene may have an adverse effect on reproductive outcomes. No data were located on reproductive effects following oral exposure to benzene in humans. Negative effects on reproductive outcome have been reported in one oral study in animals (Exxon 1986). NTP (1986) reported neoplastic changes in the reproductive tissues of female rats and male and female mice after chronic oral exposure. Given the paucity of available data across all exposure routes, it would be useful to have additional 90-day studies conducted by the oral and inhalation routes that assess reproductive organs histologically or cytogenetically. Although dermal contact is not likely to be the most relevant route of exposure for humans at hazardous waste sites, data on dermal exposure would also be useful. If the results of the suggested inhalation or oral studies indicate reproductive toxicity, multigeneration or continuous breeding studies for oral and inhalation exposures would help clarify the potential for benzene to cause reproductive effects in humans.

Developmental Toxicity.   Based on epidemiological studies at hazardous waste sites, an increased susceptibility to benzene of pregnant women or their offspring has not been demonstrated (Budnick et al. 1984; Goldman et al. 1985; Heath 1983; Olsen 1983). However, these studies have several limitations that make it impossible to assess the effect of benzene on the human fetus. For example, the few studies that do exist are limited by a lack of control incidences for end points, problems in identifying exposed populations, a lack of data on exposure levels, and/or exposure to multiple substances (Budnick et al. 1984; Forni et al. 1971a; Funes-Cravioto et al. 1977; Goldman et al. 1985; Heath 1983; Olsen 1983). In the occupational setting, however, there may be stronger evidence of increased susceptibility to benzene of pregnant women and/or their offspring (Forni et al. 1971a; Funes-Cravioto et al. 1977). For example, severe pancytopenia and increased chromosomal aberrations occurred in a pregnant worker exposed to benzene (levels not reported) during the entire pregnancy. She gave birth to a healthy boy (Forni et al. 1971b). On the other hand, increased frequency of chromatid and isochromatid breaks and sister chromatid exchanges was found in lymphocytes from 14 children of female workers exposed to benzene (levels not reported) and other organic solvents during pregnancy (Funes-Cravioto et al. 1977). Irrespective of the hematological effects reported in the pregnant worker or the 14 children of female workers, the lack of complete and detailed medical records and the lack of follow-up limit the significance of effects with regard to post exposure morbidity.

A number of investigations have evaluated the developmental/maternal toxicity of benzene in animals following inhalation exposures. In these investigations, fetotoxicity was evidenced by reduced fetal

weight and/or minor skeletal variations at concentrations 47 ppm (Coate et al. 1984; Green et al. 1978; Kimmel and Wilson 1973; Kuna and Kapp 1981; Murray et al. 1979; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985). However, persistently altered fetal hematopoiesis occurred in mice at 20 ppm (Keller and Snyder 1986, 1988). Additional information designed to assess the hematopoietic system of the developing fetus (human or animal) following low-level *in utero* exposures to benzene is needed. Oral data are limited to two animal studies in which benzene was shown to reduce pup body weight when mice were administered a high single oral dose (Seidenberg et al. 1986) or have no effect after gestational exposure (Exxon 1986). Additional oral studies designed to assess the developmental effects (human or animal) of low-level exposures to benzene would be useful. No data are available on the developmental toxicity of benzene following dermal exposure. Although these data would be useful, the most likely routes of exposure for humans at hazardous waste sites are the inhalation and oral routes.

Immunotoxicity. The immune system is known to be a target for benzene toxicity. The effects do not appear to be route- or species-specific. The evidence for immunotoxicity in humans comes from workers exposed by inhalation of intermediate and chronic durations. A series of studies demonstrated decreases in numbers of circulating leukocytes (Aksoy et al. 1971, 1972, 1974), but levels of exposure were not well documented. In other studies, alterations in human serum immunoglobulins were observed, but there was concomitant exposure to xylene and toluene (Lange et al. 1973a, 1973b). There is a need to test for subtle alterations in the immune system and immune competence in workers with intermediate- and chronic-duration exposure to benzene. Animal studies support the findings of immune dysfunction and indicate that additional parameters of the immune system are affected by exposure to benzene in the air for acute (Aoyama 1986; Chertkov et al. 1992; Cronkite 1986; Cronkite et al. 1982, 1985, 1989; Gill et al. 1980; Green et al. 1981a; Li et al. 1986; Neun et al. 1992; Rozen et al. 1984; Rosenthal and Snyder 1985; Toft et al. 1982; Ward et al. 1985; Wells and Nerland 1991), intermediate (Baarson et al. 1984; Cronkite et al. 1982, 1985, 1989; Dow 1992; Gill et al. 1980; Green et al. 1981a, 1981b; Li et al. 1992; Plappert et al. 1994b; Rosenthal and Snyder 1987; Rozen and Snyder 1985; Seidel et al. 1990; Snyder et al. 1980; Stoner et al. 1981; Ward et al. 1985; Wolf et al. 1956; Songnian et al. 1982), and chronic (Snyder et al. 1980, 1984, 1988) periods. An acute-duration inhalation study found depressions of proliferative responses of bone-marrow-derived B-cells and splenic T-cells in mice at 10 ppm (Rozen et al. 1984), and an acute-duration inhalation MRL has been derived based on this study. An intermediate-duration inhalation study found delayed splenic lymphocyte reaction to foreign antigens evaluated by *in vitro* mixed lymphocyte culture following exposure of mice to benzene vapors at a concentration of 10 ppm (Rosenthal and Snyder 1987), and an intermediate-duration inhalation MRL has been derived based on this study. No human data on the oral route were available, but animal data showed immunological

effects after intermediate (Fan 1992; Hsieh et al. 1988b, 1990, 1991; Shell 1992; Wolf et al. 1956) and chronic exposures (Huff et al. 1989; Maltoni et al. 1983, 1985; NTP 1986) via the oral route. Since the immune system is known to be a target organ, it is important to have information on subtle alterations in immune competence in people exposed to benzene in the air and in the drinking water. A decrease in cell-mediated immune functions, including alloantigen response and cytotoxicity, was reported in mice following intermediate inhalation exposure to 100 ppm of benzene (Rosenthal and Snyder 1987). This impaired cell-mediated immune function was also apparent in other *in vivo* studies (Stoner et al. 1981). Mice exposed to 100 ppm of benzene for a total of 100 days had reduced tumor resistance when challenged with syngeneic tumor cells and developed tumors that were lethal (Rosenthal and Snyder 1987). Further animal studies would also be useful in defining more NOAEL and LOAEL values. No data are available that document immunotoxicity in humans or animals exposed by dermal application. Dermal sensitization tests may also provide useful data on the likelihood of an allergic response in humans, since skin contact may occur in the workplace and at hazardous waste sites.

Neurotoxicity. In humans, the nervous system is a target of benzene toxicity following both inhalation and oral exposures. No data are available that demonstrate neurologic effects in humans or animals exposed dermally. There are sufficient data to suggest that it is the central nervous system which is affected following acute exposures. Neurological symptoms reported in humans following acute oral and inhalation exposures are similar and include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness (Cronin 1924; Flury 1928; Greenburg 1926; Midzenski et al. 1992; Tauber 1970; Thienes and Haley 1972). Acute- and intermediate-duration inhalation and oral animal studies provide supportive evidence that benzene effects the central nervous system (Carpenter et al. 1944; Cornish and Ryan 1965; Evans et al. 1981; Frantik et al. 1994). Effects observed include narcosis, hyperactivity, tremors, tonic-clonic convulsions, decreased evoked electrical activity in the brain, and slight nervous system depression. Behavioral changes were found in mice after 1 week of exposure to 300 ppm, and decreased grip strength was found after three exposures to 1,000 ppm (Dempster et al. 1984). An intermediate-duration inhalation study found increased rapid response in mice at 0.78 ppm (Li et al. 1992), but this study was limited due to apparent discrepancies between reported and actual benzene exposure levels, since neurological effects reported in other animal studies occurred only at much higher exposure levels. Acute-duration oral exposures of 950 mg/kg benzene altered neuro-transmitter levels in the brains of rats (Kanada et al. 1994). Intermediate-duration oral exposures of 8 mg/kg/day resulted in changes in the levels of monoamine transmitters in the brain without treatment-related behavioral changes (Hsieh et al. 1988a).

One chronic-duration occupational study reported neurological abnormalities of the peripheral nervous system (global atrophy of lower extremities and distal neuropathy of upper extremities) in four of six benzene-exposed patients with aplastic anemia (Baslo and Aksoy 1982). Additional studies are needed to verify the peripheral nervous system effects that might occur following chronic exposures to low doses of benzene.

Although there are sufficient data to indicate that the nervous system is a target of benzene toxicity, the neurotoxicity of benzene has not been extensively studied. Additional studies in animals are needed to identify the thresholds of neurotoxicity following acute-, intermediate-, and chronic-duration inhalation and oral exposures.

Epidemiological and Human Dosimetry Studies.   A large segment of the U.S. population is exposed to benzene. This exposure occurs primarily as a result of benzene emitted to the air from tobacco smoke, gasoline stations, and automobile exhaust. Benzene has been found in about a third of the NPL hazardous waste sites. The magnitude of exposure is greater for those occupationally exposed.

The predominance of available epidemiological data comes from occupational studies and associated analysis (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974; Baslo and Aksoy 1982; Ciccone et al. 1993; Cody et al. 1993; Dosemeci et al. 1994, 1996; Doskin 1971; EPA 1986, 1995, 1998; Erf and Rhoads 1939; Goldwater 1941; Greenburg et al. 1939; Hayes et al. 1997; IARC 1982, 1987; Infante 1978; Infante et al. 1977; IRIS 2007; Kipen et al. 1989; Lan et al. 2004a, 2004b; Li et al. 1994; Ott et al. 1978; Paustenbach et al. 1992; Paxton et al. 1994a, 1994b; Qu et al. 2002, 2003a, 2003b; Rinsky et al. 1981, 1987, 2002; Rothman et al. 1996a, 1996b; Townsend et al. 1978; Travis et al. 1994; Utterback and Rinsky 1995; Ward et al. 1996; Yin et al. 1987c, 1989, 1994, 1996a, 1996b). While these studies provide data on hematological and neurotoxic effects and evidence of carcinogenicity, they only offer information on the effects of inhalation exposure. Retrospective and prospective studies of populations that have been identified as being exposed to contaminated groundwater or drinking water could provide information on long-term health effects from oral exposures. The Benzene Subregistry Baseline Technical Report of the National Exposure Registry contains information on 1,143 persons who had documented exposure to benzene in their drinking water and were exposed for at least 30 days (Agency for Toxic Substances and Disease Registry 2001). No causal relationship has been proposed for health conditions identified in the base subregistry (Agency for Toxic Substances and Disease Registry 1995) or continued follow-up of the population (Agency for Toxic Substances and Disease Registry 2001).

Few well-conducted epidemiological studies of exposed populations exist. An epidemiological study of a population with chronic exposure to two NPL hazardous waste sites illustrates the problems inherent in assessing adverse health effects from waste site exposure (Dayal et al. 1995). Benzene was only 1 of 20 compounds or groups of compounds identified as being chemicals of concern in the waste site. The authors note that assessment of long-term exposure to a dumpsite is problematic because of a lack of history of materials deposited, the complexity of the waste components, the interaction of the components over time, and the emphasis on statistically significant versus biologically significant effects. The study had certain limitations, including lifestyle characteristics, the use of mailed-in responses, subjective symptom-reports without independent confirmation, and various problems with exposure estimation. A relationship was detected between exposed individuals and neurological symptoms, including learning difficulties, nervousness, numbness in fingers or toes, sleeping difficulties, unusual fatigue, and decreased sense of smell. Chest pains, irregular heartbeat, skin rashes, and detection of a peculiar odor or taste also occurred more often in the exposed population. However, no effort was made to relate the symptoms to particular chemicals in the waste site. Clearly, more studies of exposed populations surrounding waste sites would be warranted. Other studies include analyses of workers in China (Dosemeci et al. 1994, 1996; Lan et al. 2004a, 2004b; Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b; Yin et al. 1987c, 1994), reanalysis of data from the Pliofilm cohort (Paxton et al. 1994a, 1994b; Utterback and Rinsky 1995; Ward et al. 1996), and assessment of clinical manifestations of benzene hematotoxicity in U.S. industries with potential for benzene exposure (Collins et al. 1991, 1997; Tsai et al. 1983, 2004). A publication by Lioy and Pellizzari (1995) outlines an appropriate framework for the National Human Exposure Assessment Survey, using benzene as a candidate compound. Other articles also address population-based exposure models and measurements (MacIntosh et al. 1995; Pellizzari et al. 1995).

Analysis of benzene dosimetry in animal studies suggests that the metabolic pathways leading to the production of the putative toxic metabolites appear to be low-capacity, high-affinity pathways that are saturated at relatively low-exposure concentrations (Henderson et al. 1992). The authors suggest that this could also be true for humans. Additional dosimetry studies are necessary to further define the role of toxic metabolites in the development of benzene-related leukemia.

Studies of DNA damage have primarily been performed on workers with preexisting hematological effects and cancer (Ding et al. 1983; Forni et al. 1971a; Picciano 1979; Yardley-Jones et al. 1988) or as biomonitoring of benzene exposure (Andreoli et al. 1997; Bogadi-Šare et al. 1997; Hallberg et al. 1996; Kašuba et al. 2000; Liu et al. 1996; Nilsson et al. 1996; Pitarque et al. 1996; Sul et al. 2002; Surrallés et al. 1997; Zhang et al. 1999). Additional data on benzene-induced DNA damage could be useful in

monitoring possible adverse effects in individuals living near hazardous waste sites if more quantitative

dose-response data were available for clastogenicity.

Studies to determine whether benzene causes solid tumors and hematological malignancies other than

acute nonlymphocytic leukemia in humans have been suggested as a data need (Goldstein and Warren

1993). These data could be obtained by revisiting already established cohorts of exposed individuals, or

by examining new cohorts.

Biomarkers of Exposure and Effect.

Exposure. There is no clinical disease state unique to benzene toxicity. However, the effects on the

hematopoietic and immune systems are well recognized, and analytical methodologies exist for

monitoring benzene levels in expired breath and blood (Brugnone et al. 1989, 1992; DeLeon and Antoine

1985; Pellizzari et al. 1988). Several biomarkers of exposure to benzene exist, including unmetabolized

benzene in expired air and urine (Farmer et al. 2005; Fustinoni et al. 2005; Ghittori et al. 1993;

Nomiyama and Nomiyama 1974a, 1974b; Sherwood 1988; Srbova et al. 1950; Waidyanatha et al. 2001),

urinary phenol levels (Astier 1992; Inoue et al. 1986, 1988b; Jongeneelen et al. 1987; Karacic et al. 1987;

Pagnotto et al. 1961; Pekari et al. 1992), and urinary *trans,trans*-muconic acid and *S*-phenylmercapturic

acid levels (Boogaard and van Sittert 1995, 1996; Ducos et al. 1990, 1992; Farmer et al. 2005; Inoue et al.

1989b, 2000; Lee et al. 1993; Melikian et al. 1993, 1994; Pezzagno et al. 1999; Popp et al. 1994; Qu et al.

2005; Rothman et al. 1998; Ruppert et al. 1997; Sanguinetti et al. 2001; van Sittert et al. 1993; Weaver et

al. 2000). Estimates of benzene exposure can be made by comparing the ratio of inorganic to organic

sulfates in the urine (Hammond and Hermann 1960). However, urinary sulfate levels are variable, and

they have not been used to identify exposure levels of benzene associated with minimal toxic effect.

Furthermore, benzene-derived adducts with hemoglobin and albumin may be useful as biomarkers

(Bechtold et al. 1992a, 1992b; Bechtold and Henderson 1993; Farmer et al. 2005; Fustinoni et al. 2005;

Ghittori et al. 1993; Hedli et al. 1991; Lutz and Schlatter 1977; Nomiyama and Nomiyama 1974a, 1974b;

Pekari et al. 1992; Reddy et al. 1989; Schad et al. 1992; Sherwood 1988; Smith and Rothman 2000;

Srbova et al. 1950; Waidyanatha et al. 2001; Yeowell-O'Connell et al. 1998, 2001).

Phenol measurements have routinely been used for monitoring occupational exposures, and there is

evidence that urinary phenol levels can be correlated with exposure levels (Astier 1992; Inoue et al. 1986;

Pekari et al. 1992). Correlating urinary phenol with benzene exposure is complicated by high and

variable background levels in nonexposed persons. The data suggest that variations in urinary phenol will

obscure phenol formed from low levels of benzene (Ong and Lee 1994; Perbellini et al. 1988). For

exposures to 1 ppm or less, the current workplace standard, urinary phenol is not an adequate assay to

determine the extent of benzene exposure. In addition, urinary excretion of phenol is lowered by

coexposure to toluene (Inoue et al. 1988b). In many exposure situations (e.g., occupational settings and

hazardous waste sites), toluene and other chemicals are present and could interfere with the metabolism

and elimination of benzene. Studies that attempt to identify another biomarker that is specific to benzene,

such as *S*-phenylmercapturic acid, the proposed urinary BEI (ACGIH 1996b), may be helpful for medical

surveillance.

The metabolism of benzene to ring-opened compounds (e.g., muconic acid) may be pertinent to the

development of a biomarker of benzene exposure and effect (Ducos et al. 1990, 1992; Inoue et al. 1989b;

Lee et al. 1993; Melikian et al. 1993; Ong and Lee 1994; Witz et al. 1990b). However, the dose-response

curve for metabolism of benzene to urinary muconic acid in humans needs to be determined. If urinary

muconic acid increases in humans at low benzene exposures, as it does in mice, then it may be a valid

biomarker of benzene exposure. Excretion of urinary muconic acid in humans has also been shown to be

lowered by coexposure to toluene (Inoue et al. 1989b). The effect of coexposure to toluene and other

chemicals on muconic acid formation needs to be assessed in order to determine the usefulness of

muconic acid as a biomarker of benzene exposure.

Breath levels of benzene have been used as a measure of exposure (Brugnone et al. 1989; Money and

Gray 1989; Nomiyama and Nomiyama 1974a, 1974b; Ong and Lee 1994; Pekari et al. 1992). However,

the amount of benzene lost in expired air will vary not only with the dose, duration, and time from

exposure, but also with the extent of metabolism in the body. Benzene levels in blood have been

measured. However, blood levels rapidly decrease after exposure (Brugnone et al. 1989; DeLeon and

Antoine 1985; Schrenk et al. 1941).

Benzene metabolites also form DNA adducts (Popp et al. 1992). The use of hemoglobin adducts formed

from benzene metabolites as a biomarker of benzene exposure has been developed by Sun et al. (1990).

Bechtold and colleagues have conducted further studies using this model (Bechtold et al. 1992a; Bechtold

and Henderson 1993). Further refinement of this model would be useful to quantitate cumulative low-

level exposures to benzene. Development of specific markers of exposure to use in epidemiological

studies to clarify the shape of the dose-response curve in the low-dose region has been suggested as a data

need (Goldstein and Warren 1993). Recent reports indicate that urinary benzene may serve as a sensitive

biomarker of exposure to benzene concentrations well below 1 ppm (Farmer et al. 2005; Fustinoni et al. 2005).

Effect.   Monitoring of benzene workers has included monthly blood counts. Toxic effects occur in the bone marrow and arise either from benzene via a solvent effect, or via its metabolites (Gad-El-Karim et al. 1985; Irons et al. 1980). Hematological tests could be used as markers of hematotoxicity, but medical laboratories lack tests specific to benzene hematotoxicity. The tests cannot be relied upon to find preclinical disease but can identify the subtle changes that are early indicators of effects. Furthermore, these markers of effect may not be useful for long periods following cessation of exposure, nor do they distinguish between acute and chronic exposures. As stated above, additional studies are needed to define the role of benzene-related leukopenia in the disease process initiated by benzene exposure, to determine if it is a biomarker of effect, or an intermediate end point in the development of leukemia (Hayes 1992).

Absorption, Distribution, Metabolism, and Excretion.   Data from both humans and animals consistently indicate that benzene is rapidly absorbed through the lungs (Eutermoser et al. 1986; Nomiyama and Nomiyama 1974a; Sabourin et al. 1987; Schrenk et al. 1941; Srbova et al. 1950; Yu and Weisel 1996). Although experimentally-acquired data are not available on oral absorption of benzene in humans, case reports of accidental or intentional poisoning suggest that benzene is rapidly absorbed from the gastrointestinal tract (Thienes and Haley 1972). The efficient absorption of oral doses in animals is well documented (Cornish and Ryan 1965; Parke and Williams 1953a; Sabourin et al. 1987). Benzene can be absorbed through the skin, but the rate of absorption is much lower than that for inhalation (Maibach and Anjo 1981; Susten et al. 1985; Tsuruta 1989). Following absorption into the body, benzene is widely distributed to tissues, with the relative uptake dependent on the perfusion of the tissue by blood, and the total potential uptake dependent on fat content and metabolism (Sato et al. 1975; Tauber 1970).

There is no evidence to suggest that the route of administration has any substantial effect on the subsequent metabolism of benzene, either in humans or animals. Benzene is metabolized primarily in the liver and to a lesser extent, in the bone marrow. Benzene is a preferential substrate of CYP2E1, which also metabolizes alcohol. The induction of CYP2E1 by benzene (and some of its metabolites) with subsequent generation of reactive metabolites, oxygen radicals, circulating lipid peroxides, and hydroxyl radicals could be associated with hematopoietic toxicity and carcinogenicity of benzene (Irons 2000; Parke 1989; Ross 1996, 2000; Smith 1996a, 1996b; Snyder 2000a, 2000b, 2002; Snyder and Hedli 1996; Snyder and Kalf 1994). CYP2E1 is not confined to the liver, but has also been detected in bone marrow. Andrews et al. (1979) demonstrated that rabbit bone marrow is capable of metabolizing benzene. Schnier

et al. (1989) subsequently found that rabbit bone marrow contains CYP2E1. Irons et al. (1980) demonstrated that benzene metabolism by rat bone marrow (*in situ*) was complete and independent of metabolism by the liver, with concentrations of phenol greater than catechol and hydroquinone. Although the total metabolism by bone marrow was limited (total metabolites present were 25% of those in blood), the concentration of metabolites in the bone marrow exceeded that in the blood. Similar studies have been conducted in mice (Ganousis et al. 1992). Benzene metabolism in bone marrow is not well understood; additional data regarding the initial oxidation step and the comparatively low levels of CYP2E1 activity in bone marrow would be useful in identifying the mechanisms of benzene's hematotoxicity. This aspect of metabolism may have implications for long-term exposures, which could be explored in chronic exposure studies. The intermediary metabolites of benzene are responsible for many of the toxic effects observed (Eastmond et al. 1987; Gad-El-Karim et al. 1985). Biotransformation is believed to be essential for benzene-induced bone marrow damage.

However, there is disagreement as to whether benzene is activated in the marrow, activated elsewhere and transported to the marrow, or metabolized in the liver and the metabolites activated in the marrow. Further studies on the metabolism of benzene would help define its mechanism of action. Additionally, more information is needed on the pathways of metabolism in humans, the chemical nature of the toxic metabolites, and the mechanism of toxicity. Recently published data comparing urinary metabolite profiles of orally administered benzene and phenol in mice suggest that zonal differences in metabolism in the liver may be responsible for relative differences in the production of hydroquinone, thus explaining the higher toxicity observed after benzene administration compared with phenol administration (Kenyon et al. 1995). Additional work in this area would aid in further understanding the kinetic determinants of benzene toxicity. Ethanol and dietary factors such as food deprivation and carbohydrate restriction enhance the hematotoxic effects of benzene. Therefore, more information regarding differences in metabolic pattern according to sex, age, nutritional status, and species, and correlation to differences in health effects would be useful.

Humans and animals both excrete inhaled benzene via expiration. Additionally, benzene metabolites are excreted primarily in the urine in both humans and animals. No studies in humans exist for excretion of oral doses of benzene. Studies in several animal species indicate that the route of excretion of benzene and/or its metabolites is a function of exposure level and the saturation of metabolic systems (Henderson et al. 1989). Data regarding excretion following dermal exposure in humans are limited. However, the major route of excretion in both humans and animals following dermal exposure is the urine.

Comparative Toxicokinetics.   Qualitatively, absorption, distribution, metabolism, and excretion
appear to be similar in humans and laboratory animals. However, quantitative variations in the
absorption, distribution, metabolism, and excretion of benzene have been observed with respect to
exposure routes, sex, nutritional status, and species. Further studies that focus on these differences and
their implications for human health would be useful. Additionally, *in vitro* studies using human tissue
and further research into PBPK modeling in animals would contribute significantly to the understanding
of the kinetics of benzene and would aid in the development of pharmacokinetic models of exposure in
humans. These topics are being addressed in ongoing studies (see Section 3.12.3).

Methods for Reducing Toxic Effects.   Development of methods and practices that are specific for
benzene is needed for reducing peak absorption, body burden and for interfering with the mechanism of
action following benzene exposures. Since benzene metabolites are thought to play the major role in the
toxicity and carcinogenicity, more information is needed about their covalent binding to nucleic acids and
cellular macromolecules. This information would help the development of methods for possible
prevention of benzene-induced toxicity. Related lines of investigation include the use of non-steroidal
anti-inflammatory drugs to block prostaglandin and prostaglandin synthetase-mediated activity after
benzene exposure, and the role of IL-1 cytokine activity in preventing depression of hematopoiesis.

Children's Susceptibility.   Data needs relating to both prenatal and childhood exposures, and
developmental effects expressed either prenatally or during childhood, are discussed in detail in the
Developmental Toxicity subsection above.

No clear evidence of age-related differences in susceptibility to benzene toxicity was located. Benzene
crosses the placenta (Dowty et al. 1976). Nursing infants can be exposed to benzene in the breast milk
(Fabietti et al. 2004). Limited animal studies indicate that *in utero* exposure to benzene results in
hematological changes similar to those observed in animals exposed only as adults (Corti and Snyder
1996; Keller and Snyder 1986, 1988). There is some indication that parental occupational exposure to
benzene may play a role in childhood leukemia (Buckley et al. 1989; McKinney et al. 1991; Shaw et al.
1984; Shu et al. 1988). However, none of these studies indicate whether children may be at greater risk
than adults for benzene toxicity. Children could potentially be at increased risk for benzene toxicity via
the inhalation exposure route based on higher activity levels and ventilation rates than adults. Age-related
differences in benzene metabolism could potentially affect susceptibility. Well-designed animal studies
should be performed to adequately assess the potential for age-related increased susceptibility to benzene.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Ongoing studies pertaining to benzene have been identified and are shown in Table 3-7 (FEDRIP 2005).

Table 3-7. Ongoing Studies on the Health Effects of Benzene

Investigator	Affiliation	Research description	Sponsor
Conaway CC	University of California Lawrence Livermore National Laboratory	DNA binding of radiolabeled benzene	National Center for Research Resources
French JE	National Institute of Environmental Health Sciences	Carcinogen inactivation of tumor suppressor genes in p53 mice	National Institute of Environmental Health Sciences
French JE	National Institute of Environmental Health Sciences	Mechanisms of leukemogenesis in genetically-altered mice	National Institute of Environmental Health Sciences
Sabri MI	Oregon Health and Science University	Biomarkers of exposure and effect in neurotoxicants including neurotic benzene derivatives	National Institute of Environmental Health Sciences
Monks TJ	University of Texas at Austin	Benzene metabolites and hematotoxicity	National Institute of Environmental Health Sciences
Ross D	Texas A&M University System	Protective effect of NQ01 against benzene toxicity	National Institute of Environmental Health Sciences
Smith MT	University of California Berkeley	Biomarkers of benzene exposure and genotoxicity	National Institute of Environmental Health Sciences

Source: FEDRIP 2005

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4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY
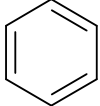
Information regarding the chemical identity of benzene is located in Table 4-1. Although the term benzol is found in older literature for the commercial product, benzene is the name presently approved by the International Union of Pure and Applied Chemistry (IUPAC), the Chemical Manufacturers Association (CMA), and the American Society for Testing and Materials (ASTM) for the pure product.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of benzene is located in Table 4-2. The major impurities found in commercial products are toluene, xylene, phenol, thiophene, carbon disulfide, acetylnitrile, and pyridine (NIOSH 1974). Commercial refined benzene-535 is free of hydrogen sulfide and sulfur dioxide, but contains a maximum of 1 ppm thiophene and a maximum of 0.15% nonaromatics. Refined nitration-grade benzene is free of hydrogen sulfide and sulfur dioxide. Benzene is also commercially available as thiophene-free, 99 mole%, 99.94 mole%, and nanograde quality (HSDB 2007).

Table 4-1. Chemical Identity of Benzene

Characteristic	Information	Reference
Chemical name	Benzene	HSDB 2007
Synonym(s)	Annulene, benzeen (Dutch), benzen (Polish), benzol, benzole; benzolo (Italian), coal naphtha, cyclohexatriene, fenzen (Czech), phene, phenyl hydride, pyrobenzol, pyrobenzole	HSDB 2007
Registered trade name(s)	Polystream	IARC 1982
Chemical formula	C_6H_6	Budavari et al. 2001
Chemical structure		Budavari et al. 2001
Identification numbers:		
CAS registry	71-43-2	HSDB 2007
NIOSH RTECS	CY-1400000	HSDB 2007
EPA hazardous waste	NA	
OHM/TADS	No Data	
DOT/UN/NA/IMCO shipping	UN1114; IMO3.2	HSDB 2007
HSDB	35	HSDB 2007
NCI	C55276	HSDB 2007
Merck	1066	Budavari et al. 2001

CAS = Chemical Abstracts Service; DOT/UN/NA/IMO=Department of Transportation/United Nations/North America/ Intergovernmental Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB=Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH= National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-2. Physical and Chemical Properties of Benzene

Property	Information	Reference
Molecular weight	78.11	Budavari et al. 2001
Color	Clear, colorless liquid	Budavari et al. 2001
Physical state	colorless to light yellow liquid	HSDB 2007
Melting point	5.5 °C	Budavari et al. 2001
Boiling point	80.1 °C	Budavari et al. 2001
Density at 15 °C, g/cm^3	0.8787	Budavari et al. 2001
Odor	Aromatic	NFPA 1994
Odor threshold:		
Water	2.0 mg/L	HSDB 2007
Air[a]	Detection range: 34–119 ppm (geometric mean: 61 ppm) Recognition: 97 ppm	AIHA 1989
Taste threshold:	0.5–4.5 mg/L	HSDB 2007
Solubility:		
Water at 25 °C	w/w: 0.188%	Budavari et al. 2001
Organic solvents	Alcohol, chloroform, ether, carbon disulfide, acetone, oils, carbon, tetrachloride, glacial acetic acid	Budavari et al. 2001
Partition coefficients:		HSDB 2007; Karickhoff 1981; Kenaga 1980
Log K_{ow}[b]	2.13	
Log K_{oc}[c]	1.8–1.9	
Vapor pressure at 20 °C	75 mm Hg	NFPA 1994
Henry's law constant at 25 °C	5.5×10^{-3} atm-m^3/mol	Mackay and Leinonen 1975
Autoignition temperature	498 °C	NFPA 1994
Flashpoint	-11 °C (closed cup)	Budavari et al. 2001
NFPA hazard classification:		HSDB 2007
Health	2.2	
Flammability	3.3	
Reactivity	0.0	
Flammability limits in air	1.2% (lower limit; 7.8% (upper limit)	NFPA 1994
Conversion factors	1 ppm=3.26 mg/m^3 at 20 °C and 1 atm pressure; 1 mg/m^3=0.31 ppm	HSDB 2007
Explosive limits	1.4% (lower limit); 8% (upper limit)	HSDB 2007

[a]Odor threshold values considered by AIHA (1989) to be acceptable based on review of peer-reviewed reports of odor thresholds for benzene (range 0.78–100 ppm).
[b]K_{ow} = octanol-water partitioning coefficient
[c]K_{oc} = soil adsorption coefficient

NFPA = National Fire Protection Association

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5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.1   PRODUCTION

In 1825, Faraday isolated benzene from a liquid condensed by compressing oil gas. Benzene was first
synthesized by Mitscherlich in 1833 by distilling benzoic acid with lime. Benzene was first commercially
recovered from light oil derived from coal tar in 1849 and from petroleum in 1941 (IARC 1982a).
Several years after the end of World War II, the rapidly expanding chemical industry created an increased
demand for benzene that the coal carbonization industry could not fulfill. To meet this demand, benzene
was produced by the petroleum and petrochemical industries by recovery from reformat and liquid
byproducts of the ethylene manufacturing process (Fruscella 1992).

Currently, benzene is commercially recovered from both coal and petroleum sources. More than 98% of
the benzene produced in the United States is derived from the petrochemical and petroleum refining
industries (OSHA 1987). These sources include refinery streams (catalytic reformats), pyrolysis gasoline,
and toluene hydrodealkylation. Catalytic reformat is the major source of benzene (Greek 1990). Between
1978 and 1981, catalytic reformats accounted for approximately 44–50% of the total U.S. benzene
production (Fishbein 1988). During catalytic reforming, cycloparaffins (also known by the obsolescent
term "naphthenes") such as cyclohexane, methyl cyclohexane, and dimethylcyclohexane are converted to
benzene by isomerization, dehydrogenation, and dealkylation, and paraffins in naphtha (such as hexane)
are converted to benzene by cyclodehydrogenation. The process conditions and the catalyst determine
which reaction will predominate. The benzene is recovered by solvent extraction (e.g., with sulpholane or
tetraethylene glycol). Pyrolysis gasoline is a liquid byproduct produced by the steam cracking of lower
paraffins (gas oil) or heavier hydrocarbons (heavy naphtha). Pyrolysis gasoline contains unsaturated
aliphatic hydrocarbons (such as ethylene and propylene) and aromatics. Several integrated pyrolysis
gasoline treatment processes are available including partial hydrogenation and extractive distillation;
hydrogenation, hydrodesulfurization, and solvent extraction; or partial hydrogenation, desulfurization,
hydrocracking, hydrodealkylation, and distillation for the optimization of benzene yield and the recovery
of benzene (Fruscella 1992; IARC 1982a). In the toluene hydrodealkylation process, toluene or
toluene/xylene mixtures are reacted with hydrogen at temperatures ranging from 500 to 595 °C with usual
pressures of 4–6 mPa (40–60 atm), and demethylated to produce benzene and methane. Another process
whereby toluene is converted to benzene and xylenes by transalkylation or disproportionation is also used
for the production of benzene (Fruscella 1992). Small quantities of benzene are also produced from
destructive distillation of coal used for coke manufacture. Benzene is derived from the light oil fraction
produced during the coking process (Greek 1990; Fruscella 1992). New coking, gasification, and

liquefaction processes for coal are all potential sources of benzene (IARC 1982a). Of the total U.S.

production capacity of 3.109 billion gallons in 2004, catalytic reformats constituted 45%, toluene and

xylene 30%, pyrolysis gasoline 23%, and coke oven <2% (SRI 2004).

Benzene ranks in the top 20 most abundantly produced chemicals in the United States (C&EN 1994;

Kirschner 1995; Reisch 1994). Production data from 1984 to 1994 indicate that the production of

benzene increased by about 4% annually (C&EN 1995).

According to the Toxics Release Inventory (TRI), 2,528 facilities in the United States produced or

processed benzene in 2004 (TRI04 2006). Table 5-1 lists the facilities in each state that manufacture or

process benzene, the intended use, and the range of maximum amounts of benzene that are stored on site.

The TRI data listed in Table 5-1 should be used with caution since only certain types of facilities were

required to report. Therefore, this is not an exhaustive list.

SRI (2004) lists the companies producing benzene in the United States in 2004. This list is summarized

in Table 5-2. Facilities listed as being responsible for producing the top 50% of regional capacity in the

United States include: Exxon Mobil Chemical Company, 518 million gallons (16%); Equistar Chemicals

LP, 330 million gallons (11%), Dow Chemical Company, 300 million gallons (10%); Flint Hills

Resources LP, 250 million gallons (8%), and BP Oil Company 230 million gallons (7%) (SRI 2004).

5.2   IMPORT/EXPORT

Benzene is imported and exported to the United States as both the pure chemical and as a mixture of

mineral fuels. The import of pure benzene into the United States is dependent on domestic production

and demand. Imports of benzene (from mineral fuels and pure benzene) into the United States were

approximately 5,252 million L (10,176 million pounds) in 2004, 6,024 million L (11,672 million pounds)

in 2003, and 5,912 million L (11,455 million pounds) in 2002 (USITC 2005). The largest exporters of

benzene to the United States during 2002-2004 were Canada, Iraq, Israel, Kuwait, Venezuela, and Saudi

Arabia (USITC 2005).

As in the case of import, the export of benzene from the United States to other countries is dependent on

domestic and world production and demand. The 2002–2004 data indicate an increase in export volumes

for benzene during this period. Exports of benzene (both pure benzene and benzene derived from mineral

fuels) to other countries were approximately 11 million L (21 million pounds) in 2002, 150 million L

Table 5-1. Facilities that Produce, Process, or Use Benzene

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AK	26	0	99,999,999	1, 2, 3, 4, 5, 7, 8, 9, 11, 12
AL	85	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
AR	24	100	9,999,999	1, 2, 3, 4, 6, 8, 9, 11, 12, 13
AS	1	100,000	999,999	9
AZ	30	100	9,999,999	1, 2, 4, 5, 7, 9, 10, 11, 12, 13, 14
CA	172	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
CO	23	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13
CT	8	1,000	9,999,999	1, 4, 6, 7, 9, 10, 12
DE	13	1,000	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13
FL	36	0	499,999,999	1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 14
GA	33	0	49,999,999	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
GU	6	100	9,999,999	2, 3, 4, 7, 9, 12
HI	15	100	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 12, 13, 14
IA	21	0	10,000,000,000	1, 2, 3, 4, 5, 7, 9, 10, 11, 12, 13, 14
ID	3	10,000	9,999,999	7, 9, 12
IL	102	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
IN	80	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
KS	59	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
KY	66	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
LA	212	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MA	15	100	99,999,999	2, 4, 7, 8, 9
MD	20	100	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12
ME	9	100	9,999,999	2, 3, 4, 7, 9, 12, 13
MI	108	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MN	36	100	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MO	30	0	9,999,999	1, 5, 7, 8, 9, 11, 12
MP	7	0	999,999	2, 3, 4, 7, 9
MS	43	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
MT	27	10,000	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14
NC	34	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13
ND	9	0	49,999,999	1, 2, 3, 4, 5, 6, 9, 12, 13
NE	11	0	9,999,999	1, 7, 10, 12, 13
NH	9	0	999,999	1, 2, 7, 9, 12, 13
NJ	61	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
NM	32	100	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14
NV	5	100	99,999	1, 2, 4, 8, 9, 12, 13
NY	48	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
OH	101	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14

Table 5-1. Facilities that Produce, Process, or Use Benzene

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
OK	48	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
OR	12	1,000	9,999,999	1, 2, 3, 4, 5, 7, 9, 12, 13
PA	100	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
PR	52	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
RI	9	10,000	9,999,999	1, 2, 4, 7, 8, 9
SC	26	0	9,999,999	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13
SD	8	100	999,999	2, 3, 4, 7, 8, 11, 12, 13, 14
TN	53	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
TX	361	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
UT	42	1,000	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
VA	36	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
VI	8	10,000	499,999,999	1, 2, 3, 4, 6, 7, 8, 9
WA	57	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14
WI	27	0	9,999,999	1, 4, 5, 7, 8, 9, 11, 12, 13, 14
WV	32	0	99,999,999	1, 2, 4, 5, 6, 7, 8, 9, 12, 13
WY	37	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 9, 12, 13, 14

[a]Post office state abbreviations used
[b]Amounts on site reported by facilities in each state
[c]Activities/Uses:

1. Produce
2. Import
3. Onsite use/processing
4. Sale/Distribution
5. Byproduct

6. Impurity
7. Reactant
8. Formulation Component
9. Article Component
10. Repackaging

11. Chemical Processing Aid
12. Manufacturing Aid
13. Ancillary/Other Uses
14. Process Impurity

Source: TRI04 2006 (Data are from 2004)

Table 5-2. Current U.S. Manufacturers of Benzene[a]

Company	Location (annual capacity millions of gallons)
ATOFINA Petrochemicals, Inc.	Port Arthur, Texas (87)
BASF FINA Petrochemicals, Inc.	Port Arthur, Texas (34)
BP America, Inc.	Texas City, Texas (230)
Ceveron Phillips Chemical Company, LP	Pascagoula, Mississippi (150)
Olefins and Polyolefins Business Unit	Port Arthur, Texas (35)
CITGO Petroleum Corporation	Corpus Christi, Texas (78)
	Lake Charles, Louisiana (55)
	Lemont, Illinois (19)
Conoco Phillips Refining Marketing and Transportation Division	Alliance Louisiana (65)
	Sweeny, Texas (39)
	Wood River Illinois (60)
Dow Chemical USA	Freeport, Texas (80)
	Plaquemine, Louisiana (317)
Equistar Chemicals, LP	Alvin, Texas (137)
	Channelveiw, Texas (90)
	Corpus Christi, Texas (103)
ExxonMobil Chemical Company	Baton Rouge Louisiana (100)
	Baytown, Texas (180)
	Beaumont, Texas (182)
	Chalmette, Louisiana (56)
Flint Hills Resources, LP	Corpus Christi, Texas (250)
Frontier El Dorado Refining Company	El Dorado, Kansas (15)
HOVENSA, LLC	St. Croix, Virgin Islands (60)
Huntsman, LLC	Port Arthur, Texas (90)
Lyondell-Citgo Refining, LP	Houston, Texas (50)
Marathon Ashland Petroleum, LLC	Catlettsburg, Kentucky (58)
	Texas City, Texas (3)
Motiva Enterprises, LLC	Delaware City, Delaware (15)
NOVA Chemicals Corp.	Bayport, Texas (15)
The Premcor Refining Group Inc.	Lima, Ohio (145)
Shell Chemical Company	Deer Park, Texas (180)
Sunoco, Inc.	Marcus Hook, Pennsylvania (60)
	Philadelphia, Pennsylvania (24)
	Toledo Ohio (20)
	Westville, New Jersey (18)
Valero Energy Corporation	Corpus Christi, Texas (82)
	Three Rivers, Texas (20)
Eagle-Pitcher Industries, Inc.	Lenexa, Kansas
Eagle-Pitcher Technologies, LLC	
Chem Syn Laboratiories	
(^{14}C-benzene)	

[a]Derived from Stanford Research Institute (SRI) 2004, receipt where otherwise noted. SRI reports production of chemicals produced in commercial quantities (defined as exceeding 5,000 pounds or $10,000 in value annually) by the companies listed.

(290 million pounds) in 2003, and 75 million L (145 million pounds) in 2004. These numbers are up

from 15 million L (29 million pounds) in 2001 and 3.3 million L (6.4 million pounds) in 1993 (USITC

2005). The largest importers of benzene from the United States are Canada, the Netherlands, Taiwan,

Spain, and Korea (USITC 2005).

5.3   USE

Benzene has been used extensively as a solvent in the chemical and drug industries, as a starting material

and intermediate in the synthesis of numerous chemicals, and as a gasoline additive (NTP 1994).

Benzene recovered from petroleum and coal sources is used primarily as an intermediate in the

manufacture of other chemicals and end products. The major uses of benzene are in the production of

ethylbenzene, cumene, and cyclohexane. Ethylbenzene (55% of benzene production volume) is an

intermediate in the synthesis of styrene, which is used to make plastics and elastomers. Cumene (24%) is

used to produce phenol and acetone. Phenols are used in the manufacture of phenolic resins and nylon

intermediates; acetone is used as a solvent and in the manufacture of pharmaceuticals. Cyclohexane

(12%) is used to make nylon resins. Other industrial chemicals manufactured from benzene include

nitrobenzene (5%), which is used in the production of aniline, urethanes, linear alkylbenzene sulfonates,

chlorobenzene, and maleic anhydride (Eveleth 1990; Greek 1990; HSDB 2007). Benzene is also a

component of gasoline since it occurs naturally in crude oil and since it is a byproduct of oil refining

processes (Brief et al. 1980; Holmberg and Lundberg 1985). Benzene is especially important for

unleaded gasoline because of its anti-knock characteristics. For this reason, the concentration of

aromatics, such as benzene, in unleaded fuels has increased (Brief et al. 1980). The percentage by

volume of benzene in unleaded gasoline is approximately 1–2% (NESCAUM 1989).

The EPA has listed benzene as a hazardous air pollutant and a hazardous waste (EPA 1977, 1981). In

addition, there is sufficient evidence to support classifying benzene as a human carcinogen (Group A)

(IRIS 2007). One result of EPA's action is that the widespread use of benzene as a solvent has decreased

in recent years. Many products that used benzene as solvents in the past have replaced it with other

organic solvents; however, benzene may still occur as a trace impurity in these products. Less than 2% of

the amount produced is used as a solvent in products such as trade and industrial paints, rubber cements,

adhesives, paint removers, artificial leather, and rubber goods. Benzene has also been used in the shoe

manufacturing and rotogravure printing industries (EPA 1978; OSHA 1977). In the past, certain

consumer products (such as some paint strippers, carburetor cleaners, denatured alcohol, and rubber

cement used in tire patch kits and arts and crafts supplies) contained small amounts of benzene (Young et al. 1978). Other consumer products that contained benzene were certain types of carpet glue, textured carpet liquid detergent, and furniture wax (Wallace et al. 1987).

The Consumer Products Safety Commission (CPSC) withdrew an earlier proposal to ban consumer products, except gasoline and laboratory reagents, that contained benzene as an intentional ingredient or as a contaminant at >0.1% by volume. The withdrawal of the rulemaking was based on CPSC findings that benzene was no longer used as an intentional ingredient and that the contaminant levels remaining in certain consumer products were unlikely to result in significant exposures (NTP 1994). Products containing >5% benzene, and paint solvents and thinners containing <10% of petroleum distillates such as benzene, are required to meet established labeling requirements. In a guidance document targeting school science laboratories, the CPSC recommended that benzene not be used or stored in schools. The document identified benzene as a carcinogen and ascertained that the hazards posed by its use in high school laboratories may be greater than its potential usefulness.

The U.S. Food and Drug Administration (FDA) regulates benzene as an indirect food additive under the Food, Drug, and Cosmetics Act (FDCA). Under the FDCA, benzene is restricted to use only as a component of adhesives used on articles intended for packaging, transport, or holding foods (FDA 1977).

5.4   DISPOSAL

Benzene-containing wastes, such as commercial chemical products, manufacturing chemical intermediates, and spent solvents, are subject to federal and/or state hazardous waste regulations (HSDB 2007). Regulations governing the treatment and disposal of benzene-containing wastes are presented in Chapter 8. Waste byproducts from benzene production processes include acid and alkali sludges, liquid-solid slurries, and solids (EPA 1982b; Saxton and Narkus-Kramer 1975). In the past, landfilling and lagooning have been the major methods of disposal of benzene-containing industrial wastes (EPA 1982b). In addition to biodegradation, a portion of the benzene is expected to be lost due to volatilization. Unfortunately benzene, along with other hazardous contaminants, also leaches into groundwater from the lagooned wastes. Currently, the recommended method of disposal is to incinerate solvent mixtures and sludges at a temperature that ensures complete combustion. The recommended methods for combustion are liquid injection incineration at a temperature range of 650–1,600 °C and a residence time of 0.1– 2 seconds; rotary kiln incineration at a temperature range of 820–1,600 °C and residence times of seconds for liquids and gases, and hours for solids; and fluidized bed incineration at a temperature range of 450–

980 °C and residence times of seconds for liquids and gases and longer for solids (HSDB 2007). Since benzene burns with a very smoky flame, dilution with alcohol or acetone is suggested to minimize smoke. Small quantities of benzene waste can be destroyed by chemical reaction. For example, treating benzene with dichromate in strong sulfuric acid for 1–2 days is sufficient for total destruction (HSDB 2007).

Underground injection also appears to be an important disposal method in some states. Approximately 436,000 pounds of benzene (6% of the total environmental release) was disposed of by underground injection. This disposal via underground injection in 2006 was higher than the amount (356,000 pounds) released in 1992 (TRI90 1992), but lower than the release in 1990 (654,000 pounds) (TRI90 1992) and 2002 (692,000 pounds) (TRI02 2005). In addition, 24,000 pounds of benzene (0.3% of the total environmental release) was disposed of via land disposal (TRI04 2006). The amount discharged to soil in 2002 was less than one quarter of the amount (724,000 pounds) discharged in 1990 (TRI90 1992) and less than one third of the amount (340,000 pounds) discharged in 1992 (TRI92 1994).

Several methods exist for the treatment of waste water that contains benzene: biological treatment (aeration or activated sludge process), solvent extraction, air and/or steam stripping, and activated carbon process (EPA 1994a; IRPTC 1985). Full-scale chemical treatability studies have demonstrated 95–100% reductions in benzene concentrations for industrial waste waters receiving biological treatments (HSDB 2007). A combination of steam stripping and air stripping, and a vapor extraction system that removes the separated benzene vapor may be suitable for the treatment of contaminated groundwater and soil (Naft 1992). An *in situ* bioremediation process has been used to decontaminate a site by delivering a controlled amount of nitrate (to accelerate biodegradation of benzene) to the site under hydraulic control (Kennedy and Hutchins 1992).

6. POTENTIAL FOR HUMAN EXPOSURE

6.1   OVERVIEW

Benzene has been identified in at least 1,000 of the 1,684 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2006). However, the number of sites evaluated for benzene is not known. The frequency of these sites can be seen in Figure 6-1. Of these sites, 995 are located within the United States, 3 are located in the Commonwealth of Puerto Rico (not shown), and 2 are located in the Virgin Islands (not shown).

Benzene is released to the environment by both natural and industrial sources, although the anthropogenic emissions are undoubtedly the most important. Emissions of benzene to the atmosphere result from gasoline vapors, auto exhaust, and chemical production and user facilities. EPA's estimate of nationwide benzene atmospheric emissions from various sources was 34,000 metric tons/year (EPA 1989). According to the Toxics Release Inventory, releases to the air from manufacturing and processing facilities were about 6,7 million pounds (3,055 metric tons) in 2004 (TRI04 2006). Releases to air accounted for about 93% of the total industry-related releases to the environment (TRI04 2006). Benzene is released to water and soil from industrial discharges, landfill leachate, and gasoline leaks from underground storage tanks.

Chemical degradation reactions, primarily reaction with hydroxyl radicals, limit the atmospheric residence time of benzene to only a few days, and possibly to only a few hours. Benzene released to soil or waterways is subject to volatilization, photooxidation, and biodegradation. Biodegradation, principally under aerobic conditions, is an important environmental fate process for water- and soil-associated benzene.

Benzene is ubiquitous in the atmosphere. It has been identified in air samples of both rural and urban environments and in indoor air. Although a large volume of benzene is released to the environment, environmental levels are low because of efficient removal and degradation processes. Benzene partitions mainly into air (99.9%) and inhalation is the dominant pathway of human exposure accounting for >99% of the total daily intake of benzene (Hattemer-Frey et al. 1990; MacLeod and MacKay 1999). The general population is exposed to benzene primarily by tobacco smoke (both active and passive smoking) and by inhaling contaminated air (particularly in areas with heavy motor vehicle traffic and around filling stations). Air around manufacturing plants that produce or use benzene and air around landfills and hazardous waste sites that contain benzene are additional sources of exposure. Exposure to benzene can

Figure 6-1. Frequency of NPL Sites with Benzene Contamination

Frequency of
NPL Sites

- 1-10
- 11-19
- 21-28
- 31-45
- 61-74
- 96
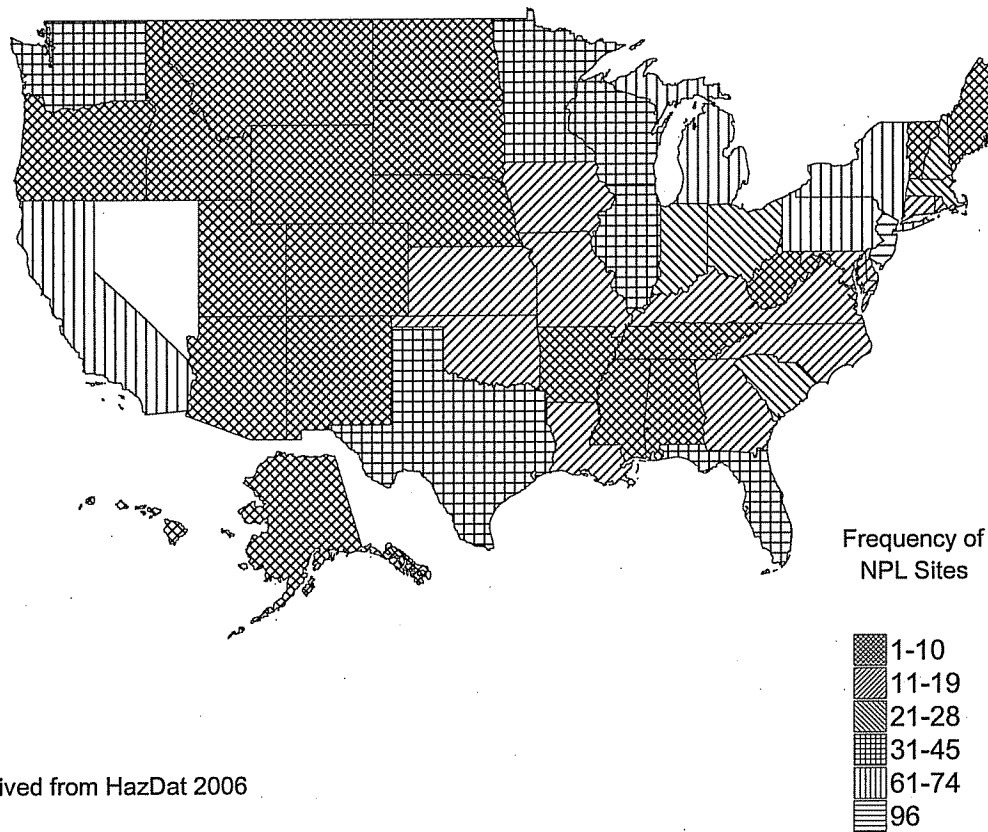
Derived from HazDat 2006

also result from ingestion of contaminated food or water. Use of contaminated tap water for cooking, showering, etc., can also be a source of inhalation exposure since benzene can volatilize from water. Compared to inhalation, dermal exposure accounts for a minor portion of the total exposure of the general population. The magnitude of exposure is greatest for those individuals occupationally exposed to benzene; however, a far greater number of individuals are exposed as a result of benzene released from smoking tobacco products, from gasoline filling stations, and from auto exhaust. Smoking was found to be the largest anthropogenic source of direct human exposure to benzene (Duarte-Davidson et al. 2001; Hattemer-Frey et al. 1990).

6.2   RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005g). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ 10 or more full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005g).

6.2.1   Air

Estimated releases of 6.7 million pounds (~ 3,055 metric tons) of benzene to the atmosphere from 968 domestic manufacturing and processing facilities in 2004, accounted for about 93% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). These releases are summarized in Table 6-1.

Benzene is released into the atmosphere from both natural and industrial sources. Natural sources include crude oil seeps, forest fires, and plant volatiles (Brief et al. 1980; Graedel 1978). Major anthropogenic sources of benzene include environmental tobacco smoke, automobile exhaust, automobile refueling

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Benzene[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AK	7	19,568	19	0	375	0	19,962	0	19,962
AL	15	290,660	290	0	280	2	290,955	277	291,232
AR	8	33,137	66	0	0	0	33,203	0	33,203
AZ	13	4,910	0	0	9	0	4,915	4	4,919
CA	65	57,611	129	228	1,292	598	57,970	1,888	59,858
CO	11	19,630	0	0	10	36	19,630	46	19,676
CT	9	2,552	2	0	0	2	2,554	2	2,556
DE	5	3,425	6,006	0	26	0	9,431	26	9,457
FL	26	396,187	41	0	0	157	396,228	157	396,385
GA	15	36,893	128	0	9	0	37,026	4	37,030
GU	3	7,106	1	0	0	401	7,107	401	7,508
HI	9	11,171	30	0	12	0	11,201	12	11,213
IA	14	42,671	1	0	0	0	42,672	0	42,672
ID	2	1,732	No data	0	0	0	1,732	0	1,732
IL	35	171,104	102	0	2,152	104	172,227	1,235	173,462
IN	30	211,476	805	14,001	4,654	29	227,079	3,886	230,965
KS	19	69,823	164	231	259	0	70,226	251	70,477
KY	22	77,345	799	0	766	20	78,904	26	78,930
LA	62	605,128	1,075	122,723	1,688	968	729,109	2,473	731,582
MA	10	5,556	55	0	26	537	5,611	563	6,173
MD	11	12,644	8	0	184	1	12,821	15	12,836
ME	5	3,027	23	0	0	467	3,049	467	3,516
MI	41	345,255	8	0	127	2,295	345,363	2,322	347,686
MN	16	16,614	2,724	0	131	1,561	19,360	1,670	21,030
MO	17	98,498	0	0	0	0	98,498	0	98,498
MP	2	710	0	0	0	0	710	0	710
MS	13	59,076	25	0	14	0	59,102	13	59,115
MT	5	17,242	5	0	15	0	17,248	14	17,262
NC	12	17,290	0	0	5	48	17,295	48	17,343
ND	2	6,400	0	0	1	0	6,400	1	6,401
NE	9	1,333	No data	0	0	6	1,333	6	1,339
NJ	20	51,054	443	0	150	169	51,510	306	51,816
NM	9	38,730	No data	7	2	0	38,739	0	38,739
NV	3	1,005	No data	0	0	0	1,005	0	1,005
NY	44	60,682	56	0	10	1,335	60,738	1,345	62,084
OH	52	183,972	67	5	730	82	184,049	807	184,856
OK	11	114,701	14	0	278	71	114,984	80	115,064

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Benzene[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
OR	6	4,651	8	0	41	0	4,659	41	4,700
PA	39	234,647	419	0	345	11,389	235,066	11,733	246,800
PR	10	49,879	7	0	0	0	49,886	0	49,886
RI	2	1,841	4	0	0	820	1,845	820	2,665
SC	6	64,627	6	0	250	1,023	64,883	1,023	65,906
SD	11	272	1	0	0	0	273	0	273
TN	17	73,926	63	0	280	0	73,989	280	74,269
TX	150	2,681,863	621	298,595	8,326	1,713	2,935,539	55,579	2,991,118
UT	11	31,314	750	0	809	233	32,069	1,038	33,107
VA	17	183,748	787	0	26	0	184,535	26	184,561
VI	3	20,576	0	0	9	0	20,576	9	20,585
WA	18	50,667	14	0	95	48	50,770	54	50,824
WI	14	141,183	0	0	251	63	141,183	314	141,496
WV	5	76,556	284	0	393	0	76,840	393	77,233
WY	7	24,986	0	0	1	0	24,986	1	24,987
Total	968	6,736,655	16,051	435,790	24,033	24,179	7,147,049	89,659	7,236,707

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other on-site landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI04 2006 (Data are from 2004)

operations, and industrial emissions. Using source exposure modeling, it was estimated that benzene emissions were highest from coke oven blast furnaces (Edgerton and Shah 1992). Other sources that contributed to emissions of benzene include automobiles, petrochemical industries, waste water treatment plants, and petroleum industries (Edgerton and Shah 1992).

Industrial and automotive sources of benzene are well monitored. EPA (1989) estimates of nationwide benzene atmospheric emissions, in metric tons/year (kkg/year), from various industrial sources include: (1) coke byproduct recovery plants (17,000 kkg/year), (2) benzene waste operations (5,300 kkg/year), (3) gasoline marketing systems, including bulk gasoline terminals and plants, service stations, and delivery tank trucks (4,800 kkg/year), (4) transfer operations at chemical production facilities, bulk terminals, and coke byproduct recovery plants (4,600 kkg/year), (5) benzene storage vessels (620–1,290 kkg/year), (6) industrial solvent use (450 kkg/year), (7) chemical manufacturing process vents (340 kkg/year), and (8) ethylbenzene/styrene process vents (135 kkg/year). Benzene is present in passenger car tailpipe emissions at compositions ranging from 2.9 to 15% of the total tailpipe hydrocarbon composition (Black et al. 1980). The 2002 benzene industrial emission inventory for California totaled 266 metric tons/year (CARB 2005); these numbers did not include motor vehicle exhaust,which accounted for 71% of emissions in 1984 (Cal EPA 1987). The contribution of mobile source hazardous air pollutant emissions has been compared to that of stationary sources in the Seattle-Tacoma area. Mobile sources were estimated to contribute approximately 83% of the benzene from stationary areas and mobile sources combined, with major stationary point sources excluded (EPA 1994d). Benzene is also released by off-gassing from particle board (Glass et al. 1986), vaporization from oil spills, and emissions from landfills (Bennett 1987; Wood and Porter 1987). While all of these sources release more benzene into the environment, a large percentage of the benzene inhaled by humans comes from cigarette smoke. Exhaled breath of smokers contains benzene (Wallace 1989a, 1989b; Wallace and Pellizzari 1986; Wester et al. 1986).

According to the Texas Natural Resource Conservation Commission (TNRCC), of the 93 districts in Texas reporting benzene emissions from industrial sources, in 1980, 1985, and 1988, 10 districts reported 0 tons/year, 79 districts reported 0.1–100 tons/year, 4 districts reported 100–400 tons/year, 2 districts reported 400–1,000 tons/year, and 1 district had emissions projected at above 1,000 tons/year (Pendleton 1995).

The natural sources most monitored for benzene released to air are fires (Austin et al. 2001; Lowry et al. 1985). A study of nine municipal fires in Canada found a mean concentration of 3.45 ppm of benzene

and had very high relative concentration of other volatile organic compounds (VOCs) monitored (Austin et al. 2001).

There is a potential for atmospheric release of benzene from hazardous waste sites. Benzene has been detected in air in 200 of the 1,684 current and former NPL sites where it has been detected in some medium (HazDat 2006).

6.2.2   Water

Estimated releases of 16,051 pounds (~7 metric tons) of benzene to surface water from 968 domestic manufacturing and processing facilities in 2004, accounted for about 0.2% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). These releases are summarized in Table 6-1.

Benzene is released to water from the discharges of both treated and untreated industrial waste water, gasoline leaks from underground storage tanks, accidental spills during marine transportation of chemical products, and leachate from landfills and other contaminated soils, (CDC 1994; Crawford et al. 1995; EPA 1979; NESCAUM 1989; Staples et al. 1985). A fire in a tire dump site in western Frederick County, Virginia, produced a free-flowing oily tar containing benzene among other chemicals. The seepage from this site contaminated nearby surface water (EPA 1993). Between 1986 and 1991, 3,000 gallons of benzene were accidentally released into Newark Bay and its major tributaries. Another 3,000 gallons were released in 1991 (Crawford et al. 1995).

There is a potential for release of benzene to water from hazardous waste sites. Benzene has been detected in groundwater samples collected at 832 of the 1,684 current and former NPL sites and in surface water samples collected at 208 of the 1,684 sites (HazDat 2006).

6.2.3   Soil

Estimated releases of 24,033 pounds (~11 metric tons) of benzene to soils from 968 domestic manufacturing and processing facilities in 2004, accounted for about 0.3% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). An additional 435,000 pounds (~197 metric tons), constituting about 6% of the total environmental emissions, were released via underground injection (TRI04 2006). These releases are summarized in Table 6-1.

Benzene is released to soils through industrial discharges, land disposal of benzene-containing wastes, and gasoline leaks from underground storage tanks. In northern Virginia, approximately 200,000 gallons of liquid hydrocarbons were released from a fuel-storage terminal into the underlying soil (Mushrush et al. 1994).

There is a potential for release of benzene to soil from hazardous waste sites. Benzene has been detected in soil samples collected at 436 of the 1,684 sites, and in sediment samples collected at 145 of the 1,684 sites where benzene has been detected in some medium (HazDat 2006).

6.3 ENVIRONMENTAL FATE

6.3.1 Transport and Partitioning

The high volatility of benzene is the controlling physical property in the environmental transport and partitioning of this chemical. Benzene is considered to be highly volatile with a vapor pressure of 95.2 mm Hg at 25 °C. Benzene is moderately soluble in water, with a solubility of 1,780 mg/L at 25 °C, and the Henry's law constant for benzene (5.5×10^{-3} atm-m^3/mole at 25 °C) indicates that benzene partitions readily to the atmosphere from surface water (Mackay and Leinonen 1975). Since benzene is soluble in water, some minor removal from the atmosphere via wet deposition may occur. A substantial portion of any benzene in rainwater that is deposited to soil or water will be returned to the atmosphere via volatilization.

Benzene released to soil surfaces partitions to the atmosphere through volatilization, to surface water through runoff, and to groundwater as a result of leaching. The soil organic carbon sorption coefficient (K_{oc}) for benzene has been measured with a range of 60–83 (Karickhoff 1981; Kenaga 1980), indicating that benzene is highly mobile in soil and readily leaches into groundwater. Other parameters that influence leaching potential include the soil type (e.g., sand versus clay), amount of rainfall, depth of the groundwater, and extent of degradation. In a study of the sorptive characteristics of benzene to groundwater aquifer solids, benzene showed a tendency to adsorb to aquifer solids. Greater soil adsorption was observed with increasing organic matter content (Uchrin and Mangels 1987). An investigation of the mechanisms governing the rates of adsorption and desorption of benzene by dry soil grains revealed that periods of hours are required to achieve equilibrium and that adsorption is much faster than desorption (Lin et al. 1994). The rate of volatilization and leaching are the principal factors that determine overall persistence of benzene in sandy soils (Tucker et al. 1986).

Studies suggest that benzene does not bioaccumulate in marine organisms. The bioconcentration/ bioaccumulation potential of benzene in aquatic organisms of the open coastal ocean was investigated by sampling final effluent from the Los Angeles County waste water treatment plant quarterly from November 1980 to August 1981 (Gossett et al. 1983). Benzene has a relatively low octanol/water partition coefficient (log K_{ow}=2.13 or 2.15) (Gossett et al. 1983; HSDB 2007). In the alga, *Chlorella,* a bioaccumulation factor of 30 was determined experimentally (Geyer et al. 1984). An experimental bioconcentration factor (BCF) of 4.27 was measured in goldfish reared in water containing 1 ppm of benzene (Ogata et al. 1984). Based on these measured values, bioconcentration/bioaccumulation of benzene in the aquatic food chains does not appear to be important. These results are consistent with the fact that benzene has a relatively low octanol/water partition coefficient (Gossett et al. 1983; HSDB 2007), suggesting relatively low bioaccumulation. There is no evidence in the literature of biomagnification of benzene in aquatic food chain.

Evidence exists for the uptake of benzene by cress and barley plants from soil (Scheunert et al. 1985; Topp et al. 1989). BCFs for barley plants after 12, 33, 71, and 125 days were 17, 2.3, 2.9, and 4.6, respectively. BCFs for cress plants after 12, 33, and 79 days were 10, 2.3, and 1.9, respectively. The relative decrease in the BCFs with time was attributed to growth dilution (Topp et al. 1989). Since benzene exists primarily in the vapor phase, air-to-leaf transfer is considered to be the major pathway of vegetative contamination (Hattemer-Frey et al. 1990). Based on an equation to estimate vegetative contamination, the total concentration of benzene on exposed food crops consumed by humans and used as forage by animals was estimated to be 587 ng/kg, 81% of which was from air-to-leaf transfer and 19% was from root uptake (Hattemer-Frey et al. 1990).

Benzene also accumulates in leaves and fruits of plants. After 40 days, plants grown in benzene-rich environments showed bioaccumulation in the leaves and fruit that were greater than the air portioning coefficient of benzene in the atmosphere. Blackberries exposed to 0.313 ppm and apples exposed to 2.75 ppm contained about 1,000 and 36 ng/g of benzene, respectively (Collins et al. 2000).

6.3.2   Transformation and Degradation

Benzene undergoes a number of different transformation and degradation reactions in the environment as discussed in the following sections. The resulting environmental transformation products within different media are shown in Figure 6-2.

Figure 6-2. Environmental Transformation Products of Benzene in Various Media
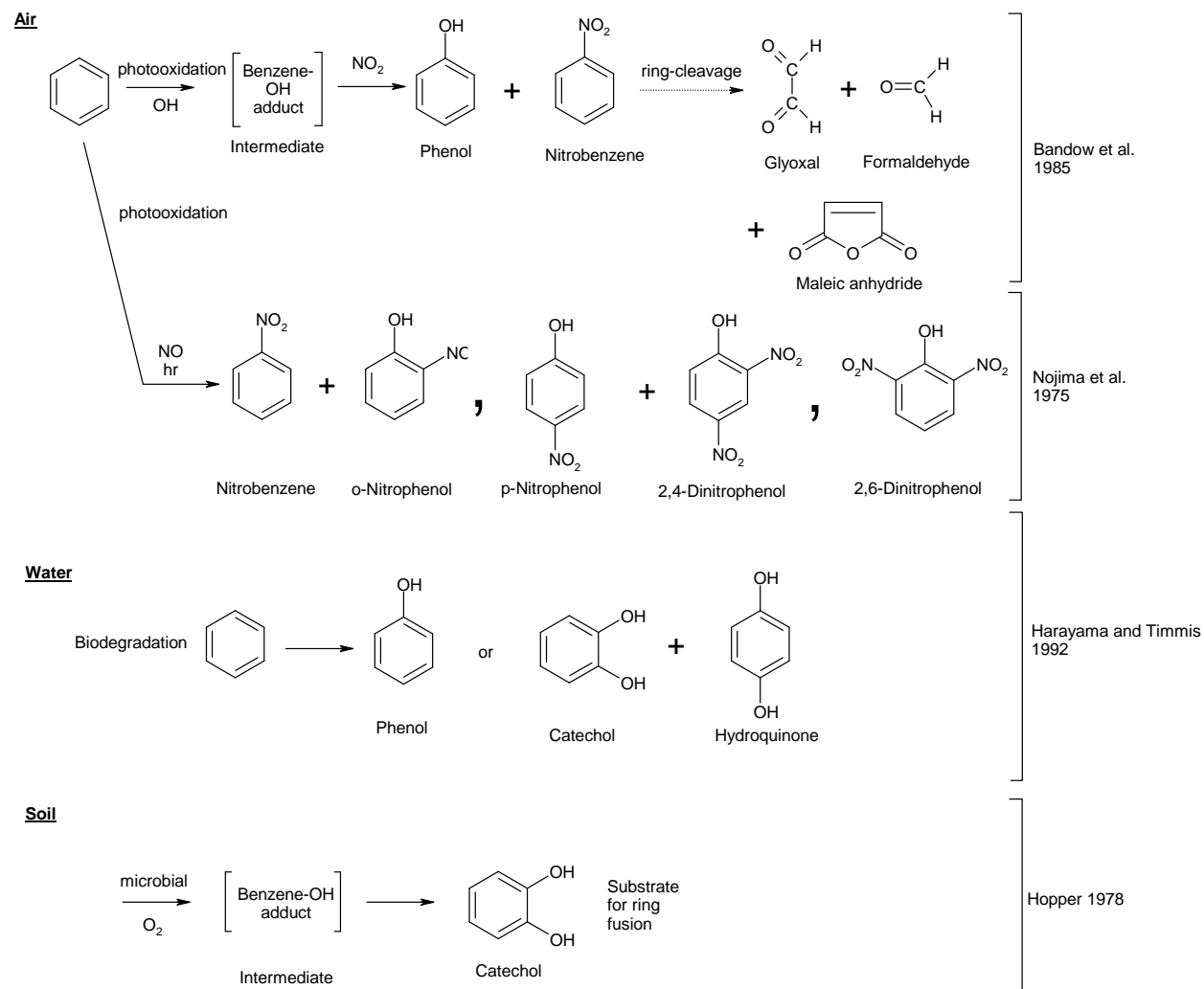
6.3.2.1   Air

Benzene in the atmosphere exists predominantly in the vapor phase (Eisenreich et al. 1981). The most significant degradation process for benzene is its reaction with atmospheric hydroxyl radicals. The rate constant for the vapor phase reaction of benzene with photochemically produced hydroxyl radicals has been determined to be $1.3x10^{-12}$ cm^3/molecule-second, which corresponds to a residence time of 8 days at an atmospheric hydroxyl radical concentration of $1.1x10^6$ molecules/cm^3 (Gaffney and Levine 1979; Lyman 1982). With a hydroxyl radical concentration of $1x10^8$ molecules/cm^3, corresponding to a polluted atmosphere, the estimated residence time is shortened to 2.1 hours (Lyman 1982). Benzene may also react with other oxidants in the atmosphere such as nitrate radicals and ozone; however, the rate of degradation is considered insignificant compared to the rate of reaction with hydroxyl radicals. Residence times of 472 years for rural atmospheres and 152 years for urban atmospheres were calculated for the reaction of benzene with ozone (O_3) using a rate constant for O_3 of $7x10^{-23}$ cm^3/molecule-second (Pate et al. 1976) and atmospheric concentrations for O_3 of $9.6x10^{11}$ molecules/cm^3 (rural) and $3x10^{12}$ molecules/cm^3 (urban) (Lyman 1982).

The reaction of benzene and nitric oxide in a smog chamber was investigated to determine the role of benzene in photochemical smog formation (Levy 1973). The results showed that benzene exhibited low photochemical smog reactivity in the four categories tested: rate of photooxidation of nitric oxide, maximum oxidant produced, eye-irritation response time, and formaldehyde formation. The authors concluded that benzene probably does not play a significant role in photochemical smog formation (Levy 1973). In the presence of active species such as nitrogen oxides and sulfur dioxide, the rate of photodegradation of benzene in the gas phase was greater than that in air alone. Its half-life in the presence of such active species (100 ppm benzene in the presence of 10–110 ppm NO_x or 10– 100 ppm SO_2) was 4–6 hours with 50% mineralization to carbon dioxide in approximately 2 days (Korte and Klein 1982). Some of the products of the reaction of benzene with nitrogen monoxide gas (e.g., nitrobenzene, *o*- and *p*-nitrophenol, and 2,4- and 2,6-dinitrophenol) may have potentially adverse effects on human health (Nojima et al. 1975); however, these species also have relatively short atmospheric lifetimes, which should limit the potential exposure to these compounds. Photooxidation of benzene in a nitrogen monoxide/nitrogen dioxide-air system formed formaldehyde, formic acid, maleic anhydride, phenol, nitrobenzene, and glyoxal (ethane-1,2-dione) (Bandow et al. 1985).

Direct photolysis of benzene in the atmosphere is not likely because the upper atmosphere effectively filters out wavelengths of light <290 nm, and benzene does not absorb wavelengths of light >260 nm (Bryce-Smith and Gilbert 1976).

6.3.2.2 Water

Benzene is subject to indirect photolysis in sunlit surface water, but does not undergo direct photolysis. For direct photolysis to occur, a substance must absorb photons of light >290 nm. During indirect photolysis, light energy is absorbed by other constituents (photosensitizers) of the media (water, soil) and the excited species can then transfer energy to benzene (indirectly promoting it to an excited electronic state), or lead to the formation of reactive species, such as singlet oxygen or hydroxy radicals, which react with benzene. Humic and fulvic acids are well-known photosensitizing agents and are practically ubiquitous in natural waters. A half-life of 16.9 days was reported for photolysis of benzene dissolved in oxygen-saturated deionized water and exposed to sunlight (Hustert et al. 1981).

Benzene is readily degraded in water under aerobic conditions. Results of a biochemical oxygen demand (BOD) test determined that benzene was completely biodegradable after the second week of static incubation at 25 °C at benzene concentrations of 5 and 10 mg/L using domestic waste water as the microbial inoculum (Tabak et al. 1981). A study of the degradation of benzene by the microbial population of industrial waste water at 23 °C using a shaker flask system showed that after 6 hours, only 8% (4 mg/L) of the initial 50 mg/L dose of benzene remained (Davis et al. 1981). Water from a petroleum production site was successfully biotreated for complete removal of benzene using a flocculated culture of *Thiobacillus denitrificans* strain F and mixed heterotrophs (Rajganesh et al. 1995).

Microbial degradation of benzene in aquatic environments is influenced by many factors including microbial population, dissolved oxygen, nutrients, other sources of carbon, inhibitors, temperature, pH, and initial concentration of benzene. Vaishnav and Babeu (1987) reported biodegradation half-lives for benzene in surface water (river water) and groundwater of 16 and 28 days, respectively. Benzene was found to be resistant to biodegradation in surface water taken from a harbor and supplemented with either nutrients (nitrogen and phosphorus) or acclimated microbes, however, biodegradation did occur, with a half-life of 8 days, in surface water enriched with both nutrients and microbes (Vaishnav and Babeu 1987). At very high levels, as may be the case of a petroleum spill, benzene (and other compounds contained in petroleum) is toxic to microorganisms and the rate of degradation is slow compared to low initial starting concentrations. In another study, Davis et al. (1994) observed rapid aerobic biodegradation

of benzene in aquifer groundwater samples and measured times for 50% disappearance ranging from
4 days for an initial benzene concentration of 1 mg/kg to 14 days for an initial benzene concentration of
10 mg/kg. Under acidic conditions (pH 5.3, 20 °C), benzene was completely microbially degraded in
16 days in groundwater taken from a shallow well (Delfino and Miles 1985).

The aerobic biodegradation of benzene is also influenced by the presence of other aromatic hydrocarbons.
A bacterial culture grown with aromatic hydrocarbons plus nitrogen-, sulfur-, and oxygen-containing
aromatic compounds was much less efficient in degrading benzene than the culture grown with aromatic
hydrocarbons alone. Pyrrole strongly inhibited benzene degradation. Benzene degradation was high
when toluene and xylene were present (Arvin et al. 1989).

Laboratory studies on microbial degradation of benzene with mixed cultures of microorganisms in
gasoline-contaminated groundwater revealed that both oxygen and nitrogen concentrations are major
controlling factors in the biodegradation of benzene. Nitrogen enhanced the biodegradation rate of
benzene 4.5-fold, over innoculum-enriched water alone. More than 95% of the benzene in groundwater
was removed through microbial action within 73.5 hours (Karlson and Frankenberger 1989).

Benzene biodegradation under anaerobic conditions does not readily occur. When dissolved oxygen is
depleted, an alternative electron acceptor such as nitrate, carbonate, or iron(III) must be available, and
microbes capable of using the alternative electron acceptor to degrade the benzene must be present
(McAllister and Chiang 1994). Using aquifer material obtained from a landfill from Norman, Oklahoma,
no significant benzene biodegradation was reported during the first 20 weeks of incubation under
anaerobic conditions at 17 °C; however, after 40 weeks of incubation, benzene concentrations were
reduced by 72 and 99% of the benzene was degraded after 120 weeks (Wilson et al. 1986). No
degradation of benzene was observed in 96 days under anaerobic conditions (20 °C) using raw water
intake from a water treatment plant (Delfino and Miles 1985).

Use of water as an oxygen source in the anaerobic degradation of benzene has been demonstrated.
Experiments indicated that incorporation of ^{18}O from ^{18}O-labeled water is the initial step in the anaerobic
oxidation of benzene by acclimated methanogenic cultures. Phenol was the first major product (Vogel
and Grbić-Galić 1986).

6.3.2.3 Sediment and Soil

Benzene is biodegraded in soil under aerobic conditions. Microbial metabolism of benzene proceeds through the formation of *cis*-dihydrodiols and, with further metabolism, to catechols, which are the substrates for ring fission (Gibson 1980; Hopper 1978). *Pseudomonas putida* oxidized benzene through *cis*-1,2-dihydroxy-1,2-dihydrobenzene (Gibson 1977; Hopper 1978). After acclimation, *Norcardia* species and *Pseudomonas* species, effectively degraded benzene to carbon dioxide within 7 days (45– 90%). A strain of *Rhodococcus* isolated from contaminated river sediment mineralized 71% of benzene at an initial concentration of 0.7 mg/L in 14 days (Malachowsky et al. 1994). The soil bacterium *Nitrosomonas europaea* catabolized benzene to phenol and hydroquinone (Keener and Arp 1994). Another new mixotrophic bacteria, a strain of *Pseudomonas sp.* isolated from contaminated soil, grew under both anaerobic and aerobic conditions and used benzene for its growth (Morikawa and Imanaka 1993). The biodegradation of 2 mg of radiolabeled benzene in 100 g of soil with a mixed microbial population transformed 47% of the added radioactivity to carbon dioxide after 10 weeks (Haider et al. 1981). The authors concluded that specific organisms that mineralize benzene were present in the soil in only small numbers.

ULTRA, a fate and transport model used to predict the environmental fate of benzene following leakage of gasoline from an underground storage tank into shallow sandy soil, indicated that only about 1% of the benzene in the gasoline would be degraded over a 17-month period, and 3% would remain in the soil (Tucker et al. 1986). According to this model, most of the benzene that would be leaked in soil would either volatilize (67%) or move into groundwater (29%).

Salanitro (1993) summarized the aerobic degradation rates for BTEX in laboratory subsoil-groundwater slurries and aquifers. The data indicate that decay rates for benzene are highest (19–52% per day) for benzene concentrations <1 ppm when initial dissolved oxygen levels are about 8 ppm. Rates are significantly reduced (0–1.1% per day) when benzene levels are 1–2 ppm, and no degradation was observed when benzene levels were >2 ppm. This is particularly relevant in the case of petroleum spills as high concentrations of petroleum compounds are toxic to organisms and decrease the rate of biodegradation.

Benzene has been shown to be anaerobically transformed by mixed methanogenic cultures derived from ferulic acid-degrading sewage sludge enrichments. In most of the experiments, benzene was the only semicontinuously supplied energy sources in the defined mineral medium (Grbić-Galić and Vogel 1987).

After an initial acclimation time of 11 days, at least 50% of the substrate was converted to CO_2 and methane. The intermediates were consistent with benzene degradation via initial oxidation by ring hydroxylation.

It has been demonstrated that when mixtures of benzene, toluene, xylenes, and ethylbenzene are present in an anaerobic environment, there is a sequential utilization of the substrate hydrocarbons, with toluene usually being the first to be degraded, followed by the isomers of xylene in varying order. Benzene and ethylbenzene tend to be degraded last, if they are degraded at all (Edwards and Grbić-Galić 1992). Similar studies using only benzene at initial concentrations ranging from 40 to 200 μM showed degradation rates ranging from 0.36 to 3.7 μM/day depending upon substrate concentration and the presence of other carbon sources (Edwards and Grbić-Galić 1992).

6.3.2.4 Other Media

Twenty-day-old spinach leaves placed in a hermetic chamber containing vapors of ^{14}C-labeled benzene were shown to assimilate benzene, which was subsequently metabolized to various nonvolatile organic acids (Ugrekhelidze et al. 1997).

6.4   LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to benzene depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of benzene in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on benzene levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for monitoring benzene in a variety of environmental media are detailed in Chapter 7.

6.4.1   Air

Benzene is ubiquitous in the atmosphere. It has been identified in outdoor air samples of both rural and urban environments and in indoor air. Table 6-2 lists benzene levels in outdoor air from various cities in the United States.

Table 6-2. Benzene Levels in Air Samples

Location	Concentration (ppb)	Comments	References
Outdoor air			
San Francisco, California	0.8–5.2 (range); 2.6±1.3[a]	Data from six different urban locations in 1984; n=25	Wester et al. 1986
Stinson Beach, California	0.38±0.39[a]	Data from remote coastal area in 1984; n=21	Wester et al. 1986
Houston, Texas	1.2–18.7 (range); 37.5[b]	EPA survey, urban area; n=14; summer 1986	EPA 1987a[c]
Houston, Texas	0.55–6.3; 1.73[b]	Semirural area; n=22; 1990–1991	Kelly et al. 1993
St. Louis, Missouri	0.63–12.1 (range); 1.85[b]	EPA survey, urban area; n=18; summer 1985	EPA 1987a[c]
Denver, Colorado	3.0–6.6 (range); 4.1[b]	EPA survey, urban area; n=13; summer 1986	EPA 1987a[c]
Philadelphia, Pennsylvania	0.32–3.0 (range); 1[b]	EPA survey, urban area; n=14; summer 1985	EPA 1987a[c]
New York (Manhattan), New York	0.88–5.3 (range); 1.75[b]	EPA survey, urban area; n=12; summer 1986	EPA 1987a[c]
Chicago, Illinois	0.63–5.05 (range); 3.45[b]	EPA survey, urban area; n=14; summer 1986	EPA 1987a[c]
Boston, Massachusetts	0.69–3.1; 1.06[b]	Urban area; n=22; 1990–1991	Kelly et al. 1993
New York (Staten Island), New York	0.1–34 (range); 4.4±6.6[a]	Urban area; spring 1984	Singh et al. 1985
73 km northwest of Denver, Colorado	0.02–0.85 (range)	Rural area; May 1981–December 1982	Roberts et al. 1985
Elizabeth and Bayonne, New Jersey	Night 2.7[d], 28.5 (max); day, 3.0[d], 13.8 (max)	Night, n=81–86; day, n=86–90; fall 1981	Wallace et al. 1985
Personal air			
Elizabeth and Bayonne, New Jersey	Night 9.7[d], 159.6 (max); day, 8.5[d], 84.5 (max)	Night, n=346–348; day, n=339–341; fall 1981	Wallace et al. 1985
Elizabeth and Bayonne, New Jersey	Winter 5.2, summer 2.7, fall 4.8		Rappaport and Kupper 2004
Los Angles, California	Winter 4.5, spring 2.2, fall 2.8		Rappaport and Kupper 2004

[a]Average±standard deviation
[b]Median
[c]Concentrations reported as ppb carbon; values presented in table represent conversion equivalents as ppb benzene.
[d]Weighted average

EPA = Environmental Protection Agency; max = maximum; N = number of samples

Volatile organic compounds, including benzene, were measured at 11 monitoring sites in Anchorage, Alaska, in a year-long study ending in April 1994 (Taylor and Morris 1995). Average annual benzene concentrations ranged from a minimum of 1.15 parts per billion by volume (ppbv) in a low density residential area to 5.44 ppbv near a major midtown intersection. In a neighborhood where residents were complaining of petroleum odors, the highest benzene concentrations (annual average 3.74 ppbv) were measured on a cliff over the petroleum tank farm, within 50 meters of a petroleum storage tank.

Ambient air samples from 44 sites in 39 U.S. urban areas were collected from 6 to 9 a.m. during June through September of 1984, 1985, and 1986. Benzene was present in every sample. The median benzene site concentrations ranged from 0.8 to 6 ppb, with the overall median being 2.1 ppb (estimated detection limit ~0.04 ppb carbon; conversion equivalent ≈0.007 ppb benzene). The data indicated that mobile sources were the major source of benzene in the vast majority of samples (EPA 1987a). Benzene concentrations were in the range of >1–<5 ppbv for all of the 13 sites tested in a 1996 study. The sites included: Baton Rouge, Louisiana; Brownsville, Texas; Brattleboro, Vermont; Burlington, Vermont; Camden, New Jersey; El Paso, Texas; Garyville, Louisiana; Galveston, Texas; Hanville, Louisiana; Port Neches, Texas; Rutland, Vermont; Underhill, Vermont; and Winooski, Vermont (Mohamed et al. 2002).

The following daily median benzene air concentrations were reported in the Volatile Organic Compound National Ambient Database (1975–1985): remote (0.16 ppb), rural (0.47 ppb), suburban (1.8 ppb), urban (1.8 ppb), indoor air (1.8 ppb), and workplace air (2.1 ppb). The outdoor air data represent 300 cities in 42 states while the indoor air data represent 30 cities in 16 states (Shah and Singh 1988). In a NHEXAS study of six states in the Great Lakes region, benzene was found in 99.7% of the 386 personal air samples taken with an average concentration of 2.35 ppb. Benzene was also found in 100% of both indoor and outdoor samples with concentrations averaging 2.25 and 1.13 ppb, respectively (Clayton et al. 1999).

An EPA study of the concentration of various pollutants in 1990 showed an average concentration of benzene of 0.948 ppb. The source of benzene included manufacturing facilities and manufacturers that emit benzene to the environment (17%), mobile (60%), and background (23%) sources (Morello-Frosch et al. 2000).

In California, motor vehicle exhaust accounted for over 70% of the nonsmoking population's exposure to ambient benzene (Cal EPA 1987). The 1984 population-weighted average benzene concentration in California was estimated to be 3.3 ppb (Cal EPA 1987). Benzene emissions in a Los Angeles roadway tunnel were measured at a concentration of 382 mg/L (118,420 ppm) (Fraser et al. 1998).

Industrial sites that produce benzene are monitored often. Analysis of ambient air samples collected in industrial areas showed benzene levels ranging from 0.13 to 5 ppb in Iberville Parish, Louisiana, an area that included many organic chemical and petroleum producer, user, and storage facilities located along the Mississippi River (Pellizzari 1982). Indoor and outdoor air measurements were made in August 1987 in the Kanawha Valley region of West Virginia, which is the center of a heavily industrialized area known for its chemical manufacturing. The mean, maximum, and median indoor concentrations of benzene were 2.1, 14.9, and 0.64 ppb, respectively. The median outdoor ambient air concentration of benzene was 0.78 ppb (Cohen et al. 1989).

An analysis of gas from 20 Class II (municipal) landfills revealed a maximum concentration of 32 ppm for benzene (Wood and Porter 1987). Benzene was measured in the vicinity of the BKK landfill, a hazardous waste landfill in California, at a maximum concentration of 1.2 ppb (Bennett 1987). Maximum estimated levels of benzene in air near uncontrolled (Superfund) hazardous waste sites were 59.5 ppb at the Kin-Buc Landfill (Edison, New Jersey) and 162.8 ppb in Love Canal basements (Niagara Falls, New York) (Bennett 1987; Pellizzari 1982).

According to the National Cancer Institute, the average concentration of benzene in 1998 was 0.58 ppb in metropolitan areas (NCI 2003). A study of New York City air found mean values of 0.80, 1.87, and 1.47 ppb for outdoor (home), indoor (home), and personal air, respectively (Kinney et al. 2002). According to the New York Department of Health, the air from about 50% of oil fuel heated homes between the years 1997 and 2003 contained benzene concentrations ≥0.69 ppb inside the home, and 0.47 ppb in the area outside their homes. New York City was not included in this study (NYSDOH 2005). Concentrations of VOCs were measured in 12 northern California office buildings with three different types of ventilation. Benzene concentrations ranged from <0.1 to 2.7 ppb, with a geometric mean of 0.98 ppb (Daisey et al. 1994).

Population-weighted personal exposures to benzene exceeded the outdoor air concentrations in data from EPA's Total Exposure Assessment Methodology (TEAM) study. The overall mean personal exposure was about 4.7 ppb, compared to an overall mean outdoor concentration of only 1.9 ppb (Wallace 1989a). The study also reported that the median level of benzene in 185 homes without smokers was 2.2 ppb, and the median level of benzene in 343 homes with one or more smokers was 3.3 ppb (Wallace 1989a). This finding points to the possible significance of passive smoking as a source of benzene exposure. Indoor air samples taken from a smoke-filled bar contained 8.08–11.3 ppb of benzene (Brunnemann et al. 1989). A

study conducted by R.J. Reynolds Tobacco Company in smoking and nonsmoking homes revealed that benzene levels were elevated in smoking homes. In 24 nonsmoking homes, the mean benzene concentration was 1.21 ppb with a maximum of 5.93 ppb. In 25 smoking homes, the mean benzene concentration was 1.73 ppb, with a maximum of 8.43 ppb. However, benzene was not significantly correlated or associated with 3-ethenylpyridine, a proposed vapor phase environmental tobacco smoke marker (Heavner et al. 1995).

A British study measured benzene concentrations from both rural and urban areas in 1995. The highest concentrations of benzene were in Southampton urban center, London roadside, and Liverpool, which contained measured benzene levels of 2.53, 1.69,, and 1.60 ppb, respectively. The lowest measured benzene concentrations were in rural Hartwell and urban Edinborough, which contained 0.4 and 0.69 ppb of benzene, respectively (Duarte-Davidson 2001).

6.4.2   Water

Data from EPA's STOrage and RETrieval (STORET) database (2003–2005) showed that benzene was positively detected in 38% of the surface water samples collected at 571 observation stations ranging from concentrations too low to quantify to 100 μg/L (100 ppb) in one Utah test site. The sampling sites in the STORET database include both ambient and pipe sites. Ambient sites include streams, lakes, ponds, wells, reservoirs, canals, estuaries, and oceans and are intended to be indicative of general U.S. waterway conditions. Pipe sites refer to municipal or industrial influents or effluents (EPA 2005f).

Benzene was found in 904 of the 8,200 samples tested by the U.S. Geological Survey across the United States and Canada (USGS 2005). The EPA found benzene in 13,919 and 1,119 groundwater and surface water samples, respectively (EPA 2001).

Benzene is found in both polluted and unpolluted waters and rainwater. Measured benzene levels in open ocean from the Gulf of Mexico in 1977 in relatively unpolluted and polluted waters were 5–15 ng/kg (5–15 parts per trillion [ppt]), and 5–40 ng/kg (5–40 ppt), respectively (Sauer 1981). Benzene levels measured in coastal surface waters of the Gulf of Mexico were 6 ng/kg (6 ppt) in relatively unpolluted waters and 50–175 ng/kg (50–175 ppt) in polluted coastal waters (Sauer 1981). Benzene has been detected in rainwater in the United Kingdom at a concentration of 87.2 ppb (Colenutt and Thorburn 1980).

Benzene levels in water in the vicinity of five industrial facilities using or producing benzene ranged from <1 ppb to a high of 179 ppb found in plant effluent. In general, benzene in plant effluents quickly dispersed in rivers or streams to levels of 1–2 ppb or less (EPA 1979). The maximum benzene levels observed in monitoring wells in plumes from fuel spills at gasoline service stations ranged from 1,200 to 19,000 ppb (Salanitro 1993). A monitoring well in the vicinity of a bulk storage facility had a maximum benzene level of 45,000 ppb (Salanitro 1993). In northern Virginia, approximately 200,000 gallons of liquid hydrocarbons were released from a fuel-storage terminal into the underlying soil. A dichloro-methane extract of groundwater from a monitoring well in the same area gave a benzene concentration of 52.1 ppm (Mushrush et al. 1994). Benzene has been detected at concentrations ranging from 16 to 110 ppb in landfill leachate from a landfill that accepted both municipal and industrial wastes (Cline and Viste 1985).

Composite data from the Comprehensive Emergency Response, Compensation, and Liability Act (CERCLA) monitoring program indicate that benzene was detected at a frequency of 11.2% in groundwater in the vicinity of 178 inactive hazardous waste disposal sites (Plumb 1987). Data from a 1980 national survey by the Council on Environmental Quality on groundwater and surface water contamination showed benzene concentrations in contaminated drinking water wells in New York, New Jersey, and Connecticut ranged from 30 to 330 ppb, with the highest concentration in drinking water from surface water sources reported to be 4.4 ppb (Burmaster 1982). Benzene has also been identified but not quantified as one of the major organic constituents in commercially bottled artisan water in the United States (Dowty et al. 1975). At detection limits ranging from 0.2 to 1.0 ppb, benzene was detected at a concentration of 2 ppb in only one sample of 182 samples of bottled drinking water (Page et al. 1993). In six states of the Great Lakes region, unspecified amounts of benzene were found in 5.7% of the 247 drinking water sites tested (Clayton et al. 1999).

Because the New Jersey Department of Environmental Protection identified serious water issues in a 930 km^2 area within metropolitan Philadelphia, tests have been done to find other water sources such as the Kirkwood-Cohansey aquifer system. A study of 57 water samples taken from the shallow ground water supply at Kirkwood Cohansey aquifer in New Jersey showed that the majority of samples (50) contained between 0.2 and 1 ppbv of benzene, three samples contained <0.1 ppbv, and four samples contained >1 ppbv (Baehr et al. 1999).

6.4.3   Sediment and Soil

A Canadian study estimated that benzene is absorbed by the soil at a rate of 100 tons/year (MacLeod and
MacKay 1999). Data from EPA's STORET database (2003–2005) showed that benzene had been
positively detected in sediment samples taken at 9% of 355 observation stations with a median level of
<5 ppb (EPA 2005f). The concentration of benzene in soil near factories where benzene was produced or
used ranged from 2 to 191 ppb (IARC 1982).

Benzene levels ranging from <2 to 191 ppb were recorded in the vicinity of five industrial facilities using
or producing benzene (EPA 1979). Sediment levels ranging from 8 to 21 ppb were detected in Lake
Pontchartrain in Louisiana (Ferrario et al. 1985). In northern Virginia, approximately 200,000 gallons of
liquid hydrocarbons were released from a fuel-storage terminal into the underlying soil. Soil about
1,000 feet from that storage terminal contained benzene gas at a concentration of 1,500 ppm at a depth of
10 feet (Mushrush et al. 1994).

6.4.4   Other Environmental Media

Benzene has been detected in a variety of food. Benzene has been reported to occur in fruits, fish,
vegetables, nuts, dairy products, beverages, and eggs (EPA 1982a). Although benzene has been detected
in dairy products, there is no evidence of the presence of benzene in either cow's milk or human breast
milk (Hattemer-Frey et al. 1990). Eggs had the highest concentrations (2,100 ppb [uncooked] and 500–
1,900 ppb [hard-boiled]), followed by haddock (100–200 ppb), Jamaican rum (120 ppb), irradiated beef
(19 ppb), heat-treated canned beef (2 ppb), and butter (0.5 ppb). Lamb, mutton, veal, and chicken all had
<10 ppb benzene (when the meats were cooked) (EPA 1980b, 1982a). A survey of more than 50 foods
collected and analyzed from 1991 to early 1992 (McNeal et al. 1993) revealed that foods (including eggs)
without added benzoates contained benzene at levels ≤2 ng/g. The level of benzene in foods containing
added benzoates in addition to ascorbates (e.g., imitation strawberry preserves, taco sauce, and duck
sauce) ranged from <1 to 38 ng/g. In many foods, the presence of benzene is likely to be due to uptake
from the air (Grob et al. 1990). This conclusion was supported by the fact that the uptake decreased with
a decrease in exposed surface area of foods and contact time with air (Grob et al. 1990).

The U.S. Food and Drug Administration sponsored a 5-year study to determine the amount of volatile
organics in food from 1996 to 2000. The results are shown in Table 6-3 (Fleming-Jones and Smith 2003).
Benzene was found in cheddar cheese, cream cheese, margarine, butter, sour cream, ground beef,
bologna, hamburger, cheeseburger, pork, beef frankfurters, tuna canned in oil, chicken nuggets, chocolate

6. POTENTIAL FOR HUMAN EXPOSURE

Table 6-3. Benzene in Food[a]

Food	Number of cases found (n=14)	Concentration minimum-maximum in ppb
Cheddar cheese	2	20–47
Mixed nuts	3	1–6
Ground beef	12	9–190
Pork bacon	6	2–17
Banana, raw	13	11–132
Frankfurters, beef	4	2–11
Cream cheese	3	1–17
Chocolate cake icing	2	2–23
Tuna canned in oil	7	4–13
Fruit flavored cereal	5	2–21
Eggs scrambled	4	2–40
Peanut butter	5	2–25
Avocado, raw	10	3–30
Popcorn, popped in oil	3	4–22
Blueberry muffin	3	3–8
Strawberries, raw	1	1
Cola carbonated	3	1–138
Orange, raw	2	11–15
Coleslaw with dressing	14	11–102
Sweet roll danish	1	3–3
Potato chips	2	2–7
Fruit flavored sherbet	3	3–61
Quarter pound hamburger cooked	11	4–47
Margarine	1	7
Sandwich cookies	3	1–39
Butter	6	4-22
Chocolate chip cookies	2	1–8
Sour cream	2	3–15
Apple pie fresh/frozen	4	2–11
Chicken nuggets fast food	4	2–5
Graham crackers	2	1–9
French fries fast food	3	2–56
Cheeseburger quarter pound	8	5–44
Cheese pizza	2	1–2
Bologna	4	2–44
Cheese and pepperoni pizza	4	8–30
Olive/safflower oil	6	1–46
Sugar cookies	3	8–30
Cake doughnuts with icing	2	3
Popscicle	4	1–10

[a]Derived from Fleming-Jones and Smith 2003

cake icing, sandwich cookie, chocolate chip cookies, graham crackers, sugar cookies, cake doughnuts with icing, french fries, apple pie, cola carbonated beverages, sweet roll Danish, potato chips, cheese pizza, cheese and pepperoni pizza, mixed nuts, fruit-flavored cereal, fruit flavored sorbet, popsicles, olive/safflower oil, scrambled eggs, peanut butter, popcorn popped in oil, blueberry muffins, coleslaw with dressing, raw banana, avocado, oranges, and strawberries. American cheese was the only food tested that did not contain benzene. Foods with the greatest maximum concentration of benzene included ground beef (maximum 190 ppb), raw bananas (maximum 132 ppb), carbonated cola (maximum 138 ppb), and coleslaw with dressing (maximum 102 ppb).

As part of a program to identify possible exposures that may be important in the high incidence of lung cancer among women in Shanghai, China, Pellizzari et al. (1995) qualitatively identified the volatile components emitted during heating of cooking oils to 265 °C. This study found that, on a relative basis, the intensity of the benzene peak in the total ion current chromatogram of vapors from Chinese rapeseed oil (commonly used in wok cooking) was 14-, 6.6-, and 1.7-fold greater than in vapors from peanut, soybean, and other canola (rapeseed) oils, respectively.

Cigarette smoke remains an important source of human exposure to benzene. The amount of benzene measured in mainstream smoke ranged from 5.9 to 73 µg/cigarette (Brunnemann et al. 1990). Larger amounts of benzene were found in sidestream smoke, ranging from 345 to 653 µg/cigarette (Brunnemann et al. 1990). Benzene has been found in vapor from cigarette smoke in concentrations ranging from 3.2 to 61.2 µg/cigarette depending on the brand of cigarette. The amount of tar in the cigarette was not directly related to the benzene concentration (Darrall et al. 1998).

6.5   GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Exposure to low levels of environmental benzene is unavoidable due to the ubiquitous presence of benzene in the environment from a variety of anthropogenic sources. Benzene can be detected in blood samples. In a study designed to establish reference blood concentrations of benzene and other selected volatile organic compounds in the general population of the United States, Ashley et al. (1994) measured blood benzene levels in 883 people (not occupationally exposed) in the United States who had participated in the Third National Health and Nutrition Examination Survey (NHANES III). Within this group of nonoccupationally exposed subjects, the mean and median blood benzene levels were 0.13 and 0.061 ppb, respectively. Seven nonsmoking subjects from this study group were assessed for blood benzene levels just prior to entering examination vans with limited potential for benzene exposure and at

the end of a 3-hour period in this environment. Mean blood benzene levels prior to van entry and after

3 hours in the van measured 0.046 ppb (range from not detected to 0.061 ppb) and 0.033 ppb (range from

not detected to 0.055 ppb), respectively. Lemire et al. (2004) reported a median blood benzene level of

0.047 ppb in a subgroup of 546 nonsmoking and nonoccupationally exposed subjects from the NHANES

III study group. Elevated blood benzene levels may be expected among subjects with potential for

elevated exposure to benzene, such as smokers, commuters, gas station attendants, and people who use

products that emit benzene.

The Total Exposure Assessment Monitoring (TEAM) studies, carried out by the EPA between 1980 and

1990, suggested that for many chemicals, including benzene, the most important sources of pollution are

small and close to the person, and that exposures are not clearly correlated with emissions. For example,

the TEAM study findings indicated that nearly 85% of atmospheric benzene in outdoor air is produced by

cars burning petroleum products and the remaining 15% is produced by industry. Despite the fact that

petroleum products contribute to the majority of benzene in the atmosphere, half of the total national

personal exposure to benzene comes from cigarette smoke (Wallace 1995). In fact, breath measurements

of benzene provided by the TEAM study between 1979 and 1988 identified smoking as the single most

important source of benzene exposure for about 40 million U.S. smokers (Wallace 1989b). Even passive

exposure to cigarette smoke is responsible for more benzene exposure (about 5% of the total) than the

emissions from the entire industrial capacity of the United States (about 3% of the total) (Wallace 1995).

A breakdown of the emissions and exposure sources for benzene that was derived from the Los Angeles

TEAM study data (Wallace et al. 1991) is given in Figure 6-3. The reason that a relatively small source

of emissions can have such a large effect on exposure is the efficiency of delivery. Wallace (1995)

reports that one cigarette delivers an average of 55 μg of benzene with nearly 100% efficiency to the

smoker. Benzene from industrial sources is dissipated into the atmosphere.

Smokers (n=200) in the TEAM study had a mean breath concentration of 15 μg/m^3 (4.7 ppb), almost

10 times the level of 1.5–2 μg/m^3 (0.47–0.63 ppb) observed in more than 300 nonsmokers (Wallace

1989b). Smokers also had about 6–10 times as much benzene in their blood as non-smokers (Wallace

1995). In another study, benzene concentrations were compared in the breath of smokers and nonsmokers

and in ambient air in both an urban area of San Francisco and in a more remote area of Stinson Beach,

California (Wester et al. 1986). In the urban area, benzene in smokers' breath (6.8±3.0 ppb) was greater

than in nonsmokers' breath (2.5±0.8 ppb) and smokers' ambient air (3.3±0.8 ppb). In the remote area, the

same pattern was observed. This suggests that smoking represented an additional source of benzene

above that of outdoor ambient air (Wester et al. 1986). In 10 of 11 homes inhabited by tobacco smokers,

Figure 6-3. Benzene Emissions and Exposures

Benzene Emissions

Industry, 14%

Personal and
Home, 3%

Cigarettes,
0.1%

Cars, 82%

Benzene Exposures

Cigarettes,
40%

Car Exhaust,
18%

Industry 3%,

ETS, 5%

Home
Sources, 16%
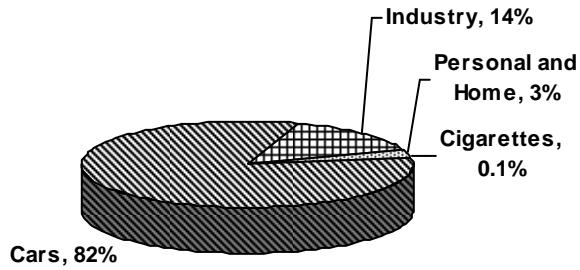
Personal
Activities,
18%
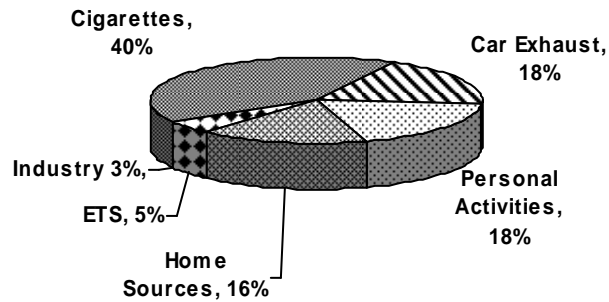
Note: A comparison of benzene emission sources (home sources include paints and petroleum products; personal activities include driving and use of consumer products that contain benzene). Data taken from Wallace et al. (1991).

mean indoor and personal benzene concentrations were 2–5 times higher than outdoor levels (Thomas et al. 1993).

Assuming that the average sales-weighted tar and nicotine cigarette yields 57 µg benzene in mainstream smoke, Wallace (1989a) estimated that the average smoker (32 cigarettes per day) takes in about 1.8 mg benzene per day from smoking. This is nearly 10 times the average daily intake of nonsmokers (Wallace 1989a). On the assumption that intake of benzene from each cigarette is 30 µg, Fishbein (1992) has calculated that a smoker who consumes two packs of cigarettes per day will have an additional daily intake of 1,200 µg.

A British study of rural and urban environments suggests that benzene exposure is greatly affected by proximity to smokers (Duarte-Davidson et al. 2001). Air concentrations of benzene at an urban center in South Hampton averaged about 2.5 ppb, while in a rural location in Hartwell, the average amount of benzene in the air was 0.41 ppb. Air at a smoky pub was found to contain 22 ppb of benzene. Comparing the daily doses of rural nonsmokers, urban nonsmokers, urban passive smokers, and urban smokers, very little difference between the rural nonsmokers' 24 ppb daily dose and the urban nonsmokers' 30 ppb daily dose was found. Passive urban smokers, on average, have a daily benzene exposure dose of 38 ppb of benzene while smokers have a daily exposure dose of benzene of 163 ppb. On average, nonsmokers in urban and rural environments have estimated benzene intakes of 1.15 and 1.5 µg/kg body weight/day.

Women tend to intake more of benzene per kg body weight than men. Passive smokers' estimated daily intake averages are 2.10 and 1.74 µg/kg body weight/day for women and men, respectively. Urban women and men smokers' estimated intakes are estimated at 9.00 and 7.46 µg/kg body weight/day, respectively; this is equivalent to an atmospheric concentration of 8.2 ppb (Duarte-Davidson et al. 2001).

In 1990, a study in Germany analyzed factors that predicted people's exposures to VOCs and found that while smoking was the most significant determinant of benzene exposure, automobile-related activities, such as refueling and driving, were also significant (Hoffmann et al. 2000). Virtually all (99.9%) of the benzene released into the environment finally distributes itself into the air. The general population may be exposed to benzene through inhalation of contaminated air, particularly in areas of heavy motor vehicle traffic and around gas stations. Compared to inhalation, dermal exposure probably constitutes a minor portion of benzene exposure for the general population. Personal sources account for 18% of the total exposure of the general population to benzene. The main personal sources (other than smoking

cigarettes) are driving or riding in automobiles and using products that emit benzene (paints, adhesives, marking pens, rubber products, and tapes) (Wallace 1989a).

Benzene constitutes 1–2% of most blends of gasoline and is released as a vapor from vehicular emissions. Since benzene is a constituent of auto exhaust and fuel evaporation, people who spend more time in cars or in areas of heavy traffic have increased personal exposure to benzene. Assuming an average benzene concentration of 40 $\mu g/m^3$ (12.5 ppb) for a moving automobile and an exposure duration of 1 hour/day, the calculated intake for driving or riding in an automobile is 40 μg/day (Wallace 1989a). In an investigation of exposure to methyl *tertiary*-butyl ether (MTBE) in oxygenated gasoline in Stamford, Connecticut, venous blood samples were collected from 14 commuters and from 30 other persons who worked in the vicinity of traffic or automobiles. In addition to MTBE, the samples were analyzed for five chemicals, including benzene. Levels of benzene in the blood of 11 nonsmoking men and women commuters ranged from 0.10 to 0.20 $\mu g/L$ (median=0.12 $\mu g/L$). Blood benzene levels of 0.29, 0.14, and 0.58 $\mu g/L$ were measured in one female and two male smoking commuters, respectively. In 12 nonsmoking male car repair workers, blood benzene levels ranged from 0.11 to 0.98 $\mu g/L$ (median=0.19 $\mu g/L$); in 8 smoking male car repair workers, levels ranged from 0.17 to 0.67 $\mu g/L$ (median=0.42 $\mu g/L$). Three nonsmoking male gasoline attendants had blood benzene levels ranging from 0.32 to 0.47 $\mu g/L$ (median=0.36 $\mu g/L$) (White et al. 1993).

Pumping gasoline can also be a significant source of exposure to benzene. A study conducted between July 1998 and March 1999 that comprised of 39 customers of gasoline self service stations from North Carolina, measured the benzene level in the air around the station as well as the levels of benzene in customers' breath prior to and immediately after fueling (Egeghy et al. 2000). Benzene levels in the air around the station ranged from <0.02 to 11.16 ppm, with a mean (±1 standard deviation) of 0.91 (±1.8) ppm. The range of benzene levels in the breath of customers prior to fueling was <0.001–0.022 ppm with a mean (±1 standard deviation) of 0.0027 (±0.0034) ppm while the range of benzene levels in the breath of customers after re-fueling was <0.001–0.434 ppm with a mean (±1 standard deviation) of 0.05 (±0.081) ppm (Egeghy et al. 2000). Another study reported a benzene concentration of 1 ppm at the breathing-level of a person pumping gas (EPA 1986). Using this concentration and an estimated 70 minutes/year of time spent pumping gasoline, a benzene intake of 10 μg/day has been calculated (Wallace 1989a). In a group of 26 subjects who were not occupationally exposed to benzene, but were exposed to benzene during refueling in Fairbanks, Alaska, median blood benzene levels prior to and immediately following refueling were 0.19 ppb (range 0.08–0.65 ppb) and 0.54 ppb (range 0.13–1.70 ppb), respectively (Backer et al. 1997).

Gasoline vapors vented into the home from attached garages can also increase indoor air exposure to benzene (Wallace 1989a, 1989b). Depending on airflow from garage to living areas, mean indoor benzene concentrations in houses with a garage were 2–5 times higher than outdoor levels in most homes (Thomas et al. 1993). Benzene levels in four garages during different times in a day ranged from 0.94 to 61.3 ppb. The higher concentrations of benzene in these garages were not only from vehicular activity, but also in varying proportions from stored gasoline, paints, and benzene-containing consumer products (Thomas et al. 1993). Inhalation exposure to off-gassing from benzene-containing products and to evaporative emissions from automobiles in attached garages has been estimated to be 150 μg/day (Wallace 1989a).

A study of human exposure to benzene in the California South Coast Air Basin showed that benzene exposure in that area has decreased greatly since 1989. Adult smokers saw a 28% decrease in benzene exposure from 1989 to 1997, while adult nonsmokers and adolescents saw a decrease in benzene exposure of 67 and 55%, respectively. Where people were being exposed to benzene also changed during that time. Ambient air was the source of 49% of the benzene that nonsmokers were exposed to in 1997, which is less than the 59% from 1987. For smokers in the region, 85% of their benzene exposure was from smoking and 4% was from the ambient air in 1997. This shows an increase in benzene exposure from smoking since 1987 (78%) and a decrease in exposure from the ambient air (12%) (Fruin et al. 2001). The main sources credited with this decrease in exposure are reformulated gasoline and stricter air emission laws as well as smoking control measures.

Another source of exposure to benzene for the general population is the use of domestic wood stoves. It has been estimated that approximately 10% of the space heating in urban areas of the northern United States is from wood burning, with up to 50% in smaller, rural towns (Larson and Koenig 1994). Benzene has been found to be a major component of the emissions from wood burning, especially from efficient flame combustion, and constituted roughly 10–20% by weight of total non-methane hydrocarbons (Barrefors and Petersson 1995). It should be noted, however, that chimney emissions result in much lower human exposure than equally large emissions at the ground level.

Other sources of inhalation exposure to benzene include air around hazardous waste sites, industrial facilities, off-gassing from particle board, and off-gassing from contaminated water during showering and cooking. Based on the TEAM study findings, it appears that the following are not important sources of

exposure to benzene on a nationwide basis: chemical manufacturing facilities, petroleum refining operations, oil storage tanks, drinking water, food, and beverages (Wallace 1989a).

Average water intake of benzene (assuming a typical drinking water concentration of 0.1 ppb and a consumption of 2 L/day) is 0.2 μg/day (HSDB 2007). According to another estimate, the daily intakes of benzene for a nonsmoking individual (not exposed to secondary smoke) are 1–550 μg (Fishbein 1992).

While most human exposure to benzene is believed to be through inhalation, studies show that benzene can permeate skin with a permeability factor of about 0.14–0.18 cm/hour at 25 °C. The permeability factor was not affected by moisturizer, baby oil, or insect repellent; however, it was affected by temperature (50 °C) and sunscreen with the permeability factors increasing to 0.26 and 0.24 cm/hour, respectively (Nakai et al. 1997).

Individuals employed in industries that use or make benzene or products containing benzene may be exposed to the highest concentrations of benzene. The National Occupational Exposure Survey (NOES), conducted by NIOSH from 1981 to 1983, estimated that approximately 272,300 workers employed in various professions were potentially exposed to benzene in the United States. Approximately half of these workers were employed in general medical and surgical hospitals, and their occupations included nurses and aides, physicians, technicians, technologists, therapists, dieticians, pharmacists, and janitors (NIOSH 1990). The NOES database does not contain information on the frequency, concentration, or duration of exposure; the survey provides only estimates of workers potentially exposed to chemicals in the workplace. The current OSHA permissible limit for an 8-hour TWA exposure to benzene is 1 ppm and a short-term exposure limit in any 15-minute period is 5 ppm (OSHA 2003). The NIOSH recommended exposure limit is 0.1 ppm for an 8-hour TWA and 1 ppm for short-term exposure (NIOSH 1992b). In 1987, OSHA estimated that approximately 238,000 workers were exposed to benzene in seven major industry sectors, including petrochemical plants, petroleum refineries, coke and coal chemicals, tire manufacturers, bulk terminals, bulk plants, and transportation via tank trucks (Table 6-4) (OSHA 1987). Approximately 10,000 workers were estimated to be exposed to TWA concentrations in excess of the 1 ppm standard. This estimate did not include firms covered by the exclusions, firms under jurisdiction of other agencies, or firms involved in the use of products containing small quantities of benzene. The uptake of benzene by workers in a municipal waste incinerator in Germany was assessed by measuring benzene levels in blood (Angerer et al. 1991). No significant difference (p<0.05) in blood benzene levels between workers and controls were detected (mean 0.22 μg/L for nonsmoking workers versus 0.25 μg/L

Table 6-4. Percentage of Employees Exposed to Benzene by Exposure Level and Industry Division[a]

Industry sector	Percentage of observations in each exposure category according to range of 8-hour TWA benzene concentrations (ppm)						Total number of employees
	0.0–0.1	0.11–0.5	0.51–1.0	1.1–5.0	5.1–10	10+	
Petrochemical plants[b]		74.6		23.0	2.4	0.0	4,300
Petroleum refineries[c,d]	64.6	26.1	4.6	3.8	0.5	0.4	47,547
Coke and coal chemicals[e]	0.0	39.3	27.6	27.5	4.4	1.3	947[f]
Tire manufacturers[c]	53.4	37.5	6.3	2.8	0.0	0.0	65,000
Bulk terminals[c]	57.8	32.8	5.3	3.7	0.3	0.1	27,095
Bulk plants[c]	57.8	32.8	5.3	3.7	0.3	0.1	45,323
Transportation via tank truck[c]	68.4	23.1	5.3	2.9	0.1	0.2	47,600
Total							237,812

[a]Derived from OSHA 1987
[b]Percentages represent the portion of workers whose average exposures are in each category.
[c]Percentages represent the portion of sampling results in each category.
[d]Data do not reflect respirator use and sampling biases.
[e]Percentages represent the portion of workers whose average exposures are in each category.
[f]Excludes workers employed at the coke ovens.

TWA = time-weighted average

for nonsmoking controls). OSHA requires the use of engineering controls and/or respiratory protection in situations where compliance with the TWA is not feasible (OSHA 1987).

Certain jobs, such as gasoline station workers, firefighters, and dry cleaners, are believed to put people at a higher risk of benzene exposure. In an analysis of literature, it was estimated that workers in the area of crude petroleum and natural gas are exposed to 0.04 ppm benzene, while workers in petroleum refining, gas stations, and crude petroleum pipelines are exposed to 0.22, 0.12 and 0.25 ppm benzene, respectively. This study also showed that fire fighters are exposed to an average of 0.38 ppm benzene (van Wijngaarden and Stewart 2003). Workers from four different dry cleaning facilities in Korea had mean benzene air concentrations ranging from 2.7 to 3.2 ppb. Their exposure to benzene was dependent upon the type of solvent used for cleaning (Jo and Kim 2001). Benzene concentrations of 25.46 and 1,331.29 ppb were found near the kiln and at the rotary line, respectively, inside a hazardous waste incinerator in Turkey (Bakoglu et al. 2004).

A study comparing workers who were exposed to benzene regularly at work to people who were not exposed to benzene at work showed that while the general population in Italy had average blood benzene concentration of 165 ng/L, the people who were exposed to high benzene levels at work had an average benzene blood concentration of 186 ng/L. Immediately following their shift, the average benzene blood level samples from of benzene-exposed workers was 420 ng/L. The average blood benzene levels for smoking and nonsmoking occupationally exposed workers were 264 and 123 ng/L respectively (Brugnone et al. 1998).

6.6   EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in Section 3.7, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths,

sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

Children can be subject to increased benzene exposure by inhalation of second-hand smoke. In a study of nonsmoking rural families, urban families, and urban smoking families, infant exposure to benzene was estimated at doses of 15.3, 19.7, and 25.9 μg/day, respectively, with daily intakes of 1.68, 2.16, and 2.55 μg/kg body weight/day, respectively. For children of the same classification, benzene exposure was measured at doses of 29.3, 37.6, and 49.3 μg/day, respectively, with daily intakes of 0.71, 0.91, and 1.20 μg/kg bodyweight/day, respectively. For all infants and children, benzene exposure predominantly comes from the indoors (Duarte-Davidson et al. 2001).

Depending on a child's living environment, they may have higher exposure to benzene than adults. In a study of two lower-income areas of Minneapolis, children were found to have average personal benzene exposures of 0.66 and 0.53 ppb in the winter and spring, respectively. The highest concentration of benzene in their environment came from the home with winter and spring concentrations of 0.69 and 0.66 ppb respectively, while the outdoor and school benzene concentrations were 0.41 and 0.19 ppb, respectively (Adgate et al. 2004). In Italy, concentrations of the benzene metabolite, *trans,trans*-muconic acid (MA), was measured in the urine of children from both urban areas in Naples and rural areas in Pollica. The mean urinary concentrations of MA detected for rural and urban children were 48.4 and 98.7 μg/L (Amodio-Cocchieri et al. 2001). These studies also found no strong link between passive smoking and MA levels. The only factor that affected levels of MA in urine samples was how close the family lived to the road. A study in Rouen, France, compared benzene exposure and concentrations in nonsmoking parents and their children. Despite the fact that the children were exposed to slightly less benzene 3.47 ppb (11.1 μg/m^3) than their parents 4.51 ppb (14.4 μg/m^3), there was no significant correlation between exposure means and urinary metabolite levels (Kouniali et al. 2003).

Benzene concentrations in women and their tissue as well as breast milk have been a major concern. In a study of South Korean housewives living near service stations, indoor, outdoor and breath benzene concentrations averaged 5.73, 3.63, and 3.29 ppm, respectively (Jo and Moon 1999). Benzene has been found in mother's milk (Pellizzari 1982). Benzene was found at mean concentrations of 0.06 μg/kg in 23 samples of breast milk taken from a children's hospital in Rome (Fabietti et al. 2004). While this may provide a mechanism by which infants are exposed to benzene, these concentrations are lower than in other foods.

6.7   POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Individuals who live near hazardous waste sites or near leaking underground fuel storage tanks might be exposed to potentially high concentrations of benzene in their drinking water if they obtain tap water from wells located near these sources. In a series of experiments conducted in a single-family residence from June 11 to 13, 1991, exposure to benzene through contaminated residential water was monitored (Lindstrom et al. 1994). The residential water was contaminated with benzene and other hydrocarbons in 1986. Periodic testing conducted from 1986 to 1991 showed benzene concentrations ranging from 33 to 673 µg/L (ppb). The experiment involved an individual taking a 20-minute shower with the bathroom door closed, followed by 5 minutes for drying and dressing; then the bathroom door was opened and this individual was allowed to leave the house. Integrated 60- and 240-minute whole-air samples were collected from the bathroom, an adjacent bedroom, living room, and in ambient air. Glass, gas-tight syringe grab samples were simultaneously collected from the shower, bathroom, bedroom, and living room at 0, 10, 18, 20, 25, 25.5, and 30 minutes. Two members of the monitoring team were measured for 6 hours using personal Tenax gas GC monitors. For the first 30 minutes of each experiment, one member was based in the bathroom and the other in the living room. Benzene concentrations in the shower head ranged from 185 to 367 µg/L (ppb), while drain level samples ranged from below the detectable limit (0.6 µg/L or ppb) to 198 µg/L (ppb). Analysis of the syringe samples suggested a pulse of benzene moving from the shower stall to the rest of the house over approximately 60 minutes. Peak benzene levels were measured in the shower stall at 18–20 minutes (758–1,670 µg/m^3), in the bathroom at 10–25 minutes (366–498 µg/m^3), in the bedroom at 25.5–30 minutes (81–146 µg/m^3), and in the living room at 36–70 minutes (40–62 µg/m^3). The total benzene dose resulting from the shower was estimated to be approximately 281 µg, with 40% via inhalation and 60% via the dermal pathway (Lindstrom et al. 1994).

The major source of exposure to benzene is cigarette smoke. A smoker of 32 cigarettes per day (the U.S. average per smoker) would have a benzene intake of approximately 1.8 mg/day (at least 10 times the average nonsmoker's intake) (Wallace 1989a). Median benzene concentrations in 343 homes with smokers averaged 3.3 ppb compared to 2.2 ppb in 185 homes without smokers. This represents a 50% increase in benzene exposure for nonsmokers exposed to passive smoke compared to nonsmokers not exposed to passive smoke (Wallace 1989a). In a study in Germany, the mean benzene concentrations for frequent smokers and nonsmokers were 6.1 and 2.4 ppb, respectively (Hoffmann et al. 2000).

6.8   ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the
Administrator of EPA and agencies and programs of the Public Health Service) to assess whether
adequate information on the health effects of benzene is available. Where adequate information is not
available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research
designed to determine the health effects (and techniques for developing methods to determine such health
effects) of benzene.

The following categories of possible data needs have been identified by a joint team of scientists from
ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would
reduce the uncertainties of human health assessment. This definition should not be interpreted to mean
that all data needs discussed in this section must be filled. In the future, the identified data needs will be
evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.8.1   Identification of Data Needs

Physical and Chemical Properties.   The physical and chemical properties of benzene are well
characterized and allow prediction of the transport and transformation of the compound in the
environment.

Production, Import/Export, Use, Release, and Disposal.   According to the Emergency
Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required
to submit substance release and off-site transfer information to the EPA. The TRI, which contains this
information for 2004, became available in May of 2006. This database is updated yearly and should
provide a list of industrial production facilities and emissions.

Benzene is one of the top 20 highest volume chemicals produced in the United States. In 1994, the U.S.
production volume of benzene was 14.7 billion pounds (C&EN 1995). The production volume during the
1984–1994 period has increased by 4% annually (C&EN 1995). The United States currently has a
benzene production capacity of 11.8 billion liters (SRI 2004). Imports of benzene into the United States
have generally ranged from 10,176 to 11,672 million pounds during 2002–2004 (USITC 2005). Exports
increased from 21 million pounds in 2002 to 290 million pounds in 2003, but decreased to 145 million
pounds in 2004 (USITC 2005). The major use of benzene is in the production of other chemicals
(primarily ethylbenzene, cumene, and cyclohexane), accounting for approximately 91% of benzene

production volume. Benzene is also used in chemical laboratories as a solvent and a reactant (OSHA 1977, 1987), and as an anti-knock agent in unleaded gasoline (Brief et al. 1980; EPA 1985a). The widespread use of benzene as a solvent has decreased in recent years. Many products that used benzene as a solvent in the past have replaced it with other organic solvents; however, benzene may still occur as a trace impurity in these products. Less than 2% of the amount of benzene produced is used as a solvent in such products as trade and industrial paints, rubber cements, adhesives, paint removers, artificial leather, and rubber goods. Benzene has also been used in the shoe manufacturing and rotogravure printing industries (EPA 1978; OSHA 1977). In the past, certain consumer products (such as some paint strippers, carburetor cleaners, denatured alcohol, and rubber cement used in tire patch kits and arts and crafts supplies) contained small amounts of benzene (Young et al. 1978). Other consumer products that contained benzene were certain types of carpet glue, textured carpet liquid detergent, and furniture wax (Wallace et al. 1987). The use of benzene in certain pesticides has been canceled. Benzene-containing wastes, such as commercial chemical products, manufacturing chemical intermediates, and spent solvents, are subject to federal and/or state hazardous waste regulations (HSDB 2007). Currently, the recommended method of disposal is to incinerate solvent mixtures and sludges at a temperature that ensures complete combustion. No additional information on the production, import/export, use, release, or disposal of benzene is needed at this time.

Environmental Fate. Benzene released to the environment partitions mainly to the atmosphere (Mackay and Leinonen 1975). However, the compound can also be found in surface water and groundwater. Benzene is mobile in soil (Karickhoff 1981; Kenaga 1980); however, there is a need for more information on the leachability potential of benzene to groundwater in different soil types. Benzene is transformed in the atmosphere by photooxidation. Biodegradation, principally aerobic, is the most important fate process of benzene in water (Delfino and Miles 1985; McAllister and Chiang 1994; Salanitro 1993) and soil (Gibson 1980; Hopper 1978; Salanitro 1993). Benzene can persist in groundwater. No additional information on the environmental fate of benzene is needed at this time.

Bioavailability from Environmental Media. Benzene can be absorbed following oral exposure (Thienes and Haley 1972), dermal exposure (Blank and McAuliffe 1985; Franz 1984; Laitinen et al. 1994; Lindstrom et al. 1994; Lodén 1986; Susten et al. 1985), and inhalation exposure (Ashley et al. 1994; Avis and Hutton 1993; Boogaard and van Sittert 1995; Brunnemann et al. 1989; Byrd et al. 1990; Etzel and Ashley 1994; Fustinoni et al. 1995; Ghittori et al. 1995; Gordian and Guay 1995; Hajimiragha et al. 1989; Hanzlick 1995; HazDat 2006; Karacic et al. 1995; Kok and Ong 1994; Lagorio et al. 1994a; Laitinen et al. 1994; Lauwerys et al. 1994; Lindstrom et al. 1994; Mannino et al. 1995; Nomiyama and

Nomiyama 1974a; Ong and Lee 1994; Ong et al. 1995; Pekari et al. 1992; Popp et al. 1994; Rauscher et al. 1994; Rothman et al. 1995; Ruppert et al. 1995; Scherer et al. 1995; Shamy et al. 1994; Srbova et al. 1950). These routes of exposure may be of concern to humans because of the potential for benzene to contaminate the air (Bennett 1987; Black et al. 1980; Brief et al. 1980; Cal EPA 1987; Edgerton and Shah 1992; EPA 1989, 1994d; Glass et al. 1986; Graedel 1978; Mayer et al. 1994; TRI02 2005; Wallace 1989a, 1989b; Wallace and Pellizzari 1986; Wester et al. 1986; Wood and Porter 1987), drinking water (CDC 1994; EPA 1979), and soil (HazDat 2006; Mushrush et al. 1994; TRI02 2005). Information on inhalation exposure and on the absorption of benzene following ingestion of plants grown in contaminated environments near hazardous waste sites would be helpful in determining bioavailability of the compound in these media.

Food Chain Bioaccumulation. Benzene has an estimated low-to-moderate bioconcentration potential in aquatic organisms (Miller et al. 1985; Ogata et al. 1984) and some plants (Geyer et al. 1984). Most of the benzene accumulation on vegetation results from air-to-leaf transfer. Root uptake is not believed to be important (Hattemer-Frey et al. 1990). Biomagnification in aquatic food chains does not appear to be important (Ogata et al. 1984). No further information appears to be needed.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of benzene in contaminated media at hazardous waste sites are needed so that the information obtained on levels of benzene in the environment can be used in combination with the known body burden of benzene to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Benzene is widely distributed in the environment and has been detected in air (Clayton et al. 1999; EPA 1987a; Mohamed et al. 2002; Morello-Frosch et al. 2000), water (EPA 2001, 2005f; Sauer 1981, USGS 2005), soil (EPA 1979; Ferrario et al. 1985; MacLeod and MacKay 1999; Staples et al. 1985), sediment, and some foods (EPA 1980b, 1982a; Fleming-Jones and Smith 2003). The levels of benzene in air and water are well documented, but there is a need for more current information. Benzene is not expected to be a significant contaminant in aquatic foods (Geyer et al. 1984; Gossett et al. 1983; Miller et al. 1985; Ogata et al. 1984); however, some contamination of food crops consumed by humans may occur, primarily from air-to-leaf transfer (Hattemer-Frey et al. 1990). The total concentration of benzene on exposed food crops consumed by humans was estimated to be 587 ng/kg (Hattemer-Frey et al. 1990). Humans are at risk of exposure to benzene because of its widespread distribution in the environment, particularly in the atmosphere. Releases to the air from gasoline, smoking, and automobile exhaust constitute the major risk of potential exposure for the general population (Wallace 1995). Additional data

characterizing the concentration of benzene in drinking water, air, and soil surrounding hazardous waste sites would be helpful in assessing human exposure for populations living near these waste sites. In addition, more current data on levels of benzene in foods would be helpful in estimating intake of benzene from food.

Exposure Levels in Humans.   Benzene has been detected in human body fluids and tissues such as blood, urine, and fat (Brugnone et al. 1989; Chao et al. 1993; Karacic et al. 1987). Most of the monitoring data have come from occupational studies of specific worker populations exposed to benzene (Inoue et al. 1989b; Karacic et al. 1987; OSHA 1987; van Wijngaarden and Stewart 2003). Biological monitoring studies exist for the general population (Melikian et al. 1994). There is information for background levels in breath of smokers and nonsmokers (Wallace 1989b), baseline blood levels (Karacic et al. 1987), and levels of urinary metabolites in unexposed people (Inoue et al. 1989b). Information on exposure levels for populations living in the vicinity of hazardous waste sites would be helpful in estimating exposure in these groups.

This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children.   Benzene levels have been monitored in children and the environments in which they live. This information gives levels found for infants and children in rural and urban area as well as the levels found for children in homes of parents who smoke (Duarte-Davidson et al. 2001). There have been many studies relating oil and petroleum exposure to childhood leukemia and other diseases; however, the majority of these studies have not recorded benzene levels. More information about the exposures of children, particularly those subject to high exposures such as smoking, busy roads, and gasoline stations, are needed.

Child health data needs relating to susceptibility are discussed in Section 3.12.2, Identification of Data Needs: Children's Susceptibility.

Exposure Registries.   In 2001, 1,143 people were included in the benzene subset of the Volatile Organics Compounds subregistry of the National Exposure Registry. These people were exposed to benzene at a site in Texas. Demographic and health information was obtained on all the exposed persons; the information will be updated longitudinally. For those who were identified as exposed and were deceased, a death certificate will be obtained to ascertain cause of death. This activity was carried out by the Exposure and Disease Registry Branch (EDRB), Division of Health Studies (DHS), ATSDR. The

data became part of public-user data files maintained by ATSDR. The statistical analyses of the baseline

data was completed and published (Agency for Toxic Substances and Disease Registry 1995). The

information that was amassed in the National Exposure Registry will be used to facilitate the

epidemiological research needs to assess adverse health outcomes that may be related to the exposure to

this substance.

6.8.2   Ongoing Studies

The Federal Research in Progress (FEDRIP 2005) database provides additional information obtainable

from a few ongoing studies that may fill in some of the data needs identified in Section 6.8.1. These

studies are summarized in Table 6-5.

Table 6-5. Ongoing Studies on the Potential for Human Exposure to Benzene[a]

Investigator	Affiliation	Research description	Sponsor
Cho, CY	CHA Corporation; Laramie, Wyoming	Study of the use of microwave technology for superfund site remediation	NIEHS
Fischer, LJ		Understanding potential health hazards from groundwater and soil contamination of chemicals commonly found at hazardous waste sites	USDA
Greenberg, A		Study of critical steps in the ring-opening metabolism of the human carcinogen benzene to muconaldehyde	USDA
Gurian, PL	University of El Paso Texas; El Paso, Texas	Modeling of organic compounds in drinking water	National Institute of General Medical Sciences
Nylander-French, LA	University of North Carolina; Chapel Hill, North Carolina	Assessment of dermal exposure to benzene and naphthalene	NIEHS
Rothman, N		Study of occupational and environmental exposures	Division of cancer epidemiology and genetics
Scow, KM	University of California; Davis, California	Molecular characterization of aquifer microbial communities	NIEHS
Stenzel, PS	National Institute of Health	Study of the link between occupational exposure of carcinogens and cancer	Division of cancer epidemiology and genetics
Thrall, KD	Oregon Health and Science University; Portland, Oregon	Assessment of human volatile organic compounds exposure near Superfund sites	NIEHS
Weisel, CP		Study of the modulation of benzene metabolism by exposure to environmental mixtures	USDA

[a]FEDRIP 2005

NIEHS = National Institute of Environmental Health Sciences; USDA = U.S. Department of Agriculture

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7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring benzene, its metabolites, and other biomarkers of exposure and effect to benzene. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

7.1   BIOLOGICAL MATERIALS

Analytical methods have been developed to measure benzene levels in exhaled breath, blood, and various body tissues. The primary method of analyzing for benzene in exhaled breath, body fluids, and tissues is gas chromatography (GC) coupled with either flame ionization detection (FID), photoionization detection (PID), or mass spectrometry (MS). Rigorous sample collection and preparation methods must be followed when analyzing for benzene to prevent contamination of the sample. A summary of commonly used methods of measuring benzene in biological samples is presented in Table 7-1.

Breath samples are collected on a solid sorbent (EPA 1987b; Gruenke et al. 1986; Pellizzari et al. 1988; Wallace et al. 1985), in canisters (Thomas et al. 1991), or in a breath sampling tube and analyzed directly (Sherwood and Carter 1970). Samples collected on Tenax sorbent are subjected to a thermal desorption/cryofocussing step prior to analysis by capillary GC/MS (EPA 1987b; Pellizzari et al. 1988; Wallace et al. 1985). Techniques involving headspace analysis of benzene adsorbed on silica gel have also been used (Gruenke et al. 1986). MS detection generally provides the most sensitivity, from the low to sub-ppb. The selectivity of the methods is improved if capillary GC columns are used (Pellizzari et al. 1988). Extraction of benzene from blood is frequently accomplished by either purge-and-trap or headspace analysis. In purge-and-trap analysis, an inert gas such as helium or nitrogen is passed through the sample, and purged volatiles are trapped on an appropriate solid sorbent (Antoine et al. 1986; Ashley et al. 1992, 1994; Michael et al. 1980). Recent improvements in the method have resulted in excellent sensitivity (300 ppt) and acceptable precision and accuracy (Ashley et al. 1992, 1994). The purge-and-trap method has also been used to analyze breast milk for other volatile organic compounds and could be

Table 7-1. Analytical Methods for Determining Benzene in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Breath	Collection on Tenax GC; thermal desorption	HRGC/MS (IARC Method 5)	3 ppt	70–130 estimated	Pellizzari et al. 1988
Breath	Collection in sampling tube; direct injection of sample	GC/FID	100 ppb	100	Sherwood and Carter 1970
Breath	Collection on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC/MS	1.6 ppb (5-L sample)	86–90	Wallace et al. 1986, 1985
Breath	Collection in bags, adsorption on silica gel; desorption to headspace vial; analysis of headspace gases	GC/MS-SIM	0.1 ppb	NR	Gruenke et al. 1986
Blood	Purge and trap	HRGC/MS	30 ppt	112–128	Ashley et al. 1992; 1994
Blood	Heparinization; transfer to isotonic saline in headspace vial; equilibration with heat	HRGC/PID	0.4 µg/L	NR	Pekari et al. 1989
Blood	Collection and transfer to headspace vial; analysis of headspace gases	GC/MS-SIM	2 µg/L	NR	Gruenke et al. 1986
Blood	Purging with nitrogen; collection on Tenax GC-silica gel	GC/MS	0.5 µg/L	NR	Antoine et al. 1986
Blood	Extraction with toluene + HCl; centrifugation; analysis of toluene layer	GC/FID	100 µg/L	98–100	Jirka and Bourne 1982
Blood	Extraction device coupled to MS	ITMS	90 ppt	NR	St-Germain et al. 1995
Blood	Centrifugation; dialysis; precipitation; acidic hydrolysis; Sep Pak cartridge column purification	HPLC	20 pmol/g globin	NR	Hanway et al. 2000
Urine	Incubation; analysis of headspace gases	GC/PID	0.51 nmol/L	>90	Kok and Ong 1994
Tissues (bone marrow, fat)	Homogenization with internal standard; centrifugation; analysis of supernatant	GC/MS-SIM	NR	NR	Rickert et al. 1979
Tissues (lung, liver)	Homogenization in buffer; centrifugation; analysis of supernatant	RID-preparative HPLC/UV	20 pg/g	NR	Bechtold et al. 1988

FID = flame ionization detection; GC = gas chromatography; HCl = hydrochloric acid; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; IARC = International Agency for Research on Cancer; ITMS = ion trap mass spectrometry; MS = mass spectrometry; NR = not reported; PID = photoionization detection; RID = reverse isotope dilution; SIM = selected ion monitoring; UV = ultraviolet detection

used for analyzing benzene (Michael et al. 1980; Pellizzari 1982). For headspace analysis, the samples

are placed in a special vial, and the gas generated above the liquid sample under equilibrium conditions is

analyzed (Gruenke et al. 1986; Pekari et al. 1989). Sensitivity is in the sub- to low-ppb range. A third

method of sample preparation involves extraction of the blood sample with an organic solvent (Jirka and

Bourne 1982) and analysis of the organic fraction. These methods are generally less sensitive, with

reported detection limits usually in the low- to mid-ppb range. Selectivity is improved with use of high

resolution gas chromatography (HRGC). Accuracy and precision could not be adequately compared

given the limited data available.

Screening methods are available for analysis of benzene in feces and urine (Ghoos et al. 1994) and body

fluids (Schuberth 1994). Both employ analysis by capillary GC with an ion trap detector (ITD). Benzene

in urine has been determined by trapping benzene stripped from the urine on a Carbotrap tube, followed

by thermal desorption GC/FID. Care must be taken when preparing benzene metabolite samples from

urine and bodily fluids in order to protect against enzymatic and oxidative degradation. These samples

are often treated to denature enzymes and avoid oxidation by hydroquinone. The detection limit is

50 ng/L and the average recovery is approximately 82% (Ghittori et al. 1993). Benzene in urine has also

been determined using headspace analysis with capillary GC/PID. The detection limit is 40 ng/L (Kok

and Ong 1994).

Methods are also available for determining metabolites of benzene in urine. A summary of available

methods is shown in Table 7-2. Both GC/FID or GC/MS and high-performance liquid chromatography

(HPLC) with ultraviolet detection (UV) have been used to measure urinary metabolites.

The primary metabolite of benzene is phenol. Phenol is excreted as glucuronide and sulphate conjugates

in urine. Total phenolic metabolites (phenol, phenyl sulfate, and phenyl glucuronide) have been

determined by hydrolyzing urine samples either enzymatically or by acid, then extracting the phenol with

solvent. Phenol is then measured by GC or HPLC techniques. Enzymatic hydrolysis coupled with

GC/FID has been reported; the detection limit is 1 mg/L and recovery is excellent (92–98%) (IARC

1988). Sulfate and glucuronide conjugates have been determined directly by HPLC/UV (Ogata and

Taguchi 1987). The normal baseline levels of urinary phenolic metabolites from humans are usually 2–

18 mg/L (Ong and Lee 1994). The available methods are sensitive enough to measure these relatively

high amounts accurately.

Table 7-2. Analytical Methods for Determining Metabolites of Benzene in Urine

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (phenol, phenyl sulfate, phenyl glucuronide)	Centrifugation	HPLC/UV	4 mg/L (PS); 5 mg/L (PG)	100.5 (PS); 101.8 (PG)	Ogata and Taguchi 1987
Urine (phenol, phenyl sulfate, phenyl glucuronide)	Digestion (enzymatic and with acid); extraction with diethyl ether	GC/FID (IARC Method 6)	1 mg/L	92–98	IARC 1988
Urine (phenols and cresols)	Hydrolysis with perchloric acid; extraction with diisopropyl ether	GC/FID	NR	NR	NIOSH 1974
Urine (phenols and cresols)	Hydrolysis with perchloric acid; saturation with NaCl; extraction with diisopropyl ether	GC/FID	2 mg/L	NR	Roush and Ott 1977
Urine (phenol)	Enzymatic reaction	HPLC/fluoro-metric detection	50 ppb	97.7	Jen and Tsai 1994
Urine (*trans,trans*-muconic acid)	Mixing with methanol; centrifugation	HPLC/UV	0.1 mg/L	NR	Inoue et al. 1989
Urine (muconic acid, phenol)	Mixing with formic acid; extraction (twice) with ethyl ether; evaporation of combined extracts	GC/MS	10 µg/L	NR	Bechtold et al. 1991
Urine (*trans,trans*-muconic acid)	Cleanup on anion exchange resin; derivitization	GC/MS	0.01 mg/L	93–106	Ruppert et al. 1995
Urine (S-phenyl-mercapturic acid	Solid-phase extraction; hydrolysis; derivatization	HPLC	1 µg/L	NR	Einig and Dehnen 1995
Urine (*trans,trans*-muconic acid)	Cleanup on anion exchange resin	HPLC/UV	5 ppb	NR	Weaver et al. 2000

FID = flame ionization detection; GC = gas chromatography; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; IARC = International Agency for Research on Cancer; MS = mass spectrometry; NaCl = sodium chloride; NR = not reported; PG = phenyl glucuronide; PS = phenyl sulfate; UV = ultraviolet detection

Analysis of urinary *trans,trans*-muconic acid seems to be a better indicator than phenol for assessing
exposure to low levels of benzene (Ducos et al. 1990). However, muconic acid is a minor metabolic route
and background levels of muconic acid in urine are much lower than levels of phenolic metabolites and
are frequently below the limit of detection of the method used to determine them (Inoue et al. 1989b).
The detection of low levels of *trans,trans*-muconic acid in urine was difficult by earlier methods because
of low recovery of *trans,trans*-muconic acid (37% with ether) by the commonly used solvent extraction
method (Gad-El-Karim et al. 1985). An improved method for the determination of urinary *trans,trans*-
muconic acid utilizes solid phase extraction with SAX sorbent in combination with the HPLC/UV for
quantitation. The detection limit is 0.06–0.1 mg/L, and recovery is very good (90%) (Boogaard and van
Sittert 1995; Ducos et al. 1990). Weaver et al. (2000) used an anion exchange column to extract the urine
sample, which was then analyzed using HPLC-UV; detection limits were around 5 ppb. The relative
standard deviation of the method was 5% in the concentration range 1–20 ng/L. *trans,trans*-muconic acid
has been determined directly by HPLC/UV with similar sensitivity (detection limit=0.1 mg/L) (Inoue et
al. 1989b). The detection limit and specificity for the determination of urinary *trans,trans*-muconic acid
may be improved by using HPLC with diode array detector, GC/FID of the methylated product, or
GC/MS of trimethylsilyated product (Bartczak et al. 1994). Both GC/FID and HPLC/diode array
detection are capable of detecting urinary *trans,trans*-muconic acid at concentrations above 40 µg/L, but
GC/MS is capable of detecting the metabolite at concentrations below 40 µg/L (Bartczak et al. 1994).

The metabolite, S-phenyl-mercaptic acid, may be an indicator of exposure to benzene. It can be detected
at low levels (1 µg/L) in urine using solid phase extraction and determination by HPLC (Einig and
Dehnen 1995). After purification by reverse phase cartridge chromatography, S-phenyl cysteine was
detected with sensitivity of about 20 pmol/g globin using HPLC (Hanway et al. 2000).

7.2   ENVIRONMENTAL SAMPLES

Methods exist for determining benzene in air (ambient, occupational, and industrial), water, sediment,
soil, foods, cigarette smoke, gasoline, and jet fuel. Most involve separation by GC with detection by FID,
PID, or MS. HPLC/UV and spectrophotometry have also been used. Table 7-3 summarizes several of
the methods that have been used to analyze for benzene in environmental samples.

Numerous methods exist for detecting and measuring benzene in ambient air. Air samples for benzene
analysis may be preconcentrated by passing the sample through a trap containing a solid adsorbent (Bayer
et al. 1988; EPA 1979, 1980a; Fung and Wright 1986; Gruenke et al. 1986; Harkov et al. 1985; Reineke

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Sample trapped on silica gel; thermal desorption	GC/MS	0.1 ppb	88–105	Gruenke et al. 1986
Air	Cryogenically trap; thermal desorption	GC/FID	NR	85–115	Singh et al. 1985
Air	Direct on-line analysis	GC/FID	NR	NR	Bayer et al. 1988
Air (ambient)	Direct injection of ambient air	GC/PID	0.25 ppb	NR	Clark et al. 1984
Air (ambient)	Direct analysis of ambient air	Electrochemical	NR	NR	Stetter et al. 1986
Air (ambient)	Sample collection in Tedlar bag; Cryogenically trap; thermally desorb	GC/PID	0.5 ppb	NR	Kowalski et al. 1985
Air (ambient)	Sample collection in stainless steel canisters or sorbent tubes; trap cryogenically; thermal desorption	HRGC/PID or HRGC/FID	5 ppt (PID) 24 ppt (FID)	97–104 (PID) 96–104 (FID)	Reineke and Bächmann 1985
Air (ambient)	Collect on charcoal (vapor badge or tube); desorb with carbon disulfide	GC/FID	0.3 ppb (estimated)	NR	Fung and Wright 1986
Air (ambient)	Cryogenically trap; thermal desorption	GC/PID/FID	1 ppt	70–130	Nutmagul and Cronn 1985
Air (ambient)	Collection in canisters; preconcentration using 2-stage trap	HRGC/ITD	sub-ppb level	8–13% bias	Kelly et al. 1993
Air (ambient)	Direct analysis	ALMS	250 ppb	NR	EPA 1985d
Air (ambient)	Sample collection onto on-column cryogenic sample loop or into stainless steel containers	GC/PID/FID	1 ppt	70–130	Nutmagul and Cronn 1985
Air (ambient)	Sample trapped on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC	<0.1 ppb	69–126	EPA 1979
Air (ambient)	Sample collected on Tenax GC; thermal desorion to on-column cryogenic trap	GC/FID	0.03 ppb	56–144	EPA 1980a
Air (ambient)	Sample trapped on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC/FID; conf. by HRGC/MS	3 ppt	NR	Roberts et al. 1984
Air (consumer products)	Sample trapped on solid sorbent; thermal desorption	GC/MS	NR	NR	Bayer et al. 1988

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (at waste sites and landfills)	Sample trapped on Tenax GC; thermal desorption	HRGC/FID/ECD; conf. by HRGC/MS	0.05 ppb	NR	Harkov et al. 1985
Air (occupational)	Sample trapped on charcoal; desorption with carbon disulfide	GC/FID (NIOSH Methods 1500 and 1501)	10–100 ppb	NR	NIOSH 1984
Air (occupational)	Sample collection on silica gel; desorption with ethanol	GC/FID	100 ppb	90	Sherwood and Carter 1970
Air (occupational)	Sample collection in Tedlar bag; direct injection	GC/PID (NIOSH Method 3700)	10 ppb	NR	NIOSH 1994
Air (occupational)	Sample collection on charcoal disk in miniature passive dosimeter; thermal desorption	GC/PID	60 ppb	85–115	Gonzalez and Levine 1986
Air (occupational; jet fuel fumes)	Sample collection on charcoal; desorption with methyl chloride-ethyl acetate	HPLC/UV	0.08 ppm	94–112	Dibben et al. 1989
Soil air	Sample collection on activated charcoal; desorption with carbon disulfide	GC/FID	NR	97–100	Colenutt and Davies 1980
Drinking water	Purge and trap	GC/MS	0.2 µg/L	NR	Brass et al. 1977
Drinking water	Purge and trap	HRGC/MS (EPA Method 524.2)	0.03–0.04 µg/L	97–99	EPA 1992a
Water	Purge and trap onto Tenax GC; thermal desorption to on-column cryogenic trap	HRGC/MS	0.1–10 µg/L	74–78	Michael et al. 1988
Water	Purge and trap on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC	<1 µg/L	69–126	EPA 1979
Water	Purge and trap onto Tenax GC; thermal desorption	GC/MS	NR	85–125	Harland et al. 1985
Water	Solvent extraction with dichloromethane; concentration	GC/MS	15 µg/L	NR	Sporstøl et al. 1985
Water	Purge and trap on adsorbent column; thermal desorption	GC/PID (EPA Method 602)	0.2 µg/L	106	NEMI 2005a
Water	Purge and trap on activated carbon; desorption with carbon disulfide	GC/FID; conf. by GC/MS	NR	96–99	Colenutt and Thorburn 1980
Water	Purge and trap on Tenax; thermal desorption	GC/FID	0.001 µg/L	94–111	Hammers and Bosman 1986

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Permeation of benzene through a silicone polycarbonate membrane into an inert gas stream	GC/FID	7.2 µg/L	NR	Blanchard and Hardy 1986
Waste water	Purge and trap onto adsorbent column; thermal desorption	GC/MS (EPA Method 624)	4.4 µg/L	113	NEMI 2005b
Waste water	Addition of isotopically labeled benzene analog; purge and trap onto adsorbent column; thermal desorption	GC/IDMS (EPA Method 1624)	10 µg/L	65–141	NEMI 2005c
Water, industrial effluents	Purge and trap on Tenax; thermal desorption	GC/MS	<5 µg/L	95–106	Pereira and Hughes 1980
Landfill leachate	Purge sample and trap on Tenax-silica gel; thermally desorb	GC/FID/FID	1 µg/L	NR	EPA 1984b
Landfill leachate	Extract sample with pentane	GC/MS	1,000–10,000 µg/L	NR	Schultz and Kjeldsen 1986
Solid wastes	Purge and trap, direct injection, vacuum distillation, or headspace	PID (EPA Method 8021B)	0.009 µg/L	99	EPA 1994c
Soil	Sample mixed with NaOH solution; equilibration; analysis of headspace gases	HRGC/FID; conf. by HRGC/MS	0.02 ng/mL	75–98	Kiang and Grob 1986
Soil	Purge and trap on Tenax; thermal desorption to on-column cryogenic trap	HRGC	<0.1 ppb	69–126	EPA 1979
Soil	Purge and trap on Tenax; thermal desorption	GC/FID	1 ppt	52	Hammers and Bosman 1986
Soil	Supercritical fluid extraction	HRGC/FID	low ppb	77–81	Burford et al. 1994
Sediment and biota	Purge and trap on Tenax GC-silica gel; thermal desorption	HRGC/MS	NR	NR	Ferrario et al. 1985
Sediment	Purge and trap on Tenax; thermal desorption	GC/MS	NR	64	Harland et al. 1985
Fruits and vegetables	Mix with water and methanol; filter; distill azeotrope	GC/FID	NR	84–96	Kozioski 1985
Shellfish	Tissue homogenized; purge with inert gas and trap on Tenax GC-silica gel; thermally desorb	GC/MS	NR	NR	Ferrario et al. 1985

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Mainstream cigarette smoke	Collection on filters and impingers; [2H6]-benzene added to impinger	HRGC/IDMS-SIM	0.05 µg/ cigarette	75–85 (trapping efficiency)	Byrd et al. 1990
Cigarette smoke	Mainstream smoke filtered and analyzed directly; side-stream smoke and smoke-polluted air filtered and collected in cryogenic methanol-filled impingers	HRGC/MS-SIM	0.1 µg/ cigarette	NR	Brunnemann et al. 1989, 1990
Gasoline	Dilute sample with hexane	GC/FID	NR	NR	Poole et al. 1988
Gasoline	Dilute sample with methanol; elute benzene to analytical column with 50% methanol; back-flush guard column with 100% methanol	HPLC/UV	NR	NR	Ludwig and Eksteen 1988

ECD = electron capture detection; EPA = Environmental Protection Agency; FID = flame ionization detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; IARC = International Agency for Research on Cancer; IDMS = isotope dilution mass spectrometry; ITD = ion trap mass spectrometry; LRS = laser Raman spectroscopy; MS = mass spectrometry; NaOH = sodium hydroxide; NIOSH = National Institute of Occupational Safety and Health; NR = not reported; PID = photoionization detection; SIM = selected ion monitoring; UV = ultraviolet detection

and Bächmann 1985; Roberts et al. 1984). Commonly used adsorbents are Tenax resins (e.g., Tenax TA, Tenax GC), silica gel, activated carbon, and carbonaceous polymeric compounds. Benzene in ambient air can be collected in stainless steel canisters (EPA 1988a; Kelly et al. 1993) or Tedlar bags (Kowalski et al. 1985) and can be analyzed with or without preconcentration. Preconcentration of benzene can be accomplished by direct on-column cryogenic trapping (EPA 1985c; Kowalski et al. 1985; Nutmagul and Cronn 1985; Reineke and Bächmann 1985; Singh et al. 1985), or samples may be analyzed directly without preconcentration (Bayer et al. 1988; Clark et al. 1984).

The most common methods of analysis for benzene in air are GC/PID, GC/FID, and GC/MS. The limit of detection for GC/FID and GC/PID ranges from low ppb to low ppt. GC/MS is generally considered to be more reliable than GC/FID or GC/PID in identifying benzene in samples, particularly those containing multiple components having similar GC elution characteristics. Benzene has been quantified in ambient air samples at sub-ppb levels by GC/MS (Gruenke et al. 1986) and ion trap mass spectrometry (Kelly et al. 1993). The ion trap detector has the advantage of remaining largely unaffected by water vapor in the sample. A continuous monitoring instrument using Fourier transform infrared (FTIR) spectroscopy is being developed for measuring the levels of benzene or other toxic chemicals in exhaust emissions from hazardous waste incinerators (DOE 1992).

Several analytical methods are available for determining atmospheric levels of benzene in the workplace. The OSHA recommended procedure involves the collection of the sample vapors on charcoal adsorption tubes, and then desorption followed by GC/MS analysis (OSHA 1985). Samples desorbed from charcoal are also analyzed by GC/FID (NIOSH 1984) or HPLC/UV (Dibben et al. 1989). Detection limits are in the ppb range (Dibben et al. 1989; NIOSH 1984). Passive dosimeters are also utilized, with GC/PID quantitation; detection limits are in the ppb range (Gonzalez and Levine 1986). Other acceptable methods include portable direct reading instruments and real-time continuous monitoring systems; these methods generally have a sensitivity in the ppm range.

The most frequently used analytical methods for water samples containing benzene are GC/MS, GC/FID, and GC/PID (Blanchard and Hardy 1986; Colenutt and Thorburn 1980; DOI 1984; EPA 1984, 1992; Hammers and Bosman 1986; Harland et al. 1985; Lysyj et al. 1980; Michael et al. 1988; Pereira and Hughes 1980; Sporstol et al. 1985). Benzene is usually isolated from aqueous media by the purge-and-trap method (Brass et al. 1977; Colenutt and Thorburn 1980; DOI 1984; EPA 1979, 1984, 1992; Hammers and Bosman 1986; Harland et al. 1985; Michael et al. 1988). An inert gas such as nitrogen is used to purge the sample. The purged benzene is trapped on an adsorbent substance, such as Tenax GC

or activated charcoal, and thermally desorbed. Recovery, where reported, ranges from acceptable (≈70%)
(EPA 1979, 1984; Michael et al. 1988) to very good (≥90%) (Colenutt and Thorburn 1980; EPA 1984,
1992; Hammers and Bosman 1986). Detection limits in the sub-ppb to ppt range may be attained with
HRGC/MS techniques (EPA 1992a); Michael et al. 1988). Liquid-liquid extraction procedures (Harrison
et al. 1994; Schultz and Kjeldsen 1986; Sporstol et al. 1985) are less commonly used, having been
replaced by more sensitive purge-and-trap methods. Interference from contamination can occur with all
methods if extreme care is not used in the handling of samples and cleaning of all equipment.

Solid samples, such as soil, sediment, and foods, are most frequently prepared for analysis using the
purge-and-trap method (EPA 1979, 1994c; Ferrario et al. 1985; Hammers and Bosman 1986; Harland et
al. 1985), although supercritical fluid extraction has recently been utilized (Burford et al. 1994).
Detection and quantitation of benzene may be GC/FID, GC/PID, or GC/MS. Detection limits as low as
1 ppt have been reported, but recoveries and precision have frequently been low. Improvements in the
method, including analysis by HRGC/PID, have resulted in low detection limits (9 ppt) and excellent
recovery (99%) for benzene (EPA 1994c). Screening methods are available for benzene; some may be
used at field sites. Immunoassay may be used as a screening and semiquantitative tool (Van Emon and
Gerlach 1995).

Methods exist for detection of benzene in other environmental media such as cigarette smoke, gasoline,
and jet fuel and its fumes (Brunnemann et al. 1989; Byrd et al. 1990; Ludwig and Eksteen 1988; Poole et
al. 1988). HPLC/UV, GC/FID, and GC/MS separation and detection techniques have been used for these
analyses. Sensitivity and reliability of these methods cannot be compared because of the lack of data.
Few methods have been reported for measurement of benzene in foods; performance data are generally
lacking.

7.3   ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the
Administrator of EPA and agencies and programs of the Public Health Service) to assess whether
adequate information on the health effects of benzene is available. Where adequate information is not
available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research
designed to determine the health effects (and techniques for developing methods to determine such health
effects) of benzene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1   Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Methods exist for measuring benzene in breath (Gruenke et al. 1986; Pellizzari et al. 1988; Sherwood and Carter 1970; Wallace et al. 1986), blood (Antoine et al. 1986; Ashley et al. 1992, 1994; Gruenke et al. 1986; Jirka and Bourne 1982; Pekari et al. 1989), and tissues (Bechtold et al. 1988; Rickert et al. 1979). The methods for breath are sensitive and accurate for determining exposure levels of benzene at which health effects have been observed to occur, as well as for background levels in the general population. The methods are relatively precise and selective. Methods for determining benzene in blood are sensitive; based on the limited recovery data available, they appear to be accurate. More information on the performance obtained with different methods would be helpful. The application of GC/MS techniques to the analysis of blood specimens has resulted in a rapid, cost-effective, clinical screening test for common volatile organic compounds, including benzene (DeLeon and Antoine 1985). This test, the VOST (Volatile Organics Screening Test), has demonstrated the presence (down to 0.1 ppb) of a variety of toxic volatile organics in the blood of environmentally sensitive patients and has provided preliminary baseline concentration levels for the test population (DeLeon and Antoine 1985). The data on determination of benzene in urine and tissue samples are very limited. In general, the available methods have limits of detection that are too high to be useful in other than acute exposure situations. Methods that could be used to measure low levels in human tissues would be useful for determining the relationship between chronic low-level exposure and the effects observed in specific tissues.

Methods are available for measuring phenolic benzene metabolites in urine (Bechtold et al. 1991; IARC 1988; Jen and Tsai 1994; Jongeneelen et al. 1987; NIOSH 1974; Ogata and Taguchi 1987). Available methods for determining most benzene metabolites in urine are sufficiently sensitive and reliable to allow measurement of background concentrations in nonoccupationally exposed individuals. However, the phenolic metabolites are not unique to benzene. Improved methods to detect phenolic metabolites are not needed. Sensitive assays have been developed for detection of urinary *trans,trans*-muconic acid

(detection limit 10 μg/L) (Bechtold et al. 1991; Ruppert et al. 1995). Since urinary *trans,trans*-muconic acid concentration can be correlated with benzene exposure, this may provide a useful biomarker of exposure on an individual basis (Bechtold et al. 1991; EPA 1992b; Weaver et al. 2000). In addition, information is needed to assess the effect of co-exposure to other chemicals (e.g., toluene) on urinary muconic acid levels. Also needed are specific biomarkers of cumulative exposure to benzene, based on albumin or hemoglobin adducts, and lymphocyte DNA adducts of N-7-phenylguanine. It would also be useful to develop specific biomarkers of acute- and chronic-duration exposure to benzene based on adducts of muconaldehyde. The levels of such biomarkers formed *in vivo* would be useful later for correlation with toxic effects of acute- or chronic-duration exposure to benzene. S-phenyl-mercapturic acid has also been useful in biological monitoring of benzene exposure in humans and animals. S-phenyl-mercapturic acid can be measured with a sensitivity of about 20 pmol/g globin using HPLC (Hanway et al. 2000). S-phenyl-mercapturic acid levels can also be correlated to environmental benzene exposure (Popp et al. 1994), which may indicate its utility as a biomarker.

Effect. Methods for determining benzene in breath, blood, and tissues and for determining its metabolites in urine could also be used as biomarkers of effect. However, efforts to correlate these measures with observed toxic effects of benzene exposure have been unsuccessful. Other biomarkers of effect (e.g., complete blood cell counts, red and white blood cell counts, chromosomal aberrations, sister chromatid exchanges, and examination of bone marrow) have been suggested for benzene, but they are not specific for benzene exposure. Further development of methods for determining reliable unique biomarkers of effect for benzene would be useful.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media.   Methods for determining benzene in air (Clark et al. 1984; Gruenke et al. 1986) and water (Brass et al. 1977; EPA 1979, 1984; Hammers and Bosman 1986; Pereira and Hughes 1980), the media of most concern for human exposure, are sensitive enough to measure background levels in the environment and levels at which health effects might occur. Their reliability is limited primarily by the ubiquitous presence of benzene in the environment, which makes contamination a constant problem. The accuracy and precision of some methods for water analyses (e.g., GC/MS) need to be improved to produce more reliable results. Methods for soil and other solid media appear to have the same problems as those for air and water. In addition, there is a lack of information on methods for determining benzene in media such as shellfish, fish, foods, and plants. Although exposure to benzene via ingestion of food is believed to be minimal, standardized methods for these media are needed to better assess the extent of benzene contamination in the environment and the resulting risk of exposure.

7.3.2   Ongoing Studies

The information in Table 7-4 was found as a result of a search of the Federal Research in Progress database (FEDRIP 2005).

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, is developing methods for the analysis of benzene and other volatile organic compounds in blood. These methods use purge and trap methodology, high-resolution gas chromatography, and magnetic sector mass spectrometry, which give detection limits in the low parts per trillion (ppt) range.

Table 7-4. Ongoing Studies on Benzene, Analytical Methods[a]

Investigator	Affiliation	Research description	Sponsor
Fales, HM		Analysis of proteins, peptides, and metabolites for carcinogen exposure using mass spectrometry	USDA
Kalman, DA	University of Washington Seattle, Washington	Study of the use of human dosimetry for assessment of exposure to volatile compounds	NIEHS
Kavanaugh, TJ		Study of glutathione biosynthesis as a biomarker of toxic exposure	NSF
O'Brien, RJ		Assessment Environmental Health Breath using PF-GC	NIEHS
Pavel, K	VOC Technologies Inc.; Portland, Oregon	Assessment Environmental Health Breath using PF-GC	NIEHS
Rappaport, SM	University of North Carolina; Chapel Hill, North Carolina	Development and application of biomarkers in the study of exposure to carcinogens	NIEHS
Sabri, MI	Oregon Health Science University; Portland, Oregon	Study of biomarkers of neurotoxicant exposure and neurodegeneration	NIH
Smejtek, PK	VOC Technologies Inc.; Portland, Oregon	A PF-GC for Environmental Health Breath Assessment	NIEHS
Smith, MT	University of California at Berkeley	Study of biomarkers of benzene exposure and genotoxicity	NIEHS
Spencer, PS	Oregon Health Sciences University; Portland, Oregon	Study of neurotoxic chemicals and biomarkers at superfund sites	NIH
Stewart, P		Use of biomarkers to study occupational exposure of carcinogens in order to enhance exposure assessment	Division of cancer epidemiology and genetics
Turtletaub, KW	University of California at Berkeley	Study and development of biomarkers for exposure using accelerator mass spectrometry	NIEHS
Weaver, VM		Use of biomarkers to study benzene exposure in inner city residents	USDA
Wiencke, JK		Use of molecular biomarkers of occupational benzene exposure	USDA

[a]FEDRIP 2005

NIEHS = National Institute of Environmental Health Sciences; NIH = National Institutes of Health; NSF = National Science Foundation; USDA = U.S. Department of Agriculture

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8. REGULATIONS AND ADVISORIES

The international and national regulations and guidelines regarding benzene in air, water, and other media are summarized in Table 8-1.

ATSDR has derived an acute-duration inhalation MRL of 0.009 ppm for benzene based on a LOAEL of 10.2 ppm for immunological effects in mice exposed for 6 hours/day for 6 consecutive days (Rozen et al. 1984). The LOAEL of 10.2 ppm was adjusted from intermittent to continuous exposure ($LOAEL_{ADJ}$=2.55 ppm) and converted to a human equivalent concentration ($LOAEL_{HEC}$=2.55 ppm) using EPA (1994b) methodology for a category 3 gas; an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, and 10 to protect sensitive individuals) was applied.

ATSDR has derived an intermediate-duration inhalation MRL of 0.006 ppm for benzene based on a LOAEL of 10 ppm for significantly delayed splenic lymphocyte reaction to foreign antigens evaluated in *in vitro* mixed lymphocyte reaction following the exposure of male C57Bl/6 mice to benzene vapors for 6 hours/day, 5 days/week for 20 exposure days (Rosenthal and Snyder 1987). The concentration was adjusted from intermittent to continuous exposure (LOAELADJ=1.8 ppm) and converted to a human equivalent concentration ($LOAEL_{HEC}$=1.8 ppm) using EPA (1994b) methodology for a category 3 gas; an uncertainty factor of 300 (10 for the use of LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, and 10 for human variability) was applied.

ATSDR has derived a chronic-duration inhalation MRL of 0.003 ppm for benzene based on the results of benchmark dose (BMD) modeling of B cell counts in workers of shoe manufacturing industries in Tianjin, China (Lan et al. 2004a). The resulting $BMCL_{0.25sd}$ of 0.10 ppm was adjusted from intermittent to continuous exposure ($BMCL_{0.25sdADJ}$=0.03 ppm) using EPA (1994b) methodology; an uncertainty factor of 10 (to protect sensitive individuals) was applied.

ATSDR has derived a chronic-duration oral MRL of 0.0005 mg/kg/day for benzene based on estimation of equivalent chronic-duration oral dosing that would result in effects similar to those observed in the occupationally-exposed workers assessed by Lan et al. (2004a, 2004b). The $BMCL_{0.25sdADJ}$ of 0.03 ppm (0.096 mg/m^3) was converted to an equivalent $BMDL_{0.25sdADJ}$ of 0.014 mg/kg/day for ingested benzene using EPA (1988b) human reference values for inhalation rate (20 m^3/day) and body weight (70 kg) and a factor of 0.5 to adjust for differences in absorption of benzene following inhalation versus oral exposure;

Table 8-1. Regulations and Guidelines Applicable to Benzene

Agency	Description	Information	Reference
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification	Group 1[a]	IARC 1987, 2004, 2007
WHO	Air quality guidelines	6×10^{-6} unit risk	WHO 2000
	Drinking water quality guidelines	0.01 mg/L[b]	WHO 2004
NATIONAL			
Regulations and Guidelines:			
a. Air			
ACGIH	TLV (TWA)	0.5 ppm[c]	ACGIH 2006
	STEL	2.5 ppm[c]	
EPA	Hazardous air pollutant	Yes	EPA 2004b 42 USC 7412
NAS/NRC	AEGL-1[d]		EPA 2005a
	10 minutes	130 ppm	
	30 minutes	73 ppm	
	60 minutes	52 ppm	
	4 hours	18 ppm	
	8 hours	9.0 ppm	
	AEGL-2[d]		
	10 minutes	2,000 ppm[e]	
	30 minutes	1,100 ppm	
	60 minutes	800 ppm	
	4 hours	400 ppm	
	8 hours	200 ppm	
	AEGL-3[d]		
	10 minutes	9,700 ppm[f]	
	30 minutes	5,600 ppm[e]	
	60 minutes	4,000 ppm[e]	
	4 hours	2,000 ppm[e]	
	8 hours	990 ppm	
NIOSH	REL (10-hour TWA)	0.1 ppm[g]	NIOSH 2005
	STEL	1.0 ppm[g]	
	IDLH	500 ppm[g]	
OSHA	PEL (8-hour TWA) for general industry	1 ppm	OSHA 2005b, 2005e 29 CFR 1910.1000 29 CFR 1910.1028
	PEL (8-hour TWA) for construction industry	1 ppm	OSHA 2005d, 2005f 29 CFR 1926.55, Appendix A 29 CFR 1926.1128

Table 8-1. Regulations and Guidelines Applicable to Benzene

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
OSHA	PEL (8-hour TWA) for shipyard industry	1 ppm	OSHA 2005a,2005b 29 CFR 1915.1000 29 CFR 1915.1028
b. Water			
EPA	Designated as hazardous substances in accordance with Section 311(b)(2)(A) of the Clean Water Act	Yes	EPA 2005b 40 CFR 116.4
	Drinking water standards and health advisories		EPA 2004a
	1-day health advisory for a 10-kg child	0.2 mg/L	
	10-day health advisory for a 10-kg child	0.2 mg/L	
	DWEL	0.1 mg/L	
	10^{-4} Cancer risk	0.1 mg/L	
	National primary drinking water standards		EPA 2002a
	MCLG	Zero	
	MCL	0.005 mg/L	
	Reportable quantities of hazardous substances designated pursuant to Section 311 of the Clean Water Act	10 pounds	EPA 2005c 40 CFR 117.3
	Water quality criteria for human health consumption of:		EPA 2002b
	Water + organism	2.2 µg/L[h]	
	Organism only	51 µg/L[h]	
c. Food			
FDA	Bottled drinking water	0.005 mg/L	FDA 2004 21 CFR 165.110
d. Other			
ACGIH	Carcinogenicity classification	A1[i]	ACGIH 2006
	Biological exposure indices (end of shift)		
	S-phenylmercapturic acid in urine	25 µg/g creatinine	
	t,t-Muconic acid in urine	500 µg/g creatinine	
EPA	Carcinogenicity classification	Group A[j]	IRIS 2007
	Oral slope factor	1.5×10^{-2}–5.5×10^{-2} per (mg/kg)/day	
	Inhalation unit risk	2.2×10^{-6}–7.8×10^{-6} per µg/m^3	
	RfC	0.03 mg/m^3	
	RfD	4×10^{-3} mg/kg/day	

Table 8-1. Regulations and Guidelines Applicable to Benzene

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
EPA	Superfund, emergency planning, and community right-to-know		
	Designated CERCLA hazardous substance		EPA 2005d 40 CFR 302.4
	Reportable quantity	10 pounds[k]	
	RCRA hazardous waste number	U019	
	Effective date of toxic chemical release reporting	01/01/87	EPA 2005e 40 CFR 372.65
NTP	Carcinogenicity classification	Known human carcinogen	NTP 2005

[a]Group 1: carcinogenic to humans
[b]For substances that are considered to be carcinogenic, the guideline value is the concentration in drinking water associated with an upper-bound excess lifetime cancer risk of 10^{-5} (one additional cancer per 100,000 of the population ingesting drinking water containing the substance at the guideline value for 70 years). Concentrations associated with upper-bound estimated excess lifetime cancer risks of 10^{-4} and 10^{-6} can be calculated by multiplying and dividing, respectively, the guideline value by 10.
[c]Skin notation: refers to the potential significant contribution to the overall exposure by the cutaneous route, including mucous membranes and the eyes, either by contact with vapors or, of probable greater significance, by direct skin contact with the substance.
[d]AEGL-1 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory effects. AEGL-2 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape. AEGL-3 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.
[e]Values denoted as having safety considerations against the hazard of explosion, whereas the Lower Explosive Limit (LEL) =14,000 ppm and each value should be ≥10% LEL.
[f]Value denoted as having extreme safety considerations against the hazard of explosion must be taken into account, whereas the LEL =14,000 ppm and each value should be ≥50% LEL.
[g]NIOSH potential occupational carcinogen
[h]This criterion is based on carcinogenicity of 10^{-6} risk.
[i]A1: confirmed human carcinogen
[j]Group A: known human carcinogen
[k]Designated CERCLA hazardous substance pursuant to Section 311(b)(2) and 307(a) of the Clean Water Act, Section 112 of the Clean Air Act, and Section 3001 of RCRA.

ACGIH = American Conference of Governmental Industrial Hygienists; AEGL = Acute Emergency Exposure Guideline Levels; CERCLA = Comprehensive Environmetnal Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; DWEL = drinking water equivalent level; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; MCL = maximum contaminant level; MCLG = maximum contaminant level goal; NAS/NRC = National Academy of Sciences/National Research Council; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; RCRA = Resource Conservation and Recovery Act; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; STEL = short-term expsoure limit; TLV = threshold limit values; TWA = time-weighted average; USC = United States Code; WHO = World Health Organization

an uncertainty factor of 30 (10 to protect sensitive individuals and 3 for uncertainty in route-to-route extrapolation) was applied.

EPA (IRIS 2007) derived an inhalation reference concentration (RfC) for benzene of 0.03 mg/m^3 (0.009 ppm) based on the results of BMD modeling of absolute lymphocyte (ALC) data from the occupational epidemiologic study of Rothman et al. (1996a), in which workers were exposed to benzene by inhalation. The resulting BMCL of 7.2 ppm for decreased lymphocyte count was converted to 23.0 mg/m^3 and adjusted from intermittent to continuous exposure (BMCL$_{ADJ}$=8.2 mg/m^3); a total uncertainty factor of 300 (3 for effect-level extrapolation, 10 to protect sensitive individuals, 3 for subchronic-to-chronic extrapolation, and 3 for database deficiencies) was applied.

EPA (IRIS 2007) derived an oral reference dose (RfD) for benzene of 0.004 mg/kg/day, based on the results of BMD modeling of ALC data from the occupational epidemiologic study of Rothman et al. (1996a), in which workers were exposed to benzene by inhalation. The resulting BMCL of 7.2 ppm for decreased lymphocyte count was converted to 23.0 mg/m^3 and adjusted from intermittent to continuous exposure (BMCL$_{ADJ}$=8.2 mg/m^3). Route-to-route extrapolation methodology was applied to convert from inhalation to equivalent oral exposure, resulting in an equivalent oral dose rate of 1.2 mg/kg/day. This value was divided by a total uncertainty factor of 300 (3 for effect-level extrapolation, 10 to protect sensitive individuals, 3 for subchronic-to-chronic extrapolation, and 3 for database deficiencies).

The International Agency for Research on Cancer (IARC) classifies benzene as a Group 1 carcinogen (carcinogenic to humans) (IARC 2004). EPA classified benzene in Category A (known human carcinogen) based on convincing evidence in humans supported by evidence from animal studies. Under EPA's most recent guidelines for carcinogen risk assessment, benzene is characterized as a known human carcinogen for all routes of exposure based on convincing human evidence as well as supporting evidence from animal studies (IRIS 2007). The National Toxicology Programs lists benzene as a "substance known to be carcinogenic," that is, a substance for which the evidence from human studies indicates that there is a causal relationship between exposure to the substance and human cancer (NTP 2005).

The EPA has a current maximum contaminant level (MCL) of 0.005 mg/L for benzene in drinking water (EPA 2002a). The World Health Organization (WHO) has established a guideline value of 0.01 mg/L for benzene in drinking water (WHO 2004).

Benzene is on the list of chemicals in "The Emergency Planning and Community Right-to-Know Act of 1986" (EPA 2005d). Section 313 of Title III of the Superfund Amendments and Reauthorization Act (SARA) requires owners and operators of certain facilities that manufacture, import, process, or otherwise use the chemicals on this list to report annually any release of those chemicals to any environmental media over a specified threshold level (U.S. Congress 1986).

OSHA requires employers of workers who are occupationally exposed to benzene to institute engineering controls and work practices to reduce and maintain employee exposure at or below permissible exposure limits (PEL). If an employer can document that benzene is used in the workplace <30 days/year, the employer can use any combination of engineering controls, work practice controls, or respirators to reduce employee exposure to or below the PEL of 1 ppm (8-hour TWA). Respirators must be provided and used during the time period necessary to install or implement feasible engineering and work practice controls, or where controls are not yet sufficient. Respirators are also required when the employer determines that compliance with the PEL is not feasible with engineering or work practice controls, such as maintenance and repair activities, vessel cleaning, or other operations where exposures are intermittent and limited in duration, and in emergencies (OSHA 2005c).

ACGIH limits exposure to benzene to 0.5 ppm (8-hour TWA) (ACGIH 2006). The National Institute for Occupational Safety and Health (NIOSH 2005) has established a recommended exposure level (REL) of 0.1 ppm (15-minute ceiling limit).

9. REFERENCES

ACGIH. 1996. Benzene. Threshold limit values for chemical substances and physical agents. Biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists, 60, 147.

ACGIH. 2004. Benzene. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Adgate JL, Church TR, Ryan AD, et al. 2004. Outdoor, indoor and personal exposure to VOCs in children. Environ Health Perspect 112(14):1386-1392.

Adinolfi M. 1985. The development of the human blood-CSF-brain barrier. Dev Med Child Neurol 27:532-537.

Adlercreutz H. 1995. Phytoestrogens: Epidemiology and a possible role in cancer protection. Environ Health Perspect Suppl 103(7):103-112.

Adlkofer F, Scherer G, Conze C, et al. 1990. Significance of exposure to benzene and other toxic compounds through environmental tobacco smoke. J Cancer Res Clin Oncol 116(6):591-598.

Agency for Toxic Substances and Disease Registry. 1989. Decision guide for identifying substance-specific data needs related to toxicological profiles; Notice. Atlanta, GA: Agency for Toxic Substances and Disease Registry, Division of Toxicology. Fed Regist 54(174):37618-37634.

Agency for Toxic Substances and Disease Registry. 1991. Benzene groundwater exposure study Nesmith, South Carolina. Final report technical assistance to South Carolina Department of Health and Environmental Control. Atlanta, GA: Agency for Toxic Substances and Disease Registry. Division of Health Assessment and Consultation.

Agency for Toxic Substances and Disease Registry. 1995. National exposure registry benzene subregistry baseline technical report. Atlanta, GA: U.S. Department of Health and Human Services. Agency for Toxic Substances and Disease Registry. PB95255766, 1-114.

Agency for Toxic Substances and Disease Registry. 2001. National exposure registry. Benzene subregistry. Baseline through followup 4 technical report. Atlanta, GA: U.S. Department of Health and Human Services. Agency for Toxic Substances and Disease Registry. http://www.atsdr.cdc.gov/NER/BENZENE/2001/benzene2001.html. July 11, 2005.

AIHA. 1989. Odor thresholds for chemicals with established occupational health standards. Fairfax, VA: American Industrial Hygiene Association, 1, 9, 13, 41, 46.

Aksoy M. 1980. Different types of malignancies due to occupational exposure to benzene: A review of recent observations in Turkey. Environ Res 23:181-190.

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*Not cited in text

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9. REFERENCES

Aksoy M. 1987. Chronic lymphoid leukaemia and hairy cell leukaemia due to chronic exposure to benzene: Report of three cases. Br J Haematol 66:209-211.

Aksoy M. 1991. Hematotoxicity, leukemogenicity, and carcinogenicity of chronic exposure to benzene. In: Arinc E, Schenkman JB, Hodgson E, eds. Molecular aspects of monooxygenases and bioactivation of toxic compounds. NATO advanced science institutes series A: Life sciences, Vol. 202. New York, NY: Plenum Press, 415-434.

Aksoy M, Erdem S. 1978. Followup study on the mortality and the development of leukemia in 44 pancytopenic patients with chronic benzene exposure. Blood 52:285-292.

Aksoy M, Dincol K, Akgun T, et al. 1971. Haematological effects of chronic benzene poisoning in 217 workers. Br J Ind Med 28:296-302.

Aksoy M, Dincol K, Erdem S, et al. 1972. Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. Br J Ind Med 29:56-64.

Aksoy M, Erdem S, Dincol G. 1974. Leukemia in shoe-workers exposed chronically to benzene. Blood 44:837-841.

*Aksoy M, Erdem S, Erdogan G, et al. 1976. Combination of genetic factors and chronic exposure to benzene in the aetiology of leukaemia. Hum Hered 26:149-153.

Aksoy M, Özeris S, Sabuncu H, et al. 1987. Exposure to benzene in Turkey between 1983 and 1985: A haematological study on 231 workers. Br J Ind Med 44:785-787.

*Alderson M, Rushton L. 1982. Mortality patterns in eight U.K. oil refineries. Ann N Y Acad Sci 381(1):139-145.

Altman PL, Dittmer DS. 1974. Biological handbooks: Biology data book. Vol. III. 2nd ed. Bethesda, MD: Federation of American Societies for Experimental Biology, 1987-2008, 2041.

Amodio-Cocchieri R, Del Prete U, Cirillo T, et al. 2001. Evaluation of benzene exposure in children living in Campania (Italy) by urinary *trans,trans*-muconic acid assay. J Toxicol Environ Health A 63(2):79-87.

Andersen ME, Krishnan K. 1994. Relating in vitro to in vivo exposures with physiologically based tissue dosimetry and tissue response models. In: Salem H, ed. Animal test alternatives: Refinement, reduction, replacement. New York: Marcel Dekker, Inc., 9-25.

Andersen ME, Clewell HJ III, Gargas ML, et al. 1987. Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol Appl Pharmacol 87:185-205.

Anderson D, Richardson CR. 1981. Issues relevant to the assessment of chemically induced chromosome damage in vivo and their relationship to chemical mutagenesis. Mutat Res 90:261-272.

Andreoli C, Leopardi P, Crebelli R. 1997. Detection of DNA damage in human lymphocytes by alkaline single cell gel electrophoresis after exposure to benzene or benzene metabolites. Mutat Res 377(1):95-104.

9. REFERENCES

Andrews LS, Lee EW, Witmer CM, et al. 1977. Effects of toluene on metabolism, disposition, and hematopoietic toxicity of [^{3}H] benzene. Biochem Pharmacol 26:293-300.

Andrews LS, Sasame HA, Gillette JR. 1979. ^{3}H-Benzene metabolism in rabbit bone marrow. Life Sci 25:567-572.

Angerer J, Scherer G, Schaller KH, et al. 1991. The determination of benzene in human blood as an indicator of environmental exposure to volatile aromatic compounds. Fresenius J Anal Chem 339(10):740-742.

Antoine SR, DeLeon IR, O'Dell-Smith RM. 1986. Environmentally significant volatile organic pollutants in human blood. Bull Environ Contam Toxicol 36:364-371.

Aoyama K. 1986. Effects of benzene inhalation on lymphocyte subpopulations and immune response in mice. Toxicol Appl Pharmacol 85:92-101.

Appel BR, Guirguis G, Kim IS, et al. 1990. Benzene, benzo(a)pyrene, and lead in smoke from tobacco products other than cigarettes. Am J Public Health 80(5):560-564.

Arfellini G, Grilli S, Colacci A, et al. 1985. *In vivo* and *in vitro* binding of benzene to nucleic acids and proteins of various rat and mouse organs. Cancer Lett 28:159-168.

Arinc E, Adali O, Iscan M, et al. 1991. Stimulatory effects of benzene on rabbit liver and kidney microsomal cytochrome P-450 dependent drug metabolizing enzymes. Arch Toxicol 65(3):186-190.

*Armstrong MJ, Galloway SM. 1993. Micronuclei induced in peripheral blood of E μ-PIM-1 transgenic mice by chronic oral treatment with 2-acetylaminofluorene or benzene but not with diethyl-nitrosamine or 1,2-dichloroethane. Mutat Res 302(1):61-70.

Arvin E, Jensen BK, Gundersen AT. 1989. Substrate interactions during aerobic biodegradation of benzene. Appl Environ Microbiol 55:3221-3225.

Ashley DL, Bonin MA, Cardinali FL. 1992. Determining volatile organic compounds in the human blood from a large sample population by using purge and trap gas chromatography/mass spectrometry. Anal Chem 64:1021-1029.

Ashley DL, Bonin MA, Cardinali FL, et al. 1994. Blood concentrations of volatile organic compounds in a nonoccupationally exposed US population and in groups with suspected exposure. Clin Chem 40(7):1401-1404.

Astier A. 1992. Simultaneous high-performance liquid chromatographic determination of urinary metabolites of benzene, nitrobenzene, toluene, xylene and styrene. J Chromatogr 573(2):318-322.

Au WW, Anwar WA, Hanania E, et al. 1990. Antimutagenic effects of dimethyl sulfoxide on metabolism and genotoxicity of benzene *in vivo*. Basic Life Sci 52:389-393.

Au WW, Ramanujam VM, Ward JB Jr, et al. 1991. Chromosome aberrations in lymphocytes of mice after sub-acute low-level inhalation exposure to benzene. Mutat Res 260(2):219-224.

Aubrecht J, Rugo R, Schiestl RH. 1995. Carcinogens induce intrachromosomal recombination in human cells. Carcinogenesis 16(11):2841-2846.

Austin CC, Wang D, Ecobichon DJ, et al. 2001. Characterization of volatile organic compounds in smoke at municipal structural fires. J Toxicol Environ Health A 63(6):437-458.

Avis SP, Hutton CJ. 1993. Acute benzene poisoning: A report of three fatalities. J Forensic Sci 38(3):599-602.

Baarson KA, Snyder CA. 1991. Evidence for the disruption of the bone marrow microenvironment by combined exposures to inhaled benzene and ingested ethanol. Arch Toxicol 65(5):414-420.

Baarson KA, Snyder CA, Albert RE. 1984. Repeated exposures of C57B1 mice to inhaled benzene at 10 ppm markedly depressed erythropoietic colony formation. Toxicol Lett 20:337-342.

Baarson K, Snyder CA, Green J, et al. 1982. The hematotoxic effects of inhaled benzene on peripheral blood, bone marrow, and spleen cells are increased by ingested ethanol. Toxicol Appl Pharmacol 64:393-404.

Backer LC, Egeland GM, Ashley DL, et al. 1997. Exposure to regular gasoline and ethanol oxyfuel during refueling in Alaska. Environ Health Perspect 105(8):850-855.

*Bader M, Lehnert G, Angerer J. 1994. GC/MS determination of *N*-phenylvaline, a possible biomarker for benzene exposure in human hemoglobin by the "N-alkyl Edman method". Int Arch Occup Environ Health 65:411-414.

Baehr AL, Stackelberg PE, Baker RJ. 1999. Evaluation of the atmosphere as a source of volatile organic compounds in shallow groundwater. Water Resour Res 35:127-136.

Bainton DF, Ullyot JL, Farquhar MG. 1971. The development of neutrophilic polymorphonuclear leukocytes in human bone marrow. J Pharmacol Exp Ther 134:907-934.

Bakoglu M, Karademir A, Ayberk S. 2004. An elevation of the occupational health risks to workers in a hazardous waste incinerator. J Occup Health 46(2):156-164.

Bandow H, Washida N, Akimoto H. 1985. Ring-cleavage reactions of aromatic hydrocarbons studied by FT-IR spectroscopy: I. Photooxidation of toluene and benzene in the NO_x-air system. Bull Chem Soc Jpn 58:2531-2540.

Barale R, Giorgelli F, Migliore L, et al. 1985. Benzene induces micronuclei in circulating erythrocytes of chronically treated mice. Mutat Res 144:193-196.

Barnes DG, Dourson M. 1988. Reference dose (RfD): Description and use in health risk assessments. Regul Toxicol Pharmacol 8:471-486.

Barrefors G, Petersson G. 1995. Assessment by gas chromatography and gas chromatography-mass spectrometry of volatile hydrocarbons from biomass burning. J Chromatogr 710:71-77.

Barrett RH. 1985. Assays for unscheduled DNA synthesis in HeLa S3 cells. Prog Mutat Res 5:347-352.

Bartczak A, Kline SA, Yu R, et al. 1994. Evaluation of assays for the identification and quantitation of muconic acid, a benzene metabolite in human urine. J Toxicol Environ Health 42:245-258.

9. REFERENCES

Baslo A, Aksoy M. 1982. Neurological abnormalities in chronic benzene poisoning: A study of six patients with aplastic anemia and two with preleukemia. Environ Res 27:457-465.

Bauer AK, Faiola B, Abernethy DJ, et al. 2003. Male mice deficient in microsomal epoxide hydrolase are not susceptible to benzene-induced toxicity. Toxicol Sci 72(2):201-209.

Bayer CW, Black MS, Galloway LM. 1988. Sampling and analysis techniques for trace volatile organic emissions from consumer products. J Chromatogr Sci 26:168-173.

Bechtold WE, Henderson RF. 1993. Biomarkers of human exposure to benzene. J Toxicol Environ Health 40:377-386.

Bechtold WE, Lucier G, Birnbaum LS, et al. 1991. Muconic acid determinations in urine as a biological exposure index for workers occupationally exposed to benzene. Am Ind Hyg Assoc J 52:473-478.

Bechtold WE, Sabourin PJ, Henderson RF. 1988. A reverse isotope dilution method for determining benzene and metabolites in tissues. J Anal Toxicol 12:176-179.

Bechtold WE, Sun JD, Birnbaum LS, et al. 1992a. S-phenylcysteine formation in hemoglobin as a biological exposure index to benzene. Arch Toxicol 66(5):303-309.

Bechtold WE, Willis JK, Sun JD, et al. 1992b. Biological markers of exposure to benzene: S-phenylcysteine in albumin. Carcinogenesis 13(7):1217-1220.

Bennett GF. 1987. Air quality aspects of hazardous waste landfills. Haz Waste Haz Mat 4:119-138.

Berger GS. 1994. Epidemiology of endometriosis. In: Berger GS, ed. Endometriosis: Advanced management and surgical techniques. New York, NY: Springer-Verlag, 3-7.

Berlin M, Gage JC, Gullberg B, et al. 1980. Breath concentration as an index to the health risk from benzene. Scand J Work Environ Health 6:104-111.

Bernauer U, Vieth B, Ellrich R, et al. 1999. CYP2E1-dependent benzene toxicity: The role of extrahepatic benzene metabolism. Arch Toxicol 73(4-5):189-196.

Bernauer U, Vieth B, Ellrich R, et al. 2000. CYP2E1 expression in bone marrow and its intra- and interspecies variability: Approaches for a more reliable extrapolation from one species to another in the risk assessment of chemicals. Arch Toxicol 73(12):618-624.

Bezabeh S, Engel A, Morris CB, et al. 1996. Does benzene cause multiple myeloma? An analysis of the published case-control literature. Environ Health Perspect Suppl 104:1393-1398.

Bird MG, Greim H, Snyder R, et al. 2005. International symposium: Recent advances in benzene toxicity. Chem Biol Interact 153-154:1-5.

Black FM, High LE, Lang JM. 1980. Composition of automobile evaporative and tailpipe hydrocarbon emissions. J Air Pollut Control Assoc 30:1216-1221.

Blanchard RD, Hardy JK. 1986. Continuous monitoring device for the collection of 23 volatile organic priority pollutants. Anal Chem 58:1529-1532.

9. REFERENCES

Blank IH, McAuliffe DJ. 1985. Penetration of benzene through human skin. J Invest Dermatol 85:522-526.

Bleasdale C, Kennedy G, MacGregor JO, et al. 1996. Chemistry of muconaldehydes of possible relevance to the toxicology of benzene. Environ Health Perspect 104(Suppl 6):1201-1209.

Bloemen LJ, Youk A, Bradley TD, et al. 2004. Lymphohaematopoietic cancer risk among chemical workers exposed to benzene. Occup Environ Med 61(3):270-274.

Bock CW, George P, Greenberg A, et al. 1994. An *ab initio* computational molecular orbital study of the conformers of muconaldehyde, and the possible role of 2-formyl-2h-pyran in bringing about the conversion of a (z,z)-muconaldehyde structure into an (e,z)-muconaldehyde structure. Chem Res Toxicol 7(4):534-543.

Bodell WJ, Levay G, Pongracz K. 1993. Investigation of benzene-DNA adducts and their detection in human bone marrow. Environ Health Perspect 99:241-244.

Bogadi-Šare A, Brumen V, Turk R, et al. 1997. Genotoxic effects in workers exposed to benzene: With special reference to exposure biomarkers and confounding factors. Ind Health 35:367-373.

Bois FY, Paxman DG. 1992. An analysis of exposure rate effects for benzene using a physiologically based pharmacokinetic model. Regul Toxicol Pharmacol 15(2):122-136.

Bois FY, Jackson E, Pekari K. 1996. Population toxicokinetics of benzene. Environ Health Perspect 104(Suppl 6):1405-1411.

Bois FY, Smith MT, Spear RC. 1991a. Mechanisms of benzene carcinogenesis: Application of a physiological model of benzene pharmacokinetics and metabolism. Toxicol Lett 56(3):283-298.

Bois FY, Woodruff TJ, Spear RC. 1991b. Comparison of three physiologically based pharmacokinetic models of benzene disposition. Toxicol Appl Pharmacol 110(1):79-88.

*Bolcsak LE, Nerland DE. 1983. Inhibition of erythropoiesis by benzene and benzene metabolites. Toxicol Appl Pharmacol 69:363-368.

Bond GG, McLaren EA, Baldwin CL, et al. 1986. An update of mortality among chemical workers exposed to benzene. Br J Ind Med 43:685-691.

Boogaard PJ, van Sittert NJ. 1995. Biological monitoring of exposure to benzene: A comparison between S-phenylmercapturic acid, *trans,trans*-muconic acid, and phenol. Occup Environ Med 52:611-620.

Boogaard PJ, van Sittert NJ. 1996. Suitability of *S*-phenyl mercapturic acid and *trans,trans*-muconic acid as biomarkers for exposure to low concentrations of benzene. Environ Health Perspect 104(Suppl 6):1151-1157.

*Bordarier C, Robain O, Ponsot G. 1991. Bilateral porencephalic defect in a newborn after injection of benzol during pregnancy. Brain Dev 13(2):126-129.

Bradley MO. 1985. Measurement of DNA single-strand breaks by alkaline elution in rat hepatocytes. Prog Mutat Res 5:353-357.

Brass HJ, Feige MA, Halloran T, et al. 1977. The national organic monitoring survey: Samplings and analyses for purgeable organic compounds. In: Pojasek RB, ed. Drinking water quality enhancement through source protection. Ann Arbor, MI: Ann Arbor Science Publishers, 393-416.

Bratton SB, Lau SS, Monks TJ. 1997. Identification of quinol thioethers in bone marrow of hydroquinone/phenol-treated rats and mice and their potential role in benzene-mediated hematotoxicity. Chem Res Toxicol 10:859-865.

Brief RS, Lynch J, Bernath T, et al. 1980. Benzene in the workplace. Am Ind Hyg Assoc J 41:616-623.

Brondeau MT, Ducos P, Gaudin R, et al. 1992. Evaluation of the interaction of benzene and toluene on the urinary excretion of *t,t*-muconic acid in rats. Toxicol Lett 61(2-3):311-316.

Bronstein AC, Currance PL. 1988. Emergency care for hazardous materials exposure. Guideline 69. Washington, DC: The C.V. Mosby Company, 221-222.

Brown EA, Shelley ML, Fisher JW. 1998. A pharmacokinetic study of occupational and environmental benzene exposure with regard to gender. Risk Anal 18(2):205-213.

Brugnone F, Perbellini L, Faccini GB, et al. 1989. Benzene in the blood and breath of normal people and occupationally exposed workers. Am J Ind Med 16:385-399.

Brugnone F, Perbellini L, Maranelli G, et al. 1992. Reference values for blood benzene in the occupationally unexposed general population. Int Arch Occup Environ Health 64(3):179-184.

Brugnone F, Perbellini L, Romeo L, et al. 1998. Benzene in environmental air and human blood. Int Arch Occup Environ Health 71:554-559.

Brunmark A, Cadenas E. 1988. Reductive addition of glutathione to *p*-benzoquinone, 2-hydroxy-*p*-benzoquinone, and *p*-benzoquinone epoxides. Effect of the hydroxy- and glutathionyl substituents on p-benzohydyroquinone autoxidation. Chem Biol Interact 68:273-298.

Brunnemann KD, Kagan MR, Cox JE, et al. 1989. Determination of benzene, toluene and 1,3-butadiene in cigarette smoke by GC-MSD. Exp Pathol 37:108-113.

Brunnemann KD, Kagan MR, Cox JE, et al. 1990. Analysis of 1,3-butadiene and other selected gas-phase components in cigarette mainstream and sidestream smoke by gas chromatography-mass selective detection. Carcinogenesis 11:1863-1868.

Bryce-Smith D, Gilbert A. 1976. The organic photochemistry of benzene. Tetrahedron 32:1309-1326.

Buckley JD, Robison LL, Swotinsky L, et al. 1989. Occupational exposure of parents of children with acute nonlymphocytic leukemia: A report from the Children's Cancer Study Group. Cancer Res 49:4030-4037.

Budavari S, O'Neil MJ, Smith A, et al., eds. 2001. Benzene. The Merck index: An encyclopedia of chemicals, drugs, and biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 182-183.

Budnick LD, Sokal DC, Falk H, et al. 1984. Cancer and birth defects near the Drake Superfund site, Pennsylvania. Arch Environ Health 39:409-413.

*Bull RJ, Robinson M, Laurie RD. 1986. Association of carcinoma yield with early papilloma development in SENCAR mice. Environ Health Perspect 68:11-17.

Burford MD, Hawthorne SB, Miller DJ. 1994. Analysis of volatile organics by supercritical fluid extraction coupled to gas chromatography II. Quantitation of petroleum hydrocarbons from environmental sample. J Chromatogr A 685:95-111.

Burgess JL, Crutchfield CD. 1995. Tucson fire fighter exposure to products of combustion: A risk assessment. Appl Occup Environ Hyg 10(1):37-42.

Burmaster DE. 1982. The new pollution-groundwater contamination. Environ 24:6-13, 33-36.

Byrd GD, Fowler KW, Hicks RD, et al. 1990. Isotope dilution gas chromatography-mass spectrometry in the determination of benzene, toluene, styrene and acrylonitrile in mainstream cigarette smoke. J Chromatogr 503:359-368.

C&EN. 1994. Top 50 chemicals production rose modestly last year. Chem Eng News 72(15):12-13.

C&EN. 1995. Production soared in most chemical sectors. Chem Eng News 73(26):38-40.

Cal EPA. 1987. Residential population exposure to ambient benzene in California. Sacramento, CA: Air Resource Board. ARB/TS87001.

Carbonnelle P, Lison D, Leroy JY, et al. 1995. Effect of the benzene metabolite, hydroquinone, on interleukin-1 secretion by human monocytes *in vitro*. Toxicol Appl Pharmacol 132(2):220-226.

Carpenter CP, Shaffer CB, Weil CS, et al. 1944. Studies on the inhalation of 1:3-butadiene; with a comparison of its narcotic effect with benzol, toluol, and styrene, and a note on the elimination of styrene by the human. J Ind Hyg Toxicol 26:69-78.

Cassidy MK, Houston JB. 1984. *In vivo* capacity of hepatic and extra hepatic enzymes to conjugate phenol. Drug Metab Dispos 12:619-624.

CDC. 1994. Continued use of drinking water wells contaminated with hazardous chemical substances Virgin Islands and Minnesota 1981-1993. Centers for Disease Control. Morbid Mortal Weekly Rep 43(5):89-92.

Chan C-C, Lin S-H, Her G-R. 1993. Student's exposure to volatile organic compounds while commuting by motorcycle and bus in Taipei City. J Air Waste Manage Assoc 43(9):1231-1238.

Chan C-C, Lin S-H, Her G-R. 1994. Office worker's exposure to volatile organic compounds while commuting and working in Taipei City. Atmos Environ 28(14):2351-2359.

Chang D, Sui H, Pan HZ, et al. 2005. Effects of chronic benzene poisoning on DNA and antioxidase of mice. Zhongguo lin chuang kang fu 9(3):240-242.

Chang RL, Wong CQ, Kline SA, et al. 1994. Mutagenicity of *trans,trans*-muconaldehyde and its metabolites in V79 cells. Environ Mol Mutagen 24:112-115.

Chao TC, Lo DST, Koh J, et al. 1993. Glue sniffing deaths in Singapore—volatile aromatic hydrocarbons in post-mortem blood by headspace gas chromatography. Med Sci Law 33(3):253-260.

Chen H, Eastmond DA. 1995. Topoisomerase inhibition by phenolic metabolites: A potential mechanism for benzene's clastogenic effects. Carcinogenesis 16(10):2301-2307.

Chen H, Rupa DS, Tomar RR, et al. 1994. Chromosomal loss and breakage in mouse bone marrow and spleen cells exposed to benzene *in vivo*. Cancer Res 54:3533-3539.

Chen KM, El-Bayoumy K, Cunningham J, et al. 2004. Detection of nitrated benzene metabolites in bone marrow of B6C3F$_1$ mice treated with benzene. Chem Res Toxicol 17(3):370-377.

Chenna A, Hang B, Rydberg B, et al. 1995. The benzene metabolite p-benzoquinone forms adducts with DNA bases that are excised by a repair activity from human cells that differs from an ethenoadenine glycosylase. Proc Natl Acad Sci USA 92(13):5890-5894.

Chepiga TA, Yang CS, Snyder R. 1991. Benzene metabolism by two purified, reconstituted rat hepatic mixed function oxidase systems. Adv Exp Med Biol 283:261-265.

Chertkov JL, Lutton JD, Jiang S, et al. 1992. Hematopoietic effects of benzene inhalation assessed by murine long-term bone marrow culture. J Lab Clin Med 119:412-419.

Choy WN, MacGregor JT, Shelby MD, et al. 1985. Induction of micronuclei by benzene in B6C3F$_1$ mice: Retrospective analysis of peripheral blood smears from the NTP carcinogenesis bioassay. Mutat Res 143:55-59.

*Christian CLE, Werley B, Smith A, et al. 1994. Comparison of employees' white blood cell counts in a petrochemical plant by worksite and race. J Natl Med Assoc 86(8):620-623.

Ciccone G, Mirabelli D, Levis A, et al. 1993. Myeloid leukemias and myelodysplastic syndromes: Chemical exposure, histologic subtype and cytogenetics in a case-control study. Cancer Genet Cytogenet 68(2):135-139.

*Ciranni R, Barale R, Adler ID. 1991. Dose-related clastogenic effects induced by benzene in bone marrow cells and in differentiating spermatogonia of Swiss CD1 mice. Mutagenesis 6(5):417-421.

Ciranni R, Barale R, Marrazzini A, et al. 1988. Benzene and the genotoxicity of its metabolites I. Transplacental activity in mouse fetuses and in their dams. Mutat Res 208:61-67.

Clark AI, McIntyre AE, Lester JN, et al. 1984. A comparison of photoionisation detection gas chromatography with a Tenax GC sampling tube procedure for the measurement of aromatic hydrocarbons in ambient air. J Environ Anal Chem 17:315-326.

Clavel J, Conso F, Limasset JC, et al. 1996. Hairy cell leukaemia and occupational exposure to benzene. Occup Environ Med 53(8):533-539.

Clayton CA, Pellizzari ED, Whitmore RW, et al. 1999. National Human Exposure Assessment Survey (NHEXAS): Distributions and associations of lead, arsenic and volatile organic compounds in EPA Region 5. J Expo Anal Environ Epidemiol 9:381-392.

Clewell HJ III, Andersen ME. 1985. Risk assessment extrapolations and physiological modeling. Toxicol Ind Health 1(4):111-131.

Cline PV, Viste DR. 1985. Migration and degradation patterns of volatile organic compounds. Waste Manage Res 3:351-360.

Coate WB, Hoberman AM, Durloo RS. 1984. Inhalation teratology study of benzene in rats. Adv Mod Environ Toxicol 6:187-198.

Cody RP, Strawderman WW, Kipen HM. 1993. Hematologic effects of benzene. Job-specific trends during the first year of employment among a cohort of benzene-exposed rubber workers. J Occup Med 35(8):776-782.

Cohen MA, Ryan PB, Yanagisawa Y, et al. 1989. Indoor/outdoor measurements of volatile organic compounds in Kanawha Valley of West Virginia. J Air Pollut Control Assoc 39:1086-1093.

Cole CE, Tran HT, Schlosser PM. 2001. Physiologically based pharmacokinetic modeling of benzene metabolism in mice through extrapolation from in vitro to in vivo. J Toxicol Environ Health A 62(6):439-465.

Colenutt BA, Davies DN. 1980. The sampling and gas chromatographic analysis of organic vapours in landfill sites. Int J Environ Anal Chem 7:223-229.

Colenutt BA, Thorburn S. 1980. Gas chromatographic analysis of trace hydrocarbon pollutants in water samples. Int J Environ Stud 15:25-32.

Colinas RJ, Burkart PT, Lawrence DA. 1995. The effects of interleukin-1-beta and tumor necrosis factor-alpha on *in vitro* colony formation by human hematopoietic progenitor cells exposed to doxorubicin or hydroquinone. Exp Hematol 23(12):1247-1255.

Collins CD, Bell JNB, Crews C. 2000. Benzene accumulation in horticultural crops. Chemosphere 40(1):109-114.

Collins JJ, Conner P, Friedlander BR, et al. 1991. A study of the hematologic effects of chronic low-level exposure to benzene. J Occup Med 33(5):619-626.

Collins JJ, Ireland B, Buckley CF, et al. 2003. Lymphohaematopoeitic cancer mortality among workers with benzene exposure. Occup Environ Med 60(9):676-679.

Collins JJ, Ireland BK, Easterday PA, et al. 1997. Evaluation of lymphopenia among workers with low-level benzene exposure and the utility of routine data collection. J Occup Environ Med 39(3):232-237.

Cornish HH, Ryan RC. 1965. Metabolism of benzene in nonfasted, fasted, and aryl-hydroxylase inhibited rats. Toxicol Appl Pharmacol 7:767-771.

Corti M, Snyder CA. 1996. Influences of gender, development, pregnancy and ethanol consumption on the hematotoxicity of inhaled 10 ppm benzene. Arch Toxicol 70:209-217.

Costantini AS, Quinn M, Consonni D, et al. 2003. Exposure to benzene and risk of leukemia among shoe factory workers. Scand J Work Environ Health 29(1):51-59.

Cotton SC, Sharp I, Little J, et al. 2000. Glutathione S-transferase polymorphisms and colorectal cancer: A HuGE review. Am J Epidemiol 151:7-32.

Cox LA Jr. 1996. Reassessing benzene risks using internal doses and Monte-Carlo uncertainty analysis. Environ Health Perspect 104(Suppl 6):1413-1429.

Crawford DW, Bonnevie NL, Wenning RJ. 1995. Sources of pollution and sediment contamination in Newark Bay, New Jersey. Ecotoxicol Environ Safety 30:85-100.

Crebelli R, Bellincampi D, Conti G, et al. 1986. A comparative study on selected chemical carcinogens for chromosome malsegregation, mitotic crossing-over and forward mutation induction in *Aspergillus nidulans*. Mutat Res 172:139-149.

Creek MR, Mani C, Vogel JS, et al. 1997. Tissue distribution and macromolecular binding of extremely low doses of $[^{14}C]$-benzene in $B_6C_3F_1$ mice. Carcinogenesis 18(12):2421-2427.

Cronin HJ. 1924. Benzol poisoning in the rubber industry. Boston Medical and Science Journal 191:1164-1166.

Cronkite EP. 1986. Benzene hematotoxicity and leukemogenesis. Blood Cells 12:129-137.

Cronkite EP, Bullis JE, Inoue T, et al. 1984. Benzene inhalation produces leukemia in mice. Toxicol Appl Pharmacol 75:358-361.

Cronkite EP, Drew RT, Inoue T, et al. 1985. Benzene hematotoxicity and leukemogenesis. Am J Ind Med 7:447-456.

Cronkite EP, Drew RT, Inoue T, et al. 1989. Hematotoxicity and carcinogenicity of inhaled benzene. Environ Health Perspect 82:97-108.

Cronkite EP, Inoue T, Carsten AL, et al. 1982. Effects of benzene inhalation on murine pluripotent stem cells. J Toxicol Environ Health 9:411-421.

Daisey JM, Hodgson AT, Fisk WJ, et al. 1994. Volatile organic compounds in twelve California office buildings: Classes, concentrations and sources. Atmos Environ 28(22):3557-3562.

Darrall KG, Figgins JA, Brown RD, et al. 1998. Determination of benzene and associated volatile compounds in mainstream cigarette smoke. Analyst 123(5):1095-1101.

Davis EM, Murray HE, Liehr JG, et al. 1981. Technical note: Basic microbial degradation rates and chemical byproducts of selected organic compounds. Water Res 15:1125-1127.

Davis JW, Klier NK, Carpenter CL. 1994. Natural biological attenuation of benzene in ground water beneath a manufacturing facility. Ground Water 32(2):215-226.

Dayal H, Gupta S, Trieff N, et al. 1995. Symptom clusters in a community with chronic exposure to chemicals in two superfund sites. Arch Environ Health 50(2):108-111.

*Decoufle P, Blattner WA, Blair A. 1983. Mortality among Chinese workers exposed to benzene and other agents. Environ Res 30:16-25.

9. REFERENCES

Dees C, Travis C. 1994. Hyperphosphorylation of p53 induced by benzene, toluene, and chloroform. Cancer Lett 84(2):117-123.

De Flora S, Zanacchi P, Camoirano A, et al. 1984. Genotoxic activity and potency for 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutat Res 133:161-198.

de Gandarias JM, Casis O, Irazusta J, et al. 1992. Effect of subacute benzene exposure on the activity of two neuropeptide-degrading enzymes in the rat brain. Arch Environ Contam Toxicol 22(3):345-348.

DeLeon IR, Antoine SR. 1985. Clinical screening test for toxic volatile organic chemicals in blood. Clinical Ecol 3:108-109.

Delfino JJ, Miles CJ. 1985. Aerobic and anaerobic degradation of organic contaminants in Florida groundwater. Proc Soil Crop Sci Soc Fla 44:9-14.

*Dempster AM, Snyder CA. 1990. Short term benzene exposure provides a growth advantage for granulopoietic progenitor cells over erythroid progenitor cells. Arch Toxicol 64(7):539-544.

Dempster AM, Snyder CA. 1991. Kinetics of granulocytic and erythroid progenitor cells are affected differently by short-term, low-level benzene exposure. Arch Toxicol 65(7):556-561.

Dempster AM, Evans HL, Snyder CA. 1984. The temporal relationship between behavioral and hematological effects of inhaled benzene. Toxicol Appl Pharmacol 76:195-203.

Diaz M, Reiser A, Braier L, et al. 1980. Studies on benzene mutagenesis: I. The micronucleus test. Experientia 36:297-299.

Dibben MJ, Thomas TC, Anderson MP. 1989. Is benzene a problem in fuel operations? A new analytical method to overcome potential interferences in the present method. LC-GC Int 2:54-58.

Ding X-J, Li Y, Ding Y, et al. 1983. Chromosome changes in patients with chronic benzene poisoning. Chin Med J (Engl) 96:681-685.

DOE. 1992. Continuous emission monitor for incinerators. Dallas, TX: U.S. Department of Energy. ANL/CP--76118. DE92015261.

DOI. 1984. Detection and effects of aperiodic leachate discharges from landfills. Technical report. U.S. Department of the Interior. PB85242949, 23-24, 98-105.

Dor F, Le Moullec Y, Festy B. 1995. Exposure of city residents to carbon monoxide and monocyclic aromatic hydrocarbons during commuting trips in the Paris metropolitan area. J Air Waste Manage Assoc 45(2):103-110.

Dosemeci M, Li GL, Hayes RB, et al. 1994. Cohort study among workers exposed to benzene in China: II. Exposure assessment. Am J Ind Med 26(3):401-411.

Dosemeci M, Yin SN, Linet M, et al. 1996. Indirect validation of benzene exposure assessment by association with benzene poisoning. Environ Health Perspect 104:1343-1347.

Doskin VA. 1971. [Effect of age on the reaction to a combination of hydrocarbons.] Hyg Sanit 36:379-384. (Russian)

Douglas GR, Blakey DH, Liu-Lee VW, et al. 1985. Alkaline sucrose sedimentation, sister-chromatid exchange and micronucleus assays in CHO cells. Prog Mutat Res 5:359-366.

*Dow. 1980. Risks of long-term health effects of benzene absorbed via skin in laboratory animals. Dow Chemical Co. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8E. OTS0533863.

Dow. 1992. Effects of benzene vapor in the pig and rat. I. Pertaining to hematology and immunology with cover letter dated 05/14/92. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8E. OTS0539784.

Dowty BJ, Carlisle DR, Laseter JL. 1975. New Orleans drinking water sources tested by gas chromatography—mass spectrometry. Environ Sci Technol 9:762-765.

Dowty BJ, Laseter JL, Storer J. 1976. The transplacental migration and accumulation in blood of volatile organic constituents. Pediatr Res 10:696-701.

Dragan YP, Sargent L, Xu YD, et al. 1993. The initiation-promotion-progression model of rat hepatocarcinogenesis. Proc Soc Exp Biol Med 202(1):16-24.

Drew RT, Fouts JR. 1974. The lack of effects of pretreatment with phenobarbital and chlorpromazine on the acute toxicity of benzene in rats. Toxicol Appl Pharmacol 27:183-193.

Duarte-Davidson R, Courage C, Rushton L, et al. 2001. Benzene in the environment: An assessment of the potential risks to the health of the population. Occup Environ Med 58(1):2-13.

Ducos P, Gaudin R, Bel J, et al. 1992. *trans,trans*-Muconic acid, a reliable biological indicator for the detection of individual benzene exposure down to the ppm level. Int Arch Occup Environ Health 64(5):309-313.

Ducos P, Gaudin R, Robert A, et al. 1990. Improvement in HPLC analysis of urinary *trans trans*-muconic acid, a promising substitute for phenol in the assessment of benzene exposure. Int Arch Occup Environ Health 62:(7):529-534.

Eastmond DA, Mondrala ST, Hasegawa L. 2005. Topoisomerase II inhibition by myeloperoxidase-activated hydroquinone: A potential mechanism underlying the genotoxic and carcinogenic effects of benzene. Chem Biol Interact 152-154:207-216.

Eastmond DA, Rupa DS, Hasegawa LS. 1994. Detection of hyperdiploidy and chromosome breakage in interphase human lymphocytes following exposure to the benzene metabolite hydroquinone using multicolor fluorescence in situ hybridization with DNA probes. Mutat Res 322(1):9-20.

Eastmond DA, Schuler M, Frantz C, et al. 2001. Characterization and mechanisms of chromosomal alterations induced by benzene in mice and humans. Cambridge, MA: Health Effects Institute. Research Report 103.

Eastmond DA, Smith MT, Irons RD. 1987. An interaction of benzene metabolites reproduces the myelotoxicity observed with benzene exposure. Toxicol Appl Pharmacol 91:85-95.

Edgerton SA, Shah JJ. 1992. Assessing total exposures to gasoline vapor using the source exposure model. J Expos Anal Environ Epidemiol 2(1):109-115.

Edwards EA, Grbić-Galić D. 1992. Complete mineralization of benzene by aquifer microorganisms under strictly anaerobic conditions. Appl Environ Microbiol 58(8):2663-2666.

Egeghy PP, Tornero-Velez R, Rappaport SM. 2000. Environmental and biological monitoring of benzene during self-service automobile refueling. Environ Health Perspect 108(12):1195-1202.

Einig T, Dehnen W. 1995. Sensitive determination of the benzene metabolite S-phenylmercapturic acid in urine by high-performance liquid chromatography with fluorescence detection. Journal of Chromatography A 697:371-375.

Eisenreich SJ, Looney BB, Thornton JD. 1981. Airborne organic contaminants in the Great Lakes ecosystem. Environ Sci Technol 15:30-38.

Ellenhorn MJ, Barceloux DG. 1988. Medical toxicology: Diagnosis and treatment of human poisoning. New York, NY: Elsevier, 947-950.

EPA. 1978. Assessment of human exposures to atmospheric benzene. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards. EPA450378031. PB284203.

EPA. 1979. Environmental monitoring benzene. Washington, DC: U.S. Environmental Protection Agency, Office of Toxic Substances. EPA560679006.

EPA. 1980a. Ambient air monitoring for benzene: 24-hour integrated sampling in six cities. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory. EPA600480027.

EPA. 1980b. Ambient water quality criteria for benzene. Washington, DC: U.S. Environmental Protection Agency. EPA440580018. PB81117293.

EPA. 1981. Hazardous wastes from specific sources. Identification and listing of hazardous waste. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 261.32.

EPA. 1982a. An exposure and risk assessment for benzene. Washington, DC: U.S. Environmental Protection Agency. EPA440485006. PB85212017, 3-1–3-32.

EPA. 1982b. U.S. EPA management of hazardous waste leachate EPA contract No. 68-03-2766. E-42, E-96, E-111, E-142, E-143. (as cited in HSDB 2007).

EPA. 1984. Guidelines for establishing test procedures for the analysis of pollutants under the Clean Water Act. U.S. Environmental Protection Agency. Fed Regist 49:43234, 43255-43256, 432272-43280, 43373-43384, 43407-43415.

*EPA. 1985a. Drinking water criteria document on benzene. Final draft. Washington, DC: U.S. Environmental Protection Agency, Office of Drinking Water. PB86118122.

EPA. 1985b. Interim quantitative cancer unit risk estimates due to inhalation of benzene. U.S. Environmental Protection Agency, Office of air quality planning and standards. EPA600X85022.

9. REFERENCES

EPA. 1985c. Comparison of ambient air sampling techniques for volatile organic compounds. Research Triangle Park, NC: U.S. Environmental Protection Agency. Environmental monitoring systems laboratory. EPA600485067. PB86120953.

EPA. 1985d. Development of an optical monitor for toxic organic compounds in air. Research Triangle Park, NC: U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory. EPA600485043. PB85225027.

EPA. 1986. Self service station vehicle refueling exposure study. Proceedings of the 1986 EPA/APCA symposium on measurements of toxic air pollutants. Raleigh, NC: U.S. Environmental Protection Agency. Air Pollution Control Association. EPA600986013, 458-466.

*EPA. 1987a. June-September, 6-9 AM, ambient air benzene concentrations in 39 U.S. cities, 1984-1986. Research Triangle Park, NC: U.S. Environmental Protection Agency, Atmospheric Sciences Research Lab. EPA600D87160.

EPA. 1987b. The total exposure assessment methodology (TEAM) study: Elizabeth and Bayonne, New Jersey, Devils Lake, North Dakota and Greensboro, North Carolina: Vol. II. Part 1. Washington, DC: U.S. Environmental Protection Agency. PB8810078, 12-13.

EPA. 1988a. Compendium of methods for the determination of toxic organic compounds in ambient air. Research Triangle Park, NC: U.S. Environmental Protection Agency. EPA600489017. PB90127374.

EPA. 1988b. Recommendations for and documentation of biological values for use in risk assessment. Cincinnati, OH: U.S. Environmental Protection Agency. EPA600687008. PB88179874.

EPA 1989. National emission standards for hazardous air pollutants; benzene emissions from maleic anhydride plants, ethylbenzene/styrene plants, benzene storage vessels, benzene equipment leaks, and coke by-products recovery plants. U.S. Environmental Protection Agency. Fed Regist 54:38044.

EPA. 1990. Interim methods for development of inhalation reference concentrations. Washington, DC: U.S. Environmental Protection Agency, Office of Health and Environmental Assessment, Office of Research and Development, Environmental Criteria and Assessment Office. EPA600890066A. PB90238890.

EPA. 1992a. Methods for the determination of organic compounds in drinking water. Supplement II. Cincinnati, OH: U.S. Environmental Protection Agency. PB92207703.

EPA. 1992b. The time-course and sensitivity of muconic acid as a biomarker for human environmental exposure to benzene. In: Measurement of toxic and related air pollutants. Proceedings of the 1992 U.S. EPA/A&WMA International symposium. Pittsburgh, PA: U.S. Environmental Protection Agency. Air and Waste Management Association, 981-986.

EPA. 1993. Regulation of fuel and fuel additives. Renewable oxygenate requirements for reformulated gasoline: Proposed Rule. U.S. Environmental Protection Agency. Fed Regist 58:68343.

EPA. 1994a. Land disposal restrictions phase II - Universal treatment standards and treatment standards for organic toxicity characteristic wastes and newly listed wastes. U.S. Environmental Protection Agency. Fed Regist 59:47982.

9. REFERENCES

EPA. 1994b. Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Washington, DC: U.S. Environmental Protection Agency, Office of Health and Environmental Assessment, Office of Research and Development, Environmental Criteria and Assessment Office. EPA600890066F.

EPA. 1994c. Test methods for evaluating solid waste method 8021a. Halogenated volatiles by gas chromatography using photoionization and electrolytic conductivity detectors in series: Capillary column technique. U.S. Environmental Protection Agency.

EPA. 1994d. Mobile source hazardous air pollutant emissions in the Seattle-Tacoma urban area. Washington, DC: U.S. Environmental Protection Agency. PB95174660.

EPA. 1995. Federal standards for marine tank vessel loading operations and national emission standards for hazardous air pollutants for marine tank vessel loading operations. U.S. Environmental Protection Agency. Fed Regist 60:48388.

EPA. 1997. Special report on environmental endocrine disruption: An effects assessment and analysis. Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. EPA630R96012.

EPA. 1998. Carcinogenic effects of benzene: An update. Washington, DC: U.S. Environmental Protection Agency. EPA600P97001F. PB99101420.

EPA. 2001. Occurence of unregulated contaminants in public water systems - A national summary. Washington, DC: U.S. Environmental Protection Agency, Office of Water. EPA815P00002.

EPA. 2002a. National primary drinking water regulations. Washington, DC: U.S. Environmental Protection Agency, Office of Ground Water and Drinking Water. EPA816F02013. http://www.epa.gov/safewater/mcl.html. February 15, 2005.

EPA. 2002b. National recommended water quality criteria. Washington, DC: U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology. EPA822R02047. http://www.epa.gov/waterscience/pc/revcom.pdf. February 15, 2005.

EPA. 2004a. Drinking water standards and health advisories. Washington, DC: U.S. Environmental Protection Agency, Office of Water. EPA822R04005. http://www.epa.gov/waterscience/drinking/standards/dwstandards.pdf. February 15, 2005.

EPA. 2004b. Hazardous air pollutants. Washington, DC: U.S. Environmental Protection Agency. United States Code 42 USC 7412. http://www.epa.gov/ttn/atw/orig189.html. February 15, 2005.

EPA. 2005a. Acute Exposure Guideline Levels (AEGLs). Washington, DC: U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics. http://www.epa.gov/oppt/aegl/chemlist.htm. March 28, 2005.

EPA. 2005b. Designated as hazardous substances in accordance with Section 311(b)(2)(A) of the Clean Water Act. Washington, DC: U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 116.4. http://www.epa.gov/ttn/atw/orig189.html. February 15, 2005.

EPA. 2005c. Reportable quantities of hazardous substances designated pursuant to Section 311 of the Clean Water Act. Washington, DC: U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 117.3. http://www.epa.gov/epacfr40/chapt-I.info/chi-toc.htm. February 16, 2005.

9. REFERENCES

EPA. 2005d. Superfund, emergency planning, and community right-to-know programs. Designation, reportable quantities, and notifications. Washington, DC: U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 302.4. http://www.epa.gov/epacfr40/chapt-I.info/chi-toc.htm. February 15, 2005.

EPA. 2005e. Superfund, emergency planning, and community right-to-know programs. Toxic chemical release reporting. Washington, DC: U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 372.65. http://www.epa.gov/epacfr40/chapt-I.info/chi-toc.htm. February 16, 2005.

EPA. 2005f. Benzene. STORET. STOrage and RETrieval. Washington, DC: U.S. Environmental Protection Agency. http://www.epa.gov/storet. March 25, 2005.

EPA. 2005g. Toxic chemical release inventory reporting forms and instructions: Revised 2004 version. Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986). U.S. Environmental Protection Agency. Office of Environmental Information. EPA260B05001.

Erexson GL, Wilmer JL, Steinhagen WH, et al. 1986. Induction of cytogenetic damage in rodents after short-term inhalation of benzene. Environ Mutagen 8:29-40.

Erf LA, Rhoads CP. 1939. The hematological effects of benzene (benzol) poisoning. J Ind Hyg Toxicol 20:421-435.

Etzel RA, Ashley DL. 1994. Volatile organic compounds in the blood of persons in Kuwait during the oil fires. Int Arch Occup Environ Health 66:125-129.

Eutermoser M, Rusch GM, Kuna RA, et al. 1986. A method for repeated evaluation of benzene uptake in rats and mice during a six hour inhalation period. Am Ind Hyg Assoc J 47:37-40.

Evans HL, Dempster AM, Snyder CA. 1981. Behavioral changes in mice following benzene inhalation. Neurobehav Toxicol Teratol 3:481-485.

Eveleth WT. 1990. Basic and intermediate chemicals. In: Eveleth WT, eds. Kline giude to the US chemical industry. 5th ed. Fairfield, NJ: Kline & Company, Inc., 2-1 to 2-42.

Exxon. 1986. Determination of matenal toxicity and fetal toxicity of benzene in rats following oral exposure. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8E. OTS0536017.

Fabietti F, Ambruzzi A, Delise M, et al. 2004. Monitoring of the benzene and toluene contents in human milk. Environ Int 30(3):397-401.

Fan XH. 1992. Effect of exposure to benzene on natural killer (NK) cell activity and interleukin-2 (IL-2) production of C57BL/6 mice. J Nippon Med Sch 59(5):393-399.

Farmer PB, Kaur B, Roach J, et al. 2005. The use of S-phenylmercapturic acid as a biomarker in molecular epidemiology studies of benzene. Chem Biol Interact 153-154:97-102.

Farris GM, Everitt JI, Irons RD, et al. 1993. Carcinogenicity of inhaled benzene in CBA mice. Fundam Appl Toxicol 20(4):503-507.

Farris GM, Robinson SN, Gaido KW, et al. 1997a. Benzene-induced hematotoxicity and bone marrow compensation in B6C3F1 mice. Fundam Appl Toxicol 36(2):119-129.

Farris GM, Robinson SN, Wong BA, et al. 1997b. Effects of benzene on splenic, thymic, and femoral lymphocytes in mice. Toxicology 118(2-3):137-148.

Farris GM, Wong VA, Wong BA, et al. 1996. Benzene-induced micronuclei in erythrocytes: An inhalation concentration-response study in B6C3F1 mice. Mutagenesis 11(5):455-462.

FDA. 1977. Indirect food additives. Adhesives coatings and components. Food and Drug Administration. Fed Regist 42:14534-14554.

FDA. 2004. Beverages. Bottled water. Washington, DC: Food and Drug Administration. Code of Federal Regulations. 21 CFR 165.110. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm. February 15, 2005.

FEDRIP. 2005. FEDRIP literature search for benzene. Palo Alto, CA: Federal Research in Progress. Dialog Information Service.

Ferrario JB, Lawler GC, DeLeon IR, et al. 1985. Volatile organic pollutants in biota and sediments of Lake Pontchartrain. Bull Environ Contam Toxicol 34:246-255.

Finkelstein MM. 2000. Leukemia after exposure to benzene: Temporal trends and implications for standards. Am J Ind Med 38(1):1-7.

Fishbeck WA, Townsend JC, Swank MG. 1978. Effect of chronic occupational exposure to measured concentrations of benzene. J Occup Med 20:539-542.

Fishbein L. 1988. Benzene: Uses, occurrence, and exposure. In: Fishbein L, O'Neill IK, eds. Environmental carcinogens methods of analysis and exposure measurement: Vol. 10—benzene and alkylated benzene. IARC Scientific Publications No. 85. Lyon, France: World Health Organization, International Agency for Research on Cancer, 67-96.

Fishbein L. 1992. Exposure from occupational versus other sources. Scand J Work Environ Health 18(Suppl 1):5-16.

Fisher J, Mahle D, Bankston L, et al. 1997. Lactational transfer of volatile chemicals in breast milk. Am Ind Hyg Assoc J 58(6):425-431.

Fleming AF. 1990. Benzene in petrol: A continuing hazard [letter; comment]. Lancet 336(8722):1076-1077.

Fleming-Jones ME, Smith RE. 2003. Volatile organic compounds in foods: A five year study. J Agric Food Chem 51(27):8120-8127.

Flury F. 1928. [II. Toxicities in modern industry. IIa. Pharmacological-toxicological aspects of intoxicants in modern industry.] Arch Exp Pathol Pharmakol 138:65-82. (German)

Fomon SJ. 1966. Body composition of the infant: Part I: The male reference infant. In: Falkner F, ed. Human development. Philadelphia, PA:WB Saunders, 239-246.

9. REFERENCES

Fomon SJ, Haschke F, Ziegler EE, et al. 1982. Body composition of reference children from birth to age 10 years. Am J Clin Nutr 35:1169-1175.

Forni A, Moreo L. 1967. Cytogenetic studies in a case of benzene leukaemia. Eur J Cancer 3:251-255.

Forni A, Moreo L. 1969. Chromosome studies in a case of benzene-induced erythroleukaemia. Eur J Cancer 5:459-463.

Forni A, Cappellini A, Pacifico E, et al. 1971a. Chromosome changes and their evolution in subjects with past exposure to benzene. Arch Environ Health 23:385-391.

Forni AM, Pacifico E, Limonta A. 1971b. Chromosome studies in workers exposed to benzene or toluene or both. Arch Environ Health 22:373-378.

Frantik E, Hornychova M, Horvath M. 1994. Relative acute neurotoxicity of solvents: Isoeffective air concentrations of 48 compounds evaluated in rats and mice. Environ Res 66:173-185.

Franz TJ. 1984. Percutaneous absorption of benzene. In: MacFarland HN, Holdsworth CE, MacGregor JA, et al., eds. Advances in modern environmental toxicology. Vol. VI. Applied toxicology of petroleum hydrocarbons. Princeton, NJ: Princeton Scientific Publishers, Inc., 61-70.

Fraser MP, Cass GR, Simoneit BRT. 1998. Gas-phase and particle-phase organic compounds emitted from motor vehicle traffic in a Los Angeles roadway tunnel. Environ Sci Technol 32:2051-2060.

Froom P, Dyerassi L, Cassel A, et al. 1994. Erythropoietin-independent colonies of red blood cells and leukocyotisis in a worker exposed to low levels of benzene. Scand J Work Environ Health 20:306-308.

Fruin SA, St. Denis M, Winer AM, et al. 2001. Reductions in human benzene exposure in the California South Coast Air Basin. Atmos Environ 35:1069-1077.

Fruscella W. 1992. Benzene. In: Kroschwitz JI, Howe-Grant M, eds. Kirk-Othmer encyclopedia of chemical toxicology. Fourth edition. New York: John & Wiley & Sons, 73-103.

Fujie K, Ito Y, Maeda S. 1992. Acute cytogenetic effect of benzene on rat bone marrow cells in vivo and the effect of inducers or inhibitors of drug-metabolizing enzymes. Mutat Res 298(2):81-90.

Funes-Cravioto F, Zapata-Gayon C, Kolmodin-Hedman B, et al. 1977. Chromosome aberrations and sister chromatid exchange in workers in chemical laboratories and a rotoprinting factory and in children of women laboratory workers. Lancet 2:322-325.

Fung KK, Wright BJ. 1986. Monitoring of benzene in ambient air with organic vapor badges. J Air Pollut Control Assoc 36:819-821.

Fuselli S, Benigni R, Conti L. 1995. Volatile organic compounds (VOCs) and air mutagenicity: Results of one year monitoring at an urban site. Int J Environ Health Res 5(2):123-132.

Fustinoni S, Buratti M, Campo L, et al. 2005. Urinary *t,t*-muconic acid, *S*-phenylmercapturic acid and benzene as biomarkers of low benzene exposure. Chem Biol Interact 153-154:253-256.

9. REFERENCES

Fustinoni S, Buratti M, Giampiccolo R, et al. 1995. Biological and environmental monitoring of exposure to airborne benzene and other aromatic hydrocarbons in Milan traffic wardens. Toxicol Lett 77:387-392.

*Gad-El Karim MM, Ramanujam VMS, Legator MS. 1985. *trans,trans*-Muconic acid, an open chain urinary metabolite of benzene in mice: Quantification by high-pressure liquid chromatography. Xenobiotica 15:211-220.

Gad-El Karim MM, Ramanujam VMS, Legator MS. 1986. Correlation between the induction of micronuclei in bone marrow by benzene exposure and the excretion of metabolites in urine of CD-1 mice. Toxicol Appl Pharmacol 85:464-477.

Ganousis LG, Goon D, Zyglewska T, et al. 1992. Cell-specific metabolism in mouse bone marrow stroma: Studies of activation and detoxification of benzene metabolites. Mol Pharmacol 42(6):1118-1125.

Geist CR, Drew KL, Schoenheit CM, et al. 1983. Learning impairments following postnatal exposures to benzene. Percept Mot Skills 57:1083-1086.

Gerner-Smidt P, Friedrich U. 1978. The mutagenic effect of benzene, toluene and xylene studied by the SCE technique. Mutat Res 58:313-316.

Geyer H, Politzki G, Freitag D. 1984. Prediction of ecotoxicological behavior of chemicals: Relationship between n-octanol/water coefficient and bioaccumulation of organic chemicals by alga *Chlorella*. Chemosphere 13(2):269-284.

Ghantous H, Danielsson BRG. 1986. Placental transfer and distribution of toluene, xylene and benzene, and their metabolites during gestation in mice. Biol Res Pregnancy 7:98-105.

Ghittori S, Fiorentino ML, Maestri L, et al. 1993. Urinary excretion of unmetabolized benzene as an indicator of benzene exposure. J Toxicol Environ Health 38(3):233-243.

Ghittori S, Maestri L, Fiorentino ML, et al. 1995. Evaluation of occupational exposure to benzene by urinalysis. Int Arch Occup Environ Health 67:195-200.

Ghoos Y, Claus D, Geypens B, et al. 1994. Screening method for the determination of volatiles in biomedical samples by means of an off-line closed-loop trapping system and high-resolution gas chromatography-ion trap detection. J Chromatogr 665:333-345.

Gibson DT. 1977. Biodegradation of aromatic petroleum hydrocarbons. In: Wolfe DA, ed. Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms. New York, NY: Pergamon, 36-46.

Gibson DT. 1980. Microbial metabolism. In: Hutzinger O, ed. The handbook of environmental chemistry. New York, NY: Springer-Verlag, 161-192.

Gill DP, Jenkins VK, Kempen RR, et al. 1980. The importance of pluripotential stem cells in benzene toxicity. Toxicology 16:163-171.

Gill DP, Kempen RR, Nash JB, et al. 1979. Modifications of benzene myelotoxicity and metabolism by phenobarbital, SKF-525A and 3-methylcholanthrene. Life Sci 25:1633-1640.

9. REFERENCES

Gilli G, Scursatone E, Bono R. 1994. Benzene, toluene and xylenes in air, geographical distribution in the piedmont region (Italy) and personal exposure. Sci Total Environ 148(1):49-56.

Giver CR, Wong R, Moore DH II, et al. 2001. Persistence of aneuploid immature/primitive hemopoietic sub-populations in mice 8 months after benzene exposure in vivo. Mutat Res 491(1-2):127-138.

Giwercman A, Carlsen E, Keiding N, et al. 1993. Evidence for increasing incidence of abnormalities of the human testis: A review. Environ Health Perspect Suppl 101(2):65-71.

Glass LR, Connor TH, Thiess JC, et al. 1986. Genotoxic evaluation of the offgassing products of particle boards. Toxicol Lett 31:75-83.

Glatt H, Witz G. 1990. Studies on the induction of gene mutations in bacterial and mammalian cells by the ring-opened benzene metabolites *trans,trans*-muconaldehyde and *trans,trans*-muconic acid. Mutagenesis 5:263-266.

Glatt H, Padykula R, Berchtold GA, et al. 1989. Multiple activation pathways of benzene leading to products with varying genotoxic characteristics. Environ Health Perspect 82:81-89.

Glauert HP, Kennan WS, Sattler GL, et al. 1985. Assays to measure the induction of unscheduled DNA synthesis in cultured hepatocytes. Progress in Mutation Research 5:371-373.

Gofmekler VA. 1968. [Effect on embryonic development of benzene and formaldehyde in inhalation experiments.] Hyg Sanit 33:327-332. (Russian)

Goldfrank LR, Flomenbaum NE, Lewin NA, et al., eds. 1998. Goldfrank's toxicologic emergencies. 6th edition. Stamford: Appleton & Lange, 1384-1398.

Goldman LR, Paigen B, Magnant MM, et al. 1985. Low birth weight, prematurity and birth defects in children living near the hazardous waste site, Love Canal. Hazard Waste Hazard Mater 2:209-223.

Goldstein BD, Warren J. 1993. HEI's mobile air toxics project. Environ Health Perspect 101(3):256-258.

Goldwater LJ. 1941. Disturbances in the blood following exposure to benzol. J Lab Clin Med 26:957-973.

Gonasun LM, Witmer C, Kocsis J, et al. 1973. Benzene metabolism in mouse liver microsomes. Toxicol Appl Pharmacol 26:398-406.

Gonzalez J, Levine SP. 1986. The development and evaluation of a thermally-desorbable miniature passive dosimeter for the monitoring of organic vapors. Am Ind Hyg Assoc J 47:339-346.

Goon D, Cheng X, Ruth JA, et al. 1992. Metabolism of *trans,trans*-muconaldehyde by aldehyde and alcohol dehydrogenases: Identification of a novel metabolite. Toxicol Appl Pharmacol 114:147-155.

Goon D, Matsuura J, Ross D. 1993. Metabolism and cytotoxicity of *trans,trans*-muconaldehyde and its derivatives: Potential markers of benzene ring cleavage reactions. Chem Biol Interact 88(1):37-53.

Gordian ME, Guay G. 1995. Benzene in Alaska. J Alaska Med 37(1):25-36.

Gosepath J, Grebneva N, Brieger J, et al. 2003. Evaluation of inflammatory reactions and genotoxic effects after exposure of nasal respiratory epithelia to benzene. ORL J Otorhinolaryngol Relat Spec 65(6):348-352.

Gossett RW, Brown DA, Young DR. 1983. Predicting the bioaccumulation of organic compounds in marine organisms using octanol/water partition coefficients. Marine Poll Bull 14(10):387-392.

Graedel TE. 1978. Chemical compounds in the atmosphere. New York, NY: Academic Press, 105-107.

Grbić-Galić D, Vogel TM. 1987. Transformation of toluene and benzene by mixed methanogenic cultures. Appl Environ Microbiol 53(2):254-260.

Greek BF. 1990. Prices and demand for aromatics reverse earlier gains. Chem Eng News 68:11-12.

Green JD, Leong BKJ, Laskin S. 1978. Inhaled benzene fetotoxicity in rats. Toxicol Appl Pharmacol 46:9-18.

Green JD, Snyder CA, LoBue J, et al. 1981a. Acute and chronic dose/response effects of inhaled benzene on multipotential hematopoietic stem (CFU-S) and granulocyte/macrophage progenitor (GM-CFU-C) cells in CD-1 mice. Toxicol Appl Pharmacol 58:492-503.

Green JD, Snyder CA, LoBue J, et al. 1981b. Acute and chronic dose/response effect of benzene inhalation on the peripheral blood, bone marrow, and spleen cell of CD-1 male mice. Toxicol Appl Pharmacol 59:204-214.

Greenburg L. 1926. Benzol poisoning as an industrial hazard. Public Health Reports 41:1357-1375.

Greenburg L, Mayers MR, Goldwater L, et al. 1939. Benzene (benzol) poisoning in the rotogravure printing industry in New York City. J Ind Hyg Toxicol 21:395-420.

*Greenland S, Salvan A, Wegman DH, et al. 1994. A case-control study of cancer mortality at a transformer-assembly facility. Int Arch Occup Environ Health 66:49-54.

Grob K, Frauenfelder C, Artho A. 1990. Uptake by foods of tetrachloroethylene, trichlorethylene, toluene, and benzene from air. Z Lebensm Unters Forsch 191:435-441.

Grotz VL, Ji S, Kline SA, et al. 1994. Metabolism of benzene and *trans,trans*-muconaldehyde in the isolated perfused rat liver. Toxicol Lett 70(3):281-290.

Gruenke LD, Craig JC, Wester RC, et al. 1986. Quantitative analysis of benzene by selected ion monitoring/gas chromatography/mass spectrometry. J Anal Toxicol 10:225-232.

Guerra G, Lemma A, Lerda D, et al. 1995. Benzene emissions from motor vehicle traffic in the urban area of Milan: Hypothesis of health impact assessment. Atmos Environ 29(23):3559-3569.

Gulati DK, Witt K, Anderson B, et al. 1989. Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro: III: Results with 27 chemicals. Environ Mol Mutagen 13:133-193.

Gut I, Nedelcheva V, Soucek P, et al. 1996a. Cytochromes P450 in benzene metabolism and involvement of their metabolites and reactive oxygen species in toxicity. Environ Health Perspect 104(Suppl 6):1211-1218.

Gut I, Nedelcheva V, Soucek P, et al. 1996b. The role of CYP2E1 and 2B1 in metabolic activation of benzene derivatives. Arch Toxicol 71:45-56.

Gut I, Terelius Y, Frantik E, et al. 1993. Exposure to various benzene derivatives differently induces cytochromes P450 2B1 and P450 2E1 in rat liver. Arch Toxicol 67(4):237-243.

*Guy RL, Dimitriadis EA, Hu PD, et al. 1990. Interactive inhibition of erythroid ^{59}Fe utilization by benzene metabolites in female mice. Chem Biol Interact 74(1-2):55-62.

Guy RL, Hu P, Witz G, et al. 1991. Depression of iron uptake into erythrocytes in mice by treatment with the combined benzene metabolites *p*-benzoquinone, muconaldehyde and hydroquinone. J Appl Toxicol 11(6):443-446.

Guzelian PS, Henry CJ, Olin SS, eds. 1992. Similarities and differences between children and adults: Implications for risk assessment. Washington, DC: International Life Sciences Institute Press.

Haddad LM, Shannon MW, Winchester JF. 1998. Clinical management of poisoning and drug overdose. 3rd edition. Philadelphia, PA: W.B Saunders Company, 940-941.

Haider K, Jagnow G, Kohnen R, et al. 1981. Degradation of chlorinated benzene, phenols and cyclohexane derivatives by benzene- and phenol-utilizing soil bacteria under aerobic conditions. In: Overcash MR, ed. Decomposition of toxic and nontoxic organic compounds in soils. Ann Arbor, MI: Ann Arbor Science, 207-223.

Hajimiragha H, Ewers U, Brockhaus A, et al. 1989. Levels of benzene and other volatile aromatic compounds in the blood of non-smokers and smokers. Int Arch Occup Environ Health 61:513-518.

Hallberg LM, El Zein R, Grossman L, et al. 1996. Measurement of DNA repair deficiency in workers exposed to benzene. Environ Health Perspect 104:529-534.

Hamilton A. 1922. The growing menace of benzene (benzol) poisoning in American industry. J Am Med Assoc 78:627-630.

Hammond JW, Hermann ER. 1960. Industrial hygiene features of a petro-chemical benzene plant design and operation. Am Ind Hyg Assoc J 21:173-177.

Hanke J, Dutkiewicz T, Piotrowski J. 1961. [The absorption of benzene through the skin in men.] Medycyna Pracy 12:413-426. (Polish)

Hanway R, Cavicchioli A, Kaur B, et al. 2000. Analysis of S-phenol-L-cysteine in globin as a marker of benzene exposure. Biomarkers 5(4):252-262.

Hanzlick R. 1995. National association of medical examiners pediatric toxicology (PedTox) registry report 3: Case submission summary and data for acetaminophen, benzene, carboxyhemoglobin, dextromethorphan, ethanol, phenobarbital, and pseudoephedrine. Am J Forensic Med Pathol 16(4):270-277.

Harkov R, Gianti SJ Jr, Bozzelli JW, et al. 1985. Monitoring volatile organic compounds at hazardous and sanitary landfills in New Jersey. J Environ Sci Health A20:491-501.

Harland BJ, Whitby FJ, Comber MHI. 1985. Measurement of volatile aromatic hydrocarbons in an industrial estuary. Int J Environ Anal Chem 20:295-311.

Harper BL, Sadagopa Ramanujam VM, Gad-El-Karim MM, et al. 1984. The influence of simple aromatics on benzene clastogenicity. Mutat Res 128:105-114.

Harrison I, Leader RU, Higgo JJW, et al. 1994. Determination of organic pollutants in small samples of groundwaters by liquid-liquid extraction and capillary gas chromatography. J Chromatogr A688:181-188.

Hartwich G, Schwanitz G, Becker J. 1969. Chromosome anomalies in a case of benzene leukaemia. German Medical Monthly 14:449-450.

Hatakeyama Y, Nakajima E, Atai H, et al. 1992. Effects of benzene in a micronucleus test on peripheral blood utilizing acridine orange-coated slides. Mutat Res 278(2-3):193-195.

Hattemer-Frey HA, Travis CC, Land ML. 1990. Benzene: Environmental partitioning and human exposure. Environ Res 53:221-232.

Hayashi M, Norppa H, Sofuni T, et al. 1992. Flow cytometric micronucleus test with mouse peripheral erythrocytes. Mutagenesis 7(4):257-264.

Hayes RB. 1992. Biomarkers in occupational cancer epidemiology: Considerations in study design. Environ Health Perspect 98:149-154.

Hayes RB, Songnian Y, Dosemeci M, et al. 2001. Benzene and lymphohematopoietic malignancies in humans. Am J Ind Med 40(2):117-126.

Hayes RB, Yin SN, Dosemeci M, et al. 1996. Mortality among benzene-exposed workers in China. Environ Health Perspect 104(Suppl 6):1349-1352.

Hayes, RB, Yin SN, Dosemeci M, et al. 1997. Benzene and the dose-related incidence of hematologic neoplasms in China. J Natl Cancer Inst 89(14):1065-1071.

HazDat. 2006. HazDat Database: ATSDR's Hazardous Substance Release and Health Effects Database. Atlanta, GA: Agency for Toxic Substances and Disease Registry. www.atsdr.cdc.gov/hazdat.html. December 18, 2006.

*Hazel BA, O'Connor A, Niculescu R, et al. 1995. Benzene and its metabolite, hydroquinone, induce granulocytic differentiation in myeloblasts by interacting with cellular signaling pathways activated by granulocyte colony-stimulating factor. Stem Cells 13(3):295-310.

Healy LN, Pluta LJ, James RA, et al. 2001. Induction and time-dependent accumulation of micronuclei in peripheral blood of transgenic p53 +/- mice, TgAC (v-HA-*ras*) and parental wild-type (C57BL/6 and FVB/N) mice exposed to benzene by inhalation. Mutagenesis 16(2):163-168.

Heath CW. 1983. Field epidemiologic studies of populations exposed to waste dumps. Environ Health Perspect 48:3-7.

9. REFERENCES

Heavner DL, Morgan WT, Ogden MW. 1995. Determination of volatile organic compounds and ETS apportionment in 49 homes. Environ Int 21(1):3-21.

Hedli CC, Snyder R, Witmer CM. 1991. Bone marrow DNA adducts and bone marrow cellularity following treatment with benzene metabolites *in vivo*. Adv Exp Med Biol 283:745-748.

Heineman EF, Olsen JH, Pottern LM, et al. 1992. Occupational risk factors for multiple myeloma among Danish men. Cancer Causes Control 3:555-568.

Hellmér L, Bolcsfoldi G. 1992a. An evaluation of the *E. coli* K-12 *uvrB/recA* DNA repair host-mediated assay: II. In vivo results for 36 compounds tested in the mouse. Mutat Res 272(2):161-173.

Hellmér L, Bolcsfoldi G. 1992b. An evaluation of the *E. coli* k-12 *uvrB/recA* DNA repair host-mediated assay: I. In vitro sensitivity of the bacteria to 61 compounds. Mutat Res 272(2):145-160.

Henderson RF, Sabourin PJ, Bechtold WE, et al. 1989. The effect of dose, dose rate, route of administration, and species on tissue and blood levels of benzene metabolites. Environ Health Perspect 82:9-17.

Henderson RF, Sabourin PJ, Medinsky MA, et al. 1992. Benzene dosimetry in experimental animals: Relevance for risk assessment. In: D'Amato R, Slaga TJ, Farland WH, et al., eds. Relevance of animal studies to the evaluation of human cancer risk. New York, NY: Wiley-Liss Inc., 93-105.

Hendrickson HP, Sahafayen M, Bell MA, et al. 1994. Relationship of flavonoid oxidation potential and effect on rat hepatic microsomal metabolism of benzene and phenol. J Pharm Biomed Anal 12(3):335-341.

Hengstler JG, Arand M, Herrero ME, et al. 1998. Polymorphisms of *N*-acetyltransferases, glutathione *S*-transferases, microsomal epoxide hydrolase and sulfotransferases: Influence on cancer susceptibility. Recent Results Cancer Res 154:47-85.

Henschler R, Glatt HR. 1995. Induction of cytochrome p4501a1 in haemopoietic stem cells by hydroxylated metabolites of benzene. Toxicol I*n Vitro* 9(4):453-457.

Hiraku Y, Kawanishi S. 1996. Oxidative DNA damage and apoptosis induced by benzene metabolites. Cancer Res 56(22):5172-5178.

Hite M, Pecharo M, Smith I, et al. 1980. The effect of benzene in the micronucleus test. Mutat Res 77:149-155.

Hoechst. 1977. Initial submission: Mutagenicity evaluation of benzene in Salmonella typhimurium, Saccharomyces cerevisiae, and mice (final report) with attachment. Hoechst Celanese Corporation. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8E. OTS0539838.

*Hoechst. 1978. Chronic toxicity with benzene in Sprague-Dawley rats. Hoechst Celanese Corporation. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8E. OTS0539825.

Hoel DG, Davis DL, Miller AB, et al. 1992. Trends in cancer mortality in 15 industrialized countries, 1969-1986. J Natl Cancer Inst 84(5):313-320.

9. REFERENCES

Hoffmann K, Krause C, Seifert B, et al. 2000. The German Environmental Survey 1990/92 (GERES II) Sources of Personal exposure to volatile organic compounds. J Expo Anal Environ Epidemiol 10:115-125.

Holmberg B, Lundberg P. 1985. Benzene: Standards, occurrence, and exposure. Am J Ind Med 7:375-383.

Hopper DJ. 1978. Microbial degradation of aromatic hydrocarbons. In: Watkinson RJ, ed. Developments in biodegradation of hydrocarbons. London, England: Applied Science Publishers LTD, 85-112.

HSDB. 2007. Benzene. Hazardous Substances Data Bank. National Library of Medicine. http://toxnet.nlm.nih.gov. May 1, 2007.

Hsieh GC, Parker RDR, Sharma RP. 1988a. Subclinical effects of groundwater contaminants: II. Alteration of regional brain monoamine neurotransmitters by benzene in CD-1 mice. Arch Environ Contam Toxicol 17:799-805.

Hsieh GC, Parker RDR, Sharma RP, et al. 1990. Subclinical effects of groundwater contaminants. III. Effects of repeated oral exposure to combinations of benzene and toluene on immunologic responses in mice. Arch Toxicol 64(4):320-328.

Hsieh GC, Sharma RP, Parker RDR. 1988b. Subclinical effects of groundwater contaminants: I. Alteration of humoral and cellular immunity by benzene in CD-1 mice. Arch Environ Contam Toxicol 17:151-158.

Hsieh GC, Sharma RP, Parker RD. 1991. Hypothalamic-pituitary-adrenocortical axis activity and immune function after oral exposure to benzene and toluene. Immunopharmacology 21(1):23-31.

Huff JE, Haseman JK, DeMarini DM, et al. 1989. Multiple-site carcinogenicity of benzene in Fischer 344 rats and B6C3F$_1$ mice. Environ Health Perspect 82:125-163.

*Hunter CG. 1968. Solvents with reference to studies on the pharmacodynamics of benzene. Proc R Soc Med 61:913-915.

Hunting KL, Longbottom H, Kalavar SS, et al. 1995. Haematopoietic cancer mortality among vehicle mechanics. Occup Environ Med 52:673-678.

*Hurley JF, Cherrie JW, Maclaren W. 1991. Exposure to benzene and mortality from leukaemia: Results from coke oven and other coal product workers [letter]. Br J Ind Med 48(7):502-503.

Hustert K, Mansour H, Korte F. 1981. The "EPA Test": A method for the determination the photo-chemical degradation of organic compounds in aquatic systems. Chemosphere 10(9):995-998.

IARC. 1982. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol. 29: Some industrial chemical and dyestuffs. Benzene. Lyon, France: World Health Organization, International Agency for Research on Cancer, 93-148.

IARC. 1987. IARC monographs on the evaluation of carcinogenic risks to humans. Overall evaluations of carcinogenicity: An updating of IARC Monographs Volumes 1 to 42. Suppl 7. Lyons, France: World Health Organization, International Agency for Research on Cancer, 38-74.

IARC. 1988. Determination of phenol and its glucurono- and sulfoconjugates in urine by gas chromatography. In: Fishbein L, O'Neill JK, eds. Environmental carcinogens method of analysis and exposure measurement: Vol. 10. Benzene and alkylated benzene. IARC Scientific Publications No. 85. Lyon, France: World Health Organization, International Agency for Research on Cancer, 281-286.

IARC. 2004. Overall evaluations of carcinogenicity to humans: As evaluated in IARC Monographs volumes 1-82 (a total of 900 agents, mixtures and exposures). Lyon, France: International Agency for Research on Cancer. http://www-cie.iarc.fr/monoeval/crthall.html. February 15, 2005.

IARC. 2007. Agents reviewed by the IARC monographs. Volumes 1-96. (alphabetical order). Lyon, France: International Agency for Research on Cancer. http://monographs.iarc.fr/ENG/Classification/Listagentsalphorder.pdf. May 1, 2007.

Ikeda M, Ohtsuji H. 1971. Phenobarbital-induced protection against toxicity of toluene and benzene in the rat. Toxicol Appl Pharmacol 20:30-43.

Ikeda M, Ohtsuji H, Imamura T. 1972. *In vivo* suppression of benzene and styrene oxidation by co-administered toluene in rats and effects of phenobarbital. Xenobiotica 2:101-106.

Infante PF. 1978. Leukemia among workers exposed to benzene. Tex Rep Biol Med 37:153-161.

Infante PF, Rinsky RA, Wagoner JK, et al. 1977. Leukemia in benzene workers. Lancet 2:76-78.

Infante-Rivard C, Siemiatycki J, Lakhani R, et al. 2005. Maternal exposure to occupational solvents and childhood leukemia. Environ Health Perspect 113(6):787-792.

Inoue O, Kanno E, Kakizaki M, et al. 2000. Urinary phenylmercapturic acid as a marker of occupational exposure to benzene. Ind Health 38(2):195-204.

Inoue O, Seiji K, Kasahara M, et al. 1986. Quantitative relation of urinary phenol levels to breathzone benzene concentrations: A factory survey. Br J Ind Med 43:692-697.

Inoue O, Seiji K, Kasahara M, et al. 1988a. Determination of catechol and quinol in the urine of workers exposed to benzene. Br J Ind Med 45:487-492.

*Inoue O, Seiji K, Nakatsuka H, et al. 1989a. Excretion of 1,2,4-benzenetriol in the urine of workers exposed to benzene. Br J Ind Med 46:559-565.

Inoue O, Seiji K, Nakatsuka H, et al. 1989b. Urinary t,t-muconic acid as an indicator of exposure to benzene. Br J Ind Med 46:122-127.

Inoue O, Seiji K, Watanabe T, et al. 1988b. Mutual metabolic suppression between benzene and toluene in man. Int Arch Occup Environ Health 60:15-20.

Ireland B, Collins JJ, Buckley CF, et al. 1997. Cancer mortality among workers with benzene exposure. Epidemiology 8(3):318-320.

IRIS. 2007. Benzene. Integrated Risk Information System. Washington, DC: U.S. Environmental Protection Agency. http://www.epa.gov/iris/subst/index.html. May 1, 2007.

Irons RD. 2000. Molecular models of benzene leukemogenesis. J Toxicol Environ Health A 61(5-6):391-397.

Irons RD, Neptune DA. 1980. Effects of the principal hydroxy metabolism of benzene on microtubule polymerization. Arch Toxicol 45:297-305.

Irons RD, Dent JG, Baker TS, et al. 1980. Benzene is metabolized and covalently bound in bone marrow in situ. Chem Biol Interact 30:241-245.

*Irons RD, Heck Hd'A, Moore BJ, et al. 1979. Effects of short-term benzene administration on bone marrow cell cycle kinetics in the rat. Toxicol Appl Pharmacol 51:399-409.

IRPTC. 1985. Treatment and disposal methods for waste chemicals. Data Profile Series No. 5. International Register of Potentially Toxic Chemicals. Geneva, Switzerland. United Nations Environment Programme.

ITII. 1975. Toxic and hazardous industrial chemicals safety manual for handling and disposal, with toxicity and hazard data. Tokyo, Japan: International Technical Information Institute, 62-64.

*Jakobsson R, Ahlbom A, Bellander T, et al. 1993. Acute myeloid leukemia among petrol station attendants. Arch Environ Health 48(4):255-259.

Jen J-F, Tsai M-Y. 1994. Determination of phenol in urine by high-performance liquid chromatography with on-line precolumn enzymatic hydrolysis of the conjugates. J Chromatogr 658:87-92.

Jerina D, Daly J, Witkop B, et al. 1968. Role of arene oxide-oxepin system in the metabolism of aromatic substances. I. *In vitro* conversion of benzene oxide to a premercapturic acid and a dihydrodiol. Arch Biochem Biophys 128:176-183.

Jirka AM, Bourne S. 1982. Gas-chromatographic analysis for benzene in blood. Clin Chem 28:1492-1494.

Jo WK, Kim SH. 2001. Worker exposure to aromatic volatile organic compounds in dry cleaning. Am Ind Hyg Assoc J 62(4):466-471.

Jo WK, Moon KC. 1999. Housewives' exposure to volatile organic compounds relative to proximity to roadside service stations. Atmos Environ 33:2921-2928.

Johanson CE. 1980. Permeability and vascularity of the developing brain: Cerebellum vs cerebral cortex. Brain Res 190:3-16.

Johansson I, Ingelman-Sundberg M. 1988. Benzene metabolism by ethanol-, acetone-, and benzene-inducible cytochrome P-450(IIE1) in rat and rabbit liver microsomes. Cancer Res 48:5387-5390.

*Jonek J, Olkowski Z, Zieleznik B. 1965. Histochemical studies on the spinal cord of mice poisoned with benzene. Acta Histochem Suppl 20:286-296.

Jongeneelen FJ, Dirven HAAM, Leijdekkers C-M, et al. 1987. S-phenyl-n-acetylcysteine in urine of rats and workers after exposure to benzene. J Anal Toxicol 11:100-104.

9. REFERENCES

Kaden DA, Hites RA, Thilly WG. 1979. Mutagenicity of soot and associated polycyclic aromatic hydrocarbons to *Salmonella typhimurium*. Cancer Res 39:4152-4159.

Kahn H, Muzyka V. 1973. The chronic effect of benzene on porphyrin metabolism. Work Environ Health 10:140-143.

Kale PG, Baum JW. 1983. Genetic effects of benzene in Drosophila melanogaster males. Environ Mutagen 5:223-226.

Kalf GF, Rushmore T, Snyder R. 1982. Benzene inhibits RNA synthesis in mitochondria from liver and bone marrow. Chem Biol Interact 42:353-370.

Kalf GF, Schlosser MJ, Renz JF, et al. 1989. Prevention of benzene-induced myelotoxicity by nonsteroidal anti-inflammatory drugs. Environ Health Perspect 82:57-64.

Kanada M, Miyagawa M, Sato M, et al. 1994. Neurochemical profile of effects of 28 neurotoxic chemicals on the central nervous system in rats (1) effects of oral administration on brain contents of biogenic amines and metabolites. Ind Health 32:145-164.

Karacic V, Skender L, Bosner-Cucancic B, et al. 1995. Possible genotoxicity in low level benzene exposure. Am J Ind Med 27:379-388.

Karacic V, Skender L, Prpic -Majic D. 1987. Occupational exposure to benzene in the shoe industry. Am J Ind Med 12:531-536.

Karickhoff SW. 1981. Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. Chemosphere 10:833-846.

Karlson U, Frankenberger WT Jr. 1989. Microbial degradation of benzene and toluene in groundwater. Bull Environ Contam Toxicol 43:505-510.

Kasai H, Nishimura S. 1986. Hydroxylation of guanine in nucleosides and DNA at the C-8 position by heated glucose and oxygen radical-forming agents. Environ Health Perspect 67:111-116.

Kašuba V, Rozgaj R, Sentija K. 2000. Cytogenic changes in subjects occupationally exposed to benzene. Chemosphere 40(3):307-310.

Kawai T, Yamaoka K, Uchida Y, et al. 1990. Benzene exposure in a Japanese petroleum refinery. Toxicol Lett 52(2):135-139.

Kawai T, Yamaoka K, Uchida Y, et al. 1991. Exposure to vapors of benzene and other aromatic solvents in tank truck loading and delivery. Bull Environ Contam Toxicol 46(1):1-8.

Keener WK, Arp DJ. 1994. Transformations of aromatic compounds by Nitrosomonas europaea. Appl Environ Microbiol 60(6):1914-1920.

Keller KA, Snyder CA. 1986. Mice exposed in utero to low concentrations of benzene exhibit enduring changes in their colony forming hematopoietic cells. Toxicology 42:171-181.

Keller KA, Snyder CA. 1988. Mice exposed *in utero* to 20 ppm benzene exhibit altered numbers of recognizable hematopoietic cells up to seven weeks after exposure. Fundam Appl Toxicol 10:224-232.

Kelly TJ, Callahan PJ, Pleil J, Evans GF. 1993. Method development and field measurements for polar volatile organic compounds in ambient air. Environ Sci Technol 27(6):1146-1152.

Kelsey KT, Ross D, Traver RD, et al. 1997. Ethnic variation in the prevalence of a common HAD(P)H quinone oxidoreductase polymorphism and its implications for anticancer chemotherapy. Br J Cancer 76:852-854.

Kenaga EE. 1980. Predicted bioconcentration factors and soil sorption coefficients of pesticides and other chemicals. Ecotoxicol Environ Safety 4:26-38.

Kennedy LG, Hutchins SR. 1992. Applied geologic, microbiological, and engineering constraints of in-situ BTEX bioremediation. Remediation 3:83-110.

Kenyon EM, Seaton MJ, Himmelstein MW, et al. 1998. Influence of gender and acetone pretreatment on benzene metabolism in mice exposed by nose-only inhalation. J Toxicol Environ Health A 55(6):421-443.

Kenyon EM, Seeley ME, Janszen D, et al. 1995. Dose-, route-, and sex-dependent urinary excretion of phenol metabolites of B6C3F$_1$ mice. J Toxicol Environ Health 44:219-233.

*Khuder SA, Youngdale MC, Schaub EA. 1999. Assessment of complete blood count variations among workers exposed to low levels of benzene. J Occup Environ Med 41(9):821-826.

Kiang PH, Grob RL. 1986. A headspace technique for the determination of volatile compounds in soil. J Environ Sci Health, Part A 21:71-100.

Kimmel CA, Wilson JG. 1973. Skeletal deviations in rats: Malformations or variations? Teratology 8:309-316.

Kinney PL, Chillrud SN, Ramstrom S, et al. 2002. Exposures to multiple air toxics in New York City. Environ Health Perspect 110(Suppl 4):539-546.

Kipen HM, Cody RP, Goldstein BD. 1989. Use of longitudinal analysis of peripheral blood counts to validate historical reconstructions of benzene exposure. Environ Health Perspect 82:199-206.

Kirschner EM. 1995. Production of top 50 chemicals increased substantially in 1994. Chem Eng News (April 10, 1995):16-20.

Kissling M, Speck B. 1972. Further studies on experimental benzene induced aplastic anemia. Blut 25:97-103.

Kissling M, Speck B. 1973. Chromosome aberrations in experimental benzene intoxication. Helv Med Acta 36:59-66.

*Klan MJ, Adams DO, Lewis JG. 1990. Effects of exposure to benzene *in vivo* on the murine mononuclear phagocyte system. Toxicol Appl Pharmacol 103(2):198-205.

Kok PW, Ong CN. 1994. Blood and urinary benzene determined by headspace gas chromatography with photoionization detection: Application in biological monitoring of low-level nonoccupational exposure. Int Arch Occup Environ Health 66(3):195-201.

Kolachana P, Subrahmanyam VV, Meyer KB, et al. 1993. Benzene and its phenolic metabolites produce oxidative DNA damage in HL60 cells *in vitro* and in the bone marrow *in vivo*. Cancer Res 53(5):1023-1026.

Komori M, Nishio K, Kitada M, et al. 1990. Fetus-specific expression of a form of cytochrome P-450 in human livers. Biochemistry 29:4430-4433.

Koop DR, Laethem CL. 1992. Inhibition of rabbit microsomal cytochrome P-450 2E1-dependent p-nitrophenol hydroxylation by substituted benzene derivatives. Drug Metab Dispos 20(5):775-777.

*Koop DR, Laethem CL, Schnier GG. 1989. Identification of ethanol-inducible P450 isozyme 3a (P45011E1) as a benzene and phenol hydroxylase. Toxicol Appl Pharmacol 98:278-288.

Korte F, Klein W. 1982. Degradation of benzene in the environment. Ecotoxicol Environ Safety 6:311-327.

Kouniali A, Cicolella A, Gonzalez-Flesca N, et al. 2003. Environmental benzene exposure assessment for parent-child pairs in Rouen, France. Sci Total Environ 308:73-82.

Kowalski JD, Shkiya JM, Tsou G. 1985. Ambient sampling and analysis of halogenated hydrocarbons and benzene. In: APCA Annual Meeting, June 16-21. Detroit, MI: Air Pollution Control Association, 2-13.

Kozioski RP. 1985. Determination of benzene and toluene in soils and plant material by azeotropic distillation. Bull Environ Contam Toxicol 34:10-16.

Kraut A, Lilis R, Marcus M, et al. 1988. Neurotoxic effects of solvent exposure on sewage treatment workers. Arch Environ Health 43:263-268.

Krishnan K, Andersen ME. 1994. Physiologically-based pharmacokinetic modeling in toxicology. In: Hayes W, ed. Principles and methods of toxicology. 3rd ed. New York, NY: Raven Press, Ltd., 149-188.

Krishnan K, Andersen ME, Clewell HJ III, et al. 1994. Physiologically-based pharmacokinetic modeling of chemical mixtures. In: Yang RSA, ed. Toxicology of chemical mixtures. New York, NY: Academic Press, 399-437.

Kuna RA, Kapp RW. 1981. Embryotoxic/teratogenic potential of benzene vapor in rats. Toxicol Appl Pharmacol 57:1-7.

Kuna RA, Nicolich MJ, Schroeder RE, et al. 1992. A female rat fertility study with inhaled benzene. J Am Coll Toxicol 11(3):275-282.

Kunisaki TA, Augenstein WL. 1994. Drug-and toxin-induced seizures. Emerg Med Clin North Am 12(4):1027-1056.

*Laerum OD. 1973. Reticulum cell neoplasms in normal and benzene treated hairless mice. Acta Pathol Microbiol Scand Sect A 81(1):57-63.

Lagorio S, Forastiere F, Iavarone I, et al. 1994b. Mortality of filling station attendants. Scand J Work Environ Health 20:331-338.

Lagorio S, Tagesson C, Forastiere F, et al. 1994a. Exposure to benzene and urinary concentrations of 8-hydroxydeoxyguanosine, a biological marker of oxidative damage to DNA. Occup Environ Med 51:739-743.

Laitinen J, Kangas J, Pekari K, et al. 1994. Short time exposure to benzene and gasoline at garages. Chemosphere 28:197-205.

Lakhanisky TH, Hendrickx B. 1985. Induction of DNA single-strand breaks in CHO cells in culture. Prog Mutat Res 5:367-370.

Lan Q, Zhang L, Li G, et al. 2004a. Hematotoxicity in workers exposed to low levels of benzene. Science 306:1774-1776.

Lan Q, Zhang L, Li G, et al. 2004b. Hematotoxicity in workers exposed to low levels of benzene: Supporting online material. Science 306:1774-1776. http://www.sciencemag.org/cgi/data/306/5702/1774/DC1/1. August 2, 2005.

Lange A, Smolik R, Zatonski W, et al. 1973a. Leukocyte agglutinins in workers exposed to benzene, toluene and xylene. Int Arch Arbeitsmed 31:45-40.

Lange A, Smolik R, Zatonski W, et al. 1973b. Serum immunoglobulin levels in workers exposed to benzene, toluene and xylene. Int Arch Arbeitsmed 31:37-44.

Larson TV, Koenig JQ. 1994. Wood smoke: Emissions and noncancer respiratory effects. Annu Rev Public Health 15:133-156.

Latriano L, Goldstein BD, Witz G. 1986. Formation of muconaldehyde, an open-ring metabolite of benzene, in mouse liver microsomes: An additional pathway for toxic metabolites. Proc Natl Acad Sci USA 83:8356-8360.

Lauwerys RR, Buchet JP, Adrien F. 1994. Muconic acid in urine: A reliable indicator of occupational exposure to benzene. Am J Ind Med 25:297-300.

*Lee EW, Garner CD. 1991. Effects of benzene on DNA strand breaks *in vivo* versus benzene metabolite-induced DNA strand breaks *in vitro* in mouse bone marrow cells. Toxicol Appl Pharmacol 108(3):497-508.

Lee B-L, New A-L, Kok P-W, et al. 1993. Urinary *trans,trans*-muconic acid determined by liquid chromatography: Application in biological monitoring of benzene exposure. Clin Chem 39:1788-1792.

Lee E, Im H, Oh E, et al. 2005. DNA damage in T and B lymphocytes, bone marrow, spleens, and livers of rats exposed to benzene. Inhal Toxicol 17(7-8):401-408.

Lee EW, Garner CD, Johnson JT. 1988. A proposed role played by benzene itself in the induction of acute cytopenia: Inhibition of DNA synthesis. Res Commun Chem Pathol Pharmacol 60:27-46.

Lee EW, Johnson JT, Garner CD. 1989. Inhibitory effect of benzene metabolites on nuclear DNA synthesis in bone marrow cells. J Toxicol Environ Health 26:277-291.

Leeder JS, Kearns GL. 1997. Pharmcogenetics in pediatrics: Implications for practice. Pediatr Clin North Am 44(1):55-77.

Lemire S, Ashley D, Olaya P, et al. 2004. Environmental exposure of commuters in Mexico City to volatile organic compounds as assessed by blood concentrationas, 1998. Salud Pub Mexico Jan-Feb(1):32-38.

Leung H. 1993. Physiologically-based pharmacokinetic modeling. In: Ballantine B, Marro T, Turner T, eds. General and applied toxicology. Vol. I. New York, NY: Stockton Press, 153-164.

Levy A. 1973. The photochemical smog reactivity of organic solvents. In: Solvent theory and practices. Adv Chem Ser 124:70-94.

Lévay G, Bodell WJ. 1992. Potentiation of DNA adduct formation in HL-60 cells by combinations of benzene metabolites. Proc Natl Acad Sci USA 89(15):7105-7109.

Lévay G, Pathak DN, Bodell WJ. 1996. Detection of DNA adducts in the white blood cells of B6C3F1 mice treated with benzene. Carcinogenesis 17(1):151-153.

Lewis SJ, Bell GM, Cordingley N, et al. 1997. Retrospective estimation of exposure to benzene in a leukaemia case-control study of petroleum marketing and distribution workers in the United Kingdom. Occup Environ Med 54(3):167-175.

Lewis JG, Stewart W, Adams DO. 1988. Role of oxygen radicals in induction of DNA damage by metabolites of benzene. Cancer Res 48:4762-4765.

Li G-L, Linet MS, Hayes RB, et al. 1994. Gender differences in hematopoietic and lymphoproliferative disorders and other cancer risks by major occupational group among workers exposed to benzene in China. J Occup Med 36(8):875-881.

Li G-L, Yin S-N, Watanabe T, et al. 1986. Benzene-specific increase in leukocyte alkaline phosphatase activity in rats exposed to vapors of various organic solvents. J Toxicol Environ Health 19:581-589.

Li L, Sun W, Gong Z, et al. 1992. Effect of low benzene exposure on neurobehavioral function, AChE in blood and brain and bone marrow picture in mice. Biomed Environ Sci 5(4):349-354.

Li Q, Aubrey MT, Christian T, et al. 1997. Differential inhibition of DNA synthesis in human T cells by the cigarette tar components hydroquinone and catechol. Fundam Appl Toxicol 38(2):158-165.

Li Q, Kasten-Jolly J, Yen Y, et al. 1998. Reversal of hydroquinone-mediated suppression of T cell proliferation by transfection of the M2 subunit of ribonucleotide reductase. Toxicol Appl Pharmacol 150(1):154-157.

Li Y, Kuppusamy P, Zweier JL, et al. 1995. ESR evidence for the generation of reactive oxygen species from the copper-mediated oxidation of the benzene metabolite, hydroquinone: Role in DNA damage. Chem Biol Interact 94(2):101-120.

Lin T-F, Little JC, Nazaroff WW. 1994. Transport and sorption of volatile organic compounds and water vapor within dry soil grains. Environ Sci Technol 28:322-330.

9. REFERENCES

Lindstrom AB, Highsmith VR, Buckley TJ, et al. 1994. Gasoline-contaminated ground water as a source of residential benzene exposure: A case study. J Expos Anal Environ Epidemiol 4(2):183-195.

Lindstrom AB, Yeowell-O'Connell K, Waidyanatha S, et al. 1997. Measurement of benzene oxide in the blood of rats following administration of benzene. Carcinogenesis 18(8):1637-1641.

Lindstrom AB, Yeowell-O'Connell K, Waidyanatha S, et al. 1999. Investigation of benzene oxide in bone marrow and other tissues of F344 rats following metabolism of benzene in vitro and in vivo. Chem Biol Interact 122(1):45-58.

Linet MS, Harlow SD, McLaughlin JK. 1987. A case-control study of muliple myeloma in whites: Chronic antigenic stimulation, occupation, and drug use. Cancer Res 47:2978-2981.

Linet MS, Yin S-N, Travis LB, et al. 1996. Clinical features of hematopoietic malignancies and related disorders among benzene-exposed workers in China. Environ Health Perspect 104(Suppl 6):1353-1364.

Lioy PJ, Pellizzari E. 1995. Conceptual framework for designing a national survey of human exposure. J Expos Anal Environ Epidemiol 5(3):425-444.

Liu L, Zhang Q, Feng J, et al. 1996. The study of DNA oxidative damage in benzene-exposed workers. Mutat Res 370(3-4):45-50.

Livingston, AL. 1978. Forage plant estrogens. J Toxicol Environ Health 4:301-324.

Lodén M. 1986. The *in vitro* permeability of human skin to benzene, ethylene glycol, formaldehyde, and n-hexane. Acta Pharmacol Toxicol 58:382-389.

Longacre SL, Kocsis JJ, Snyder R. 1981a. Influence of strain differences in mice on the metabolism and toxicity of benzene. Toxicol Appl Pharmacol 60:398-409.

Longacre SL, Kocsis JJ, Witmer CM, et al. 1981b. Toxicological and biochemical effects of repeated administration of benzene in mice. J Toxicol Environ Health 7:223-237.

Lovern MR, Maris ME, Schlosser PM. 1999. Use of a mathematical model of rodent in vitro benzene metabolism to predict human in vitro metabolism data. Carcinogenesis 20(8):1511-1520.

Lovern MR, Turner MJ, Meyer M, et al. 1997. Identification of benzene oxide as a product of benzene metabolism by mouse, rat, and human liver microsomes. Carcinogenesis 18(9):1695-1700.

Low LK, Meeks JR, Norris KJ, et al. 1989. Pharmacokinetics and metabolism of benzene in Zymbal gland and other key target tissues after oral administration in rats. Environ Health Perspect 82:215-222.

Lowry WT, Juarez L, Petty CS, et al. 1985. Studies of toxic gas production during actual structural fires in the Dallas area. J Forensic Sci 30:59-72.

Ludwig RC, Eksteen R. 1988. LC column switching for the rapid analysis of benzene in unleaded gasoline. LC-GC 6:250-252.

Luke CA, Tice RR, Drew RT. 1988a. The effect of exposure regimen and duration on benzene-induced bone-marrow damage in mice: I. Sex comparison in DBA/2 mice. Mutat Res 203:251-271.

9. REFERENCES

Luke CA, Tice RR, Drew RT. 1988b. The effect of exposure regimen and duration on benzene-induced bone marrow damage in mice: II. Strain comparisons involving B6C3F$_1$, C57BL/6 and DBA/2 male mice. Mutat Res 203:273-295.

Lumley M, Barker H, Murray JA. 1990. Benzene in petrol [letter; comment]. Lancet 336(8726):1318-1319.

Lutz WK, Schlatter CH. 1977. Mechanism of the carcinogenic action of benzene: Irreversible binding to rat liver DNA. Chem Biol Interact 18:241-245.

Lyman WJ. 1982. Atmospheric residence time. In: Lyman WJ, Reehl WF, Rosenblatt DH, eds. Handbook of chemical property estimation methods: Environmental behavior of organic compounds. New York, NY: McGraw-Hill.

Lysyj I, Perkins G, Farlow JS. 1980. Trace analysis for aromatic hydrocarbons in natural waters. Environ Int 4:407-416.

*MacEachern L, Laskin DL. 1992. Increased production of tumor necrosis factor-α by bone marrow leukocytes following benzene treatment of mice. Toxicol Appl Pharmacol 113(2):260-266.

*MacEachern L, Snyder R, Laskin DL. 1992. Alterations in the morphology and functional activity of bone marrow phagocytes following benzene treatment of mice. Toxicol Appl Pharmacol 117(2):147-154.

MacIntosh DL, Xue J, Ozkaynak H, et al. 1995. Population-based exposure model for benzene. J Expos Anal Environ Epidemiol 5(3):375-403.

Mackay D, Leinonen PJ. 1975. Rate of evaporation of low-solubility contaminants from water bodies to atmosphere. Environ Sci Technol 9:1178-1180.

MacLeod M, Mackay D. 1999. An assessment of the environmental fate and exposure of benzene and the chlorobenzenes in Canada. Chemosphere 38(8):1777-1796.

Magos GA, Lorenzana-Jimenez M, Vidrio H. 1990. Toluene and benzene inhalation influences on ventricular arrhythmias in the rat. Neurotoxicol Teratol 12(2):119-124.

Maibach HI, Anjo DM. 1981. Percutaneous penetration of benzene and benzene contained in solvents in the rubber industry. Arch Environ Health 36:256-260.

Major J, Jakab M, Kiss G, et al. 1994. Chromosome aberration, sister-chromatid exchange, proliferative rate index, and serum thiocyanate concentration in smokers exposed to low-dose benzene. Environ Mol Mutagen 23:137-142.

Major J, Kemeny G, Tompa A. 1992. Genotoxic effects of occupational exposure in the peripheral blood lymphocytes of pesticide preparing workers in Hungary. Acta Med Hung 49(1-2):79-90.

Malachowsky KJ, Phelps TJ, Teboli AB, et al. 1994. Aerobic mineralization of trichloroethylene, vinyl chloride, and aromatic compounds by Rhodococcus species. Appl Environ Microbiol 60(2):542-548.

Maltoni C, Ciliberti A, Cotti G, et al. 1989. Benzene, an experimental multipotential carcinogen: Results of the long-term bioassays performed at the Bologna Institute of Oncology. Environ Health Perspect 82:109-124.

Maltoni C, Conti B, Cotti G. 1983. Benzene: A multipotential carcinogen: Results of long-term bioassays performed at the Bologna Institute of Oncology. Am J Ind Med 4:589-630.

Maltoni C, Conti B, Cotti G, et al. 1985. Experimental studies on benzene carcinogenicity at the Bologna Institute of Oncology: Current results and ongoing research. Am J Ind Med 7:415-446.

Maltoni C, Cotti G, Valgimigli L, et al. 1982a. Hepatocarcinomas in Sprague-Dawley rats following exposure to benzene by inhalation: First experimental demonstration. Med Lav 4:446-450.

Maltoni C, Cotti G, Valgimigli L, et al. 1982b. Zymbal gland carcinomas in rats following exposure to benzene by inhalation. Am J Ind Med 3:11-16.

Mani C, Freeman S, Nelson DO, et al. 1999. Species and strain comparisons in the macromolecular binding of extremely low doses of [^{14}C]benzene in rodents, using accelerator mass spectrometry. Toxicol Appl Pharmacol 159(2):83-90.

Mannino DM, Schreiber J, Aldous K, et al. 1995. Human exposure to volatile organic compounds: A comparison of organic vapor monitoring badge levels with blood levels. Int Arch Occup Environ Health 67:59-64.

Marrazzini A, Chelotti L, Barrai I, et al. 1994. In vivo genotoxic interactions among three phenolic benzene metabolites. Mutat Res 341(1):29-46.

Mathews JM, Etheridge AS, Matthews HB. 1998. Dose-dependent metabolism of benzene in hamsters, rats and mice. Toxicol Sci 44(1):14-21.

Mattie DR, Bates G D JR, Jepson GW, et al. 1994. Determination of skin:air partition coefficients for volatile chemicals: Experimental method and applications. Fundam Appl Toxicol 22(1):51-57.

Mayer GJ, Cheng IS, Pau P, et al. 1994. Emissions of air toxics from wastewater treatment plants. Water Environ Res 66:140-144.

Mayr U, Butsch A, Schneider S. 1992. Validation of two in vitro test systems for estrogenic activities with zearalenone, phytoestrogens and cereal extracts. Toxicology 74:135-149.

Mazzullo M, Bartoli S, Bonora B, et al. 1989. Benzene adducts with rat nucleic acids and proteins: Dose-response relationship after treatment *in vivo*. Environ Health Perspect 82:259-266.

McAllister PM, Chiang CY. 1994. A practical approach to evaluating natural attenuation of contaminants in ground water. Ground Water Monit Remed 14:161-173.

McDonald TA, Yeowell-O'Connell K, Rappaport SM. 1994. Comparison of protein adducts of benzene oxide and benzoquinone in the blood and bone marrow of rats and mice exposed to [^{14}C/^{13}C$_6$] benzene. Cancer Res 54:4907-4914.

McDougal JN, Jepson GW, Clewell HJ III, et al. 1990. Dermal absorption of organic chemical vapors in rats and humans. Fundam Appl Toxicol 14:299-308.

McKinney PA, Alexandria FE, Cartwright RA, et al. 1991. Parental occupations of children with leukaemia in West Cumbria, North Humberside, and Gateshead. Br Med J 302(6778):681-687.

McMahon TF, Birnbaum LS. 1991. Age-related changes in disposition and metabolism of benzene in male C57BL/6N mice. Drug Metab Dispos 19(6):1052-1057.

*McMurry ST, Lochmiller RL, Vestey MR, et al. 1991. Acute effects of benzene and cyclophosphamide exposure on cellular and humoral immunity of cotton rats, *Sigmodon hispidus*. Bull Environ Contam Toxicol 46(6):937-945.

McNeal TP, Nyman PJ, Diachenko GW, et al. 1993. Survey of benzene in foods by using headspace concentration techniques and capillary gas chromatography. J AOAC Int 76:1213–1219.

Medinsky MA. 1995. The application of physiologically based pharmacokinetic-pharmacodynamic (PBPK-PD) modeling to understanding the mechanism of action of hazardous substances. Toxicology Lett 79:185-191.

Medinsky MA, Sabourin PJ, Henderson RF, et al. 1989b. Differences in the pathways for metabolism of benzene in rats and mice simulated by a physiological model. Environ Health Perspect 82:43-49.

Medinsky MA, Sabourin PJ, Lucier G, et al. 1989a. A physiological model for simulation of benzene metabolism by rats and mice. Toxicol Appl Pharmacol 99:193-206.

Medinsky MA, Sabourin PJ, Lucier G, et al. 1989c. A toxicokinetic model for simulation of benzene metabolism. Exp Pathol 37:150-154.

Melikian AA, Prahalad AK, Hoffmann D. 1993. Urinary *trans,trans*-muconic acid as an indicator of exposure to benzene in cigarette smokers. Cancer Epidemiol Biomarkers Prev 2(1):47-51.

Melikian AA, Prahalad AK, Secker-Waker RH. 1994. Comparison of the levels of the urinary benzene metabolite *trans,trans*-muconic acid in smokers and nonsmokers, and the effects of pregnancy. Cancer Epidemiol 3:239-244.

Meyne J, Legator MS. 1980. Sex-related differences in cytogenetic effects of benzene in the bone marrow of Swiss mice. Environ Mutagen 2:43-50.

Michael LC, Erickson MD, Parks SP, et al. 1980. Volatile environmental pollutants in biological matrices with a headspace purge technique. Anal Chem 52:1836-1841.

Michael LC, Pellizzari ED, Wiseman RW. 1988. Development and evaluation of a procedure for determining volatile organics in water. Environ Sci Technol 22:565-570.

Michon S. 1965. [Disturbances of menstruation in women working in an atmosphere polluted with aromatic hydrocarbons.] Pol Tyg Lek 20:1648-1649. (Polish)

Midzenski MA, McDiarmid MA, Rothman N, et al. 1992. Acute high dose exposure to benzene in shipyard workers. Am J Ind Med 22:553-565.

Miller MM, Wasik SP, Huang G-L, et al. 1985. Relationships between octanol-water partition coefficients and aqueous solubility. Environ Sci Technol 19:522-529.

Mohamed MF, Kang D, Aneja VP. 2002. Volatile organic compounds in some urban locations in United States. Chemosphere 47:863-882.

9. REFERENCES

Money CD, Gray CN. 1989. Exhaled breath analysis as a measure of workplace exposure to benzene ppm. Ann Occup Hyg 33:257-262.

Morello-Frosch RA, Woodruff TJ, Axelrad DA, et al. 2000. Air toxics and health risks in California: The public health implications of outdoor concentrations. Risk Anal 20(2):273-291.

Morgan GJ, Alvares CL. 2005. Benzene and the hemopoietic stem cell. Chem Biol Interact 153-154:217-222.

Morikawa M, Imanaka T. 1993. Isolation of a new mixotrophic bacterium which can fix CO_2 and assimilate aliphatic and aromatic hydrocarbons anaerobically. J Ferment Bioengin 76(4):280-283.

Morimoto K. 1976. Analysis of combined effects of benzene with radiation on chromosomes in cultured human leukocytes. Jpn J Ind Health 18:23-34.

Morimoto K. 1983. Induction of sister chromatid exchanges and cell division delays in human lymphocytes by microsomal activation of benzene. Cancer Res 43:1330-1334.

Morselli PL, Franco-Morselli R, Bossi L. 1980. Clinical pharmacokinetics in newborns and infants: Age-related differences and therapeutic implications. Clin Pharmacokin 5:485-527.

*Moszczynski P. 1981. Organic solvents and T lymphocytes. Lancet 1:438.

Mukhametova IM, Vozovaya MA. 1972. [Reproductive power and the incidence of gynecological affections in female workers exposed to the combined effect of benzene and chlorinated hydrocarbons.] Gig Tr Prof Zabol 16:6-9. (Russian)

Mullin AH, Nataraj D, Ren JJ, et al. 1998. Inhaled benzene increases the frequency and length of *lacI* deletion mutations in lung tissue of mice. Carcinogenesis 19(10):1723-1733.

Mullin AH, Rando R, Esmundo F, et al. 1995. Inhalation of benzene leads to an increase in the mutant frequencies of a *lacI* transgene in lung and spleen tissues of mice. Mutat Res 327:121-129.

Murray FJ, John JA, Rampy LW, et al. 1979. Embryotoxicity of inhaled benzene in mice and rabbits. Am Ind Hyg Assoc J 40:993-998.

Mushrush GW, Mose DG, Sullivan KT. 1994. Soil vapor and groundwater analysis from a recent oil spill. Bull Environ Contam Toxicol 52:31-38.

Naft BN. 1992. Removing benzene from wastewater. Environ Protect 3(2):47-54.

Nahum LH, Hoff HE. 1934. The mechanism of sudden death in experimental acute benzol poisoning. J Pharmacol Exp Ther 50:336-345.

Nakai JS, Chu I, Li-Muller A, et al. 1997. Effect of environmental conditions on the penetration of benzene through human skin. J Toxicol Environ Health 51(5):447-462.

Nakajima T, Okino T, Sato A. 1987. Kinetic studies on benzene metabolism in rat liver—possible presence of three forms of benzene metabolizing enzymes in the liver. Biochem Pharmacol 36:2799-2804.

9. REFERENCES

Nakajima T, Okuyama S, Yonekura I, et al. 1985. Effects of ethanol and phenobarbital administration on the metabolism and toxicity of benzene. Chem Biol Interact 55:23-38.

NAS/NRC. 1989. Report of the oversight committee. In: Biologic markers in reproductive toxicology. Washington, DC: National Academy of Sciences, National Research Council, National Academy Press.

NCI. 2003. Cancer progress report. Prevention: Environmental. Benzene in the air. National Cancer Institute. http://progressreport.cancer.gov/doc.asp?pid=1&did=21&chid=9&coid=54&mid=vpco. February 08, 2005.

Nebert DW, Roe AL, Vandale SE, et al. 2002. NAD(P)H:quinone oxidoreductase (NQO1) polymorphism, exposure to benzene, and predisposition to disease: A huGE review. Genet Med 4(2):62-70.

Nedelcheva V, Gut I, Soucek P, et al. 1999. Metabolism of benzene in human liver microsomes: Individual variations in relation to CYP2E1 expression. Arch Toxicol 73(1):33-40.

NEMI. 2005a. Method number 602. Purgeable aromatics via GC with photoionization detector (PID) Washington, DC: U.S. Environmental Protection Agency. National Environmental Methods Index.

NEMI. 2005b. Method number 624. Purgeable organic compounds via GC/MS. Washington, DC: U.S. Environmental Protection Agency. National Environmental Methods Index.

NEMI. 2005c. Method number 1624. Volatile organic compounds by GS/MS. Washington, DC: U.S. Environmental Protection Agency. National Environmental Methods Index.

*Nerland DE, Pierce WM Jr. 1990. Identification of N-acetyl-S-(2,5-dihydroxyphenyl)-L-cysteine as a urinary metabolite of benzene, phenol, and hydroquinone. Drug Metab Dispos 18(6):958-961.

NESCAUM. 1989. Evaluation of the health effects from exposure to gasoline and gasoline vapors. Final Report. Northeast States for Coordinated Air Use Management, Air Toxics Committee. 3-1 - 3-16, 5-1 - 5-33.

Neun DJ, Penn A, Snyder CA. 1992. Evidence for strain-specific differences in benzene toxicity as a function of host target cell susceptibility. Arch Toxicol 66(1):11-17.

Neun DJ, Penn A, Snyder CA. 1994. Erythroid progenitor cells that survive benzene exposure exhibit greater resistance to the toxic benzene metabolites benzoquinone and hydroquinone. Arch Toxicol 68:535-540.

NFPA. 1994. Benzene. Fire protection guide to hazardous materials. 11th edition. Quincy, MA: National Fire Protection Association.

Nilsson RI, Nordlinder RG, Tagesson C, et al. 1996. Genotoxic effects in workers exposed to low levels of benzene from gasoline. Am J Ind Med 30(3):317-324.

*Ning H, Kado NY, Kuzmicky PA, et al. 1991. Benzene-induced micronuclei formation in mouse fetal liver blood, peripheral blood, and maternal bone marrow cells. Environ Mol Mutagen 18(1):1-5.

NIOSH. 1974. Criteria for a recommended standard. Occupational exposure to benzene. Washington, DC: U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health. NIOSH 74-137.

NIOSH. 1984. NIOSH manual of analytical methods, Vol. 2, 3rd ed. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, 1501-1506.

NIOSH. 1990. National occupational exposure survey 1981-1983. U.S. Department of Health and Human Services. Cincinnati, OH: National Institute for Occupational Safety and Health.

NIOSH. 1992. NIOSH recommendations for occupational safety and health: Compendium of policy documents and statements. Table 1. NIOSH recommended safety and health standards for hazardous agents in the workplace. U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health.

NIOSH. 1994. Method 3700. Benzene by portable GC. NIOSH manual of analytical methods. Fourth ed. National Institute for Occupational Safety and Health. http://www.cdc.gov/niosh/nmam/. July 06, 2005.

NIOSH. 2005. Benzene. NIOSH pocket guide to chemical hazards. Atlanta, GA: National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. http://www.cdc.gov/niosh/npg/npgdname.html. February 15, 2004.

*Nojima K, Fukaya K, Fukui S, et al. 1975. Studies on photochemistry of aromatic hydrocarbons: II. The formation of nitrophenols and nitrobenzene by the photochemical reaction of benzene in the presence of nitrogen monoxide. Chemosphere 2:77-82.

Nomiyama K, Nomiyama H. 1974a. Respiratory retention, uptake and excretion of organic solvents in man: Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Int Arch Arbeitsmed 32:75-83.

Nomiyama K, Nomiyama H. 1974b. Respiratory elimination of organic solvents in man: Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Int Arch Arbeitsmed 32:85-91.

Norpoth K, Stuker W, Krewet E, et al. 1988. Biomonitoring of benzene exposure by trace analyses of phenylguanine. Int Arch Occup Environ Health 60:163-168.

NRC. 1993. National Research Council. Pesticides in the diets of infants and children. Washington, DC: National Academy Press.

NTP. 1986. Toxicology and carcinogenesis studies of benzene (CAS No. 71-43-2) in F344/N rats and B6C3F$_1$ mice (gavage studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institute of Health. National Toxicology Program--technical report series no. 289. NIH publication no. 86-2545.

NTP. 1994. Seventh annual report on carcinogens: 1994 summary. National Toxicology Program. Benzene. U.S. Department of Health and Human Services, Public Health Service.

9. REFERENCES

NTP. 2005. Report on carcinogens. 11th ed. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. http://ntp-server.niehs.nih.gov/ntp/roc/toc11.html. February 15, 2004.

Nutmagul W, Cronn DR. 1985. Determination of selected atmospheric aromatic hydrocarbons at remote continental and oceanic locations using photoionization/flame-ionization detection. J Atmos Chem 2:415-433.

NYSDOH. 2005. Study of volatile organic chemicals in air of fuel oil heated homes. New York State Department of Health. http://www.health.state.ny.us/nysdoh/indoor/fuel_oil.html. March 04, 2005.

O'Bryan TR, Ross RH. 1988. Chemical scoring system for hazard and exposure identification. J Toxicol Environ Health 1:119-134.

Oberly TJ, Bewsey BJ, Probst GS. 1984. An evaluation of the L5178Y TK$^{+/-}$ mouse lymphoma forward mutation assay using 42 chemicals. Mutat Res 125:291-306.

Ogata M, Taguchi T. 1987. Quantitation of urinary metabolites of toluene, xylene, styrene, ethylbenzene, benzene and phenol by automated high performance liquid chromatography. Int Arch Occup Environ Health 59:263-272.

Ogata M, Fujisawa K, Ogino Y, et al. 1984. Partition coefficients as a measure of bioconcentration potential of crude oil compounds in fish and shellfish. Bull Environ Contam Toxicol 33:561-567.

Olsen J. 1983. Organic solvents as possible risk factors of low birthweight. J Occup Med 25:854-855.

Omiecinski CJ, Hassett, Hosagrahara V. 2000. Epoxide hydrolase–polymorphism and role in toxicology. Toxicol Lett 112-113:365-370.

Ong CN, Lee BL. 1994. Determination of benzene and its metabolites: Application in biological monitoring of environmental and occupational exposure to benzene. J Chromatogr B Biomed Appl 660(1):1-22.

Ong CN, Kok PW, Lee BL, et al. 1995. Evaluation of biomarkers for occupational exposure to benzene. Occup Environ Med 52(8):528-533.

Orzechowski A, Schwarz LR, Schwegler U, et al. 1995. Benzene metabolism in rodent hepatocytes: Role of sulphate conjugation. Xenobiotica 25(10):1093-1102.

OSHA. 1977. Final environmental impact statement: Benzene. U.S. Department of Labor, Occupational Safety and Health Administration. Fed Regist 42:22516-22529, 27460-27464.

OSHA. 1985. Occupational exposure to benzene. Proposed rule and notice of hearing. U.S. Department of Labor, Occupational Safety and Health Administration. Fed Regist 50:50512-50517, 50540, 50528-50529, 50577-50582.

OSHA. 1987. Occupational exposure to benzene. Final Rule. U.S. Department of Labor, Occupational Safety and Health Administration. Fed Regist 52:34460-34578.

OSHA. 2003. Benzene. U.S. Department of Labor, Occupational Safety and Health Administration. Code of Federal Regulations. 29CFR1910.1028.

OSHA. 2005a. Air contaminants. Occupational safety and health standards for shipyard employment. 1000 Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1915. http://www.osha.gov/comp-links.html. February 15, 2005.

OSHA. 2005b. Air contaminants. Occupational safety and health standards for shipyard employment. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1915.1028. http://www.osha.gov/comp-links.html. February 15, 2005.

OSHA. 2005c. Benzene. Limits for air contaminants. Occupational safety and health standards. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.1028. http://www.osha.gov/comp-links.html. February 15, 2005.

OSHA. 2005d. Gases, vapors, fumes, dusts, and mists. Safety and health regulations for construction. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1926.55, Appendix A. http://www.osha.gov/comp-links.html. February 15, 2005.

OSHA. 2005e. Limits for air contaminants. Occupational safety and health standards. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.1000. http://www.osha.gov/comp-links.html. February 15, 2005.

OSHA. 2005f. Safety and Health Regulations for Construction. Toxic and Hazardous Substances. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1926.1128. http://www.osha.gov/comp-links.html. March 31, 2005.

Ott MG, Townsend JC, Fishbeck WA, et al. 1978. Mortality among workers occupationally exposed to benzene. Arch Environ Health 33:3-10.

Owen GM, Brozek J. 1966. Influence of age, sex and nutrition on body composition during childhood and adolescence. In: Falkner F, ed. Human development. Philadelphia, PA: WB Saunders, 222-238.

Paci E, Buiatti E, Costantini AS, et al. 1989. Aplastic anemia, leukemia and other cancer mortality in a cohort of shoe workers exposed to benzene. Scand J Work Environ Health 15:313-318.

Page BD, Conacher HBS, Salminen J, et al. 1993. Survey of bottled drinking water sold in Canada. II. Selected volatile organic compounds. J AOAC Int 76:26-31.

Pagnotto LD, Elkins HB, Brugsch HG, et al. 1961. Industrial benzene exposure from petroleum naphtha: I. Rubber coating industry. Am Ind Hyg Assoc J 22:417-421.

Painter RB, Howard R. 1982. The HeLa DNA-synthesis inhibition test as a rapid screen for mutagenic carcinogens. Mutat Res 92:427-437.

*Pandya KP, Rao GS, Khan S, et al. 1990. Accumulation of low molecular weight (bleomycin detectable) iron in bone marrow cells of rats after benzene exposure. Arch Toxicol 64(4):339-342.

Parke DV. 1989. Introduction: Session on metabolism. Environ Health Perspect 82:7-8.

*Parke DV, Williams RT. 1953b. Studies in detoxication 54. The metabolism of benzene. (a) The formation of phenylglucuronide and phenylsulphuric acid from [^{14}C] benzene (b) the metabolism of [^{14}C$_1$]phenol. Biochem J 54:337-340.

Parke DV, Williams RT. 1953a. Studies in detoxication 49. The metabolism of benzene containing [^{14}C1] benzene. Biochem J 54:231-238.

Pate CT, Atkinson R, Pitts JN Jr. 1976. The gas phase reaction of O_3 with a series of aromatic hydrocarbons. J Environ Sci Health AII:1-10.

Paterson S, Mackay D. 1989. Correlation of tissue, blood and air partition coefficients of volatile organic chemicals. Brit J Ind Med 46:321-314.

Pathak DN, Levay G, Bodell WJ. 1995. DNA adduct formation in the bone marrow of B6C3F$_1$ mice treated with benzene. Carcinogenesis 16(8):1803-1808.

Paustenbach DJ, Price PS, Ollison W, et al. 1992. Reevaluation of benzene exposure for the pliofilm (rubberworker) cohort (1936-1976). J Toxicol Environ Health 36(3):177-231.

Pawar SS, Mungikar AM. 1975. Changes in the activities of hepatic drug metabolizing enzymes and lipid peroxidation caused by benzene and toluene. Indian J Biochem Biophys 12:133-134.

Paxton MB, Chinchilli VM, Brett SM, et al. 1994a. Leukemia risk associated with benzene exposure in the pliofilm cohort: I. mortality update and exposure distribution. Risk Analysis 14(2):147-154.

Paxton MB, Chinchilli VM, Brett SM, et al. 1994b. Leukemia risk associated with benzene exposure in the pliofilm cohort. II. Risk estimates. Risk Anal 14(2):155-161.

Pekari K, Riekkola M-L, Aitio A. 1989. Simultaneous determination of benzene and toluene in blood using head-space gas chromatography. J Chromatogr 491:309-320.

Pekari K, Vainiotalo S, Heikkila P, et al. 1992. Biological monitoring of occupational exposure to low levels of benzene. Scand J Work Environ Health 18(5):317-322.

Pellack-Walker P, Blumer JL. 1986. DNA damage in L5178YS cells following exposure to benzene metabolites. Mol Pharmacol 30:42-47.

Pellizzari ED, Lioy P, Quackenboss J, et al. 1995. Population-based exposure measurements in EPA region 5: A phase I field study in support of the national human exposure assessment survey. J Expos Anal Environ Epidemiol 15(3):327-358.

Pellizzari ED. 1982. Analysis for organic vapor emissions near industrial and chemical waste disposal sites. Environ Sci Technol 16:781-785.

Pellizzari ED, Zweidinger RA, Sheldon LS. 1988. Method 5-Determination of benzene, toluene and xylene in breath samples by gas chromatography/mass spectrometry. IARC Sci Publ 85:267-279.

Pendleton DR. 1995. Environmental health issues. Environ Health Perspect Suppl 103(supp 6):223-228.

Perbellini L, Faccini GB, Pasini F, et al. 1988. Environmental and occupational exposure to benzene by analysis of breath and blood. Br J Ind Med 45:345-352.

9. REFERENCES

Pereira WE, Hughes BA. 1980. Determination of selected volatile organic priority pollutants in water by computerized gas chromatography-quadrupole mass spectrometry. J Am Water Works Assoc 72:220-230.

Pezzagno G, Maestri L, Fiorentino ML. 1999. *Trans,trans*-muconic acid, a biological indicator to low levels of environmental benzene: Some aspects of its specificity. Am J Ind Med 35(5):511-518.

Philip P, Jensen MK. 1970. Benzene induced chromosome abnormalities in rat bone marrow cells. Acta Pathol Microbiol Scand Sect A 78:489-490.

Picciano D. 1979. Cytogenetic study of workers exposed to benzene. Environ Res 19:33-38.

Pitarque M, Carbonell E, Lape-na N, et al. 1996. No increase in micronuclei frequency in cultured blood lymphocytes from a group of filling station attendents. Mutat Res 367(3):161-167.

Pitarque M, Carbonell E, Lape-na E, et al. 1997. SCE analysis in peripheral blood lymphocytes of a group of filling station attendants. Mutat Res 390(1-2):153-159.

Plappert U, Barthel E, Raddatz K, et al. 1994a. Early effects of benzene exposure in mice. Haematological versus genotoxic effects. Arch Toxicol 68:284-290.

Plappert U, Barthel E, Seidel HJ, et al. 1994b. Reduction of benzene toxicity by toluene. Environ Mol Mutagen 24:283-292.

Plumb RH Jr. 1987. A comparison of groundwater monitoring data from CERCLA and RCRA sites. Ground Water Monit Rev 7:94-100.

Poole SK, Furton KG, Poole CF. 1988. Determination of benzene and toluene in gasoline by gas chromatography using a liquid organic salt column. J Chromatogr Sci 26:67-73.

Popp W, Rauscher D, Muller G, et al. 1994. Concentrations of benzene in blood and S-phenylmercapturic and t,t-muconic acid in urine in car mechanics. Int Arch Occup Environ Health 66(1):1-6.

Popp W, Vahrenholz C, Yaman S, et al. 1992. Investigations of the frequency of DNA strand breakage and cross-linking and of sister chromatid exchange frequency in the lymphocytes of female workers exposed to benzene and toluene. Carcinogenesis 13(1):57-61.

Post GB, Snyder R, Kalf GF. 1985. Inhibition of RNA synthesis and interleukin-2 production in lymphocytes *in vitro* by benzene and its metabolites, hydroquinone and *p*-benzoquinone. Toxicol Lett 29:161-167.

Poulin P, Krishnan K. 1995. A biologically-based algorithm for predicting human tissue:blood partition coefficients of orgasnic chemicals. Hum Exp Toxicol 14:273-280.

Powley MW, Carlson GP. 1999. Species comparison of hepatic and pulmonary metabolism of benzene. Toxicology 139:207-217.

Powley MW, Carlson GP. 2000. Cytochromes P450 involved with benzene metabolism in hepatic and pulmonary microsomes. J Biochem Mol Toxicol 14(6):303-309.

9. REFERENCES

Powley MW, Carlson GP. 2001. Hepatic and pulmonary microsomal benzene metabolism in CYP2E1 knockout mice. Toxicology 169(3):187-194.

Powley MW, Carlson GP. 2002. Benzene metabolism by the isolated perfused lung. Inhal Toxicol 14(6):569-584.

Probst GS, Hill LE. 1985. Tests for the induction of DNA-repair synthesis in primary cultures of adult rat hepatocytes. Prog Mutat Res 5:381-386.

Purcell KJ, Cason GH, Gargas ML, et al. 1990. In vivo metabolic interactions of benzene and toluene. Toxicol Lett 52(2):141-152.

Qu Q, Shore R, Li G, et al. 2002. Hematological changes among Chinese workers with a broad range of benzene exposures. Am J Ind Med 42(4):275-285.

Qu Q, Shore R, Li G, et al. 2003a. Validation and evaluation of biomarkers in workers exposed to benzene in China. Boston, MA: Health Effects Institute, 1-84. Research number 115.

Qu Q, Shore R, Li G, et al. 2003b. Appendix A: Analyses of the combined data for year 1 and year 2. Validation and evaluation of biomarkers in workers exposed to benzene in China. Boston, MA: Health Effects Institute, 1-54. Research number 115. http://pubs.healtheffects.org/getfile.php?u=34. January 18, 2007.

Qu Q, Shore R, Li G, et al. 2005. Biomarkers of benzene: Urinary metabolites in relation to individual genotype and personal exposure. Chem Biol Interact 153-154:85-95.

Raabe GK, Wong O. 1996. Leukemia mortality by cell type in petroleum workers with potential exposure to benzene. Environ Health Perspect 104(Suppl 6):1381-1392.

Rajganesh B, Selvaraj PT, Manning FS, et al. 1995. Biotreatment of produced water for removal of sulfides, organics, and toxicity. Appl Biochem Biotech 51/52:735-746.

Ranaldi R, Bassani B, Villani P, et al. 1998. Measurement and characterization of micronuclei in cultured primary lung cells of mice following inhalation exposure to benzene. Mutagenesis 13(5):453-460.

Rao GS. 1996. Glutathionyl hydroquinone: A potent pro-oxidant and a possible toxic metabolite of benzene. Toxicology 106:49-54.

Rao RR, Snyder R. 1995. Oxidative modifications produced in HL-60 cells on exposure to benzene metabolites. J Appl Toxicol 15(5):403-409.

Rappaport SM, Kupper LL. 2004. Variability of environmental exposures to volatile organic compounds. J Expo Anal Environ Epidemiol 14(1):92-107.

Rappaport SM, McDonald TA, Yeowell-O-Connell K. 1996. The use of protein adducts to investigate the disposition of reactive metabolites of benzene. Environ Health Perspect 104:1235-1237.

Rappaport SM, Waidyanatha S, Qu Q, et al. 2002a. Albumin adducts of benzene oxide and 1,4-benzoquinone as measures of human benzene metabolism. Cancer Res 62(5):1330-1337.

Rappaport SM, Yeowell-O'Connor K, Smith MT, et al. 2002b. Non-linear production of benzene oxide–albumin adducts with human exposure to benzene. J Chromatogr B Analyt Technol Biomed Life Sci 778(1-2):367-374.

Rauscher D, Lehnert G, Angerer J. 1994. Biomonitoring of occupational and environmental exposures to benzene by measuring *trans,trans*-muconic acid in urine. Clin Chem 40:1468–1470.

Reddy MV, Blackburn GR, Irwin SE, et al. 1989. A method for *in vitro* culture of rat Zymbal gland: Use in mechanistic studies of benzene carcinogenesis in combination with ^{32}P-postlabeling. Environ Health Perspect 82:239-247.

Reineke FJ, Bachmann K. 1985. Gas chromatographic determination of C_2-C_8 hydrocarbons and halocarbons in ambient air by simultaneous use of three detectors. J Chromatogr 323:323-329.

Reisch MS. 1994. Top 50 chemicals production rose modestly last year. Chem Eng News (April 11):12–15.

Renz JF, Kalf GF. 1991. Role for interleukin-1 (IL-1) in benzene-induced hematotoxicity: Inhibition of conversion of pre-IL-1 alpha to mature cytokine in murine macrophages by hydroquinone and prevention of benzene-induced hematotoxicity in mice by IL-1 alpha. Blood 78(4):938-944.

Rickert DE, Baker TS, Bus JS, et al. 1979. Benzene disposition in the rat after exposure by inhalation. Toxicol Appl Pharmacol 49:417-423.

Rinsky RA, Hornung RW, Silver SR, et al. 2002. Benzene exposure and hematopoietic mortality: A long-term epidemiologic risk assessment. Am J Ind Med 42(6):474-480.

Rinsky RA, Smith AB, Hornung R, et al. 1987. Benzene and leukemia: An epidemiological risk assessment. N Eng J Med 316:1044-1050.

Rinsky RA, Young RJ, Smith AB. 1981. Leukemia in benzene workers. Am J Ind Med 2:217-245.

Rithidech K, Au WW, Ramanujam VMS, et al. 1987. Induction of chromosome aberrations in lymphocytes of mice after subchronic exposure to benzene. Mutat Res 188:135-140.

Rithidech K, Au WW, Ramanujam VMS, et al. 1988. Persistence of micronuclei in peripheral blood normochromatic erythrocytes of subchronically benzene-treated male mice. Environ Mol Mutagen 12:319-329.

Rivedal E, Witz G. 2005. Benzene metabolites block gap junction intercellular communication. Role in hematotoxicity and leukemia? Chem Biol Interact 153-154:257-260.

Roberts JM, Fehsenfeld FC, Liu SC, et al. 1984. Measurements of aromatic hydrocarbon ratios and NO_x concentrations in the rural troposphere: Observation of air mass photochemical aging and NO_x removal. Atmos Environ 18:2421-2432.

Roberts JM, Hutte RS, Fehsenfeld FC, et al. 1985. Measurements of anthropogenic hydrocarbon concentration ratios in the rural troposphere: Discrimination between background and urban sources. Atmos Environ 19:1945-1950.

9. REFERENCES

Robertson ML, Eastmond DA, Smith MT. 1991. Two benzene metabolites, catechol and hydroquinone, produce a synergistic induction of micronuclei and toxicity in cultured human lymphocytes. Mutat Res 249(1):201-210.

Robinson SN, Shah R, Wong BA, et al. 1997. Immunotoxicological effects of benzene inhalation in male Sprague-Dawley rats. Toxicology 119(3):227-237.

Rosenthal GJ, Snyder CA. 1985. Modulation of the immune response to *Listeria monocytogenes* by benzene inhalation. Toxicol Appl Pharmacol 80:502-510.

Rosenthal GJ, Snyder CA. 1987. Inhaled benzene reduces aspects of cell-mediated tumor surveillance in mice. Toxicol Appl Pharmacol 88:35-43.

Ross D. 1996. Metabolic basis of benzene toxicity. Eur J Haematol Suppl 60:111-118.

Ross D. 2000. The role of metabolism and specific metabolites in benzene-induced toxicity: Evidence and issues. J Toxicol Environ Health A 61(5-6):357-372.

Ross D. 2005. Functions and distribution of NQO1 in human bone marrow: Potential clues to enzene toxicity. Chem Biol Interact 153-154:137-146.

Rothman N, Bechtold WE, Yin SN, et al. 1998. Urinary excretion of phenol, catechol, hydroquinone, and muconic acid by workers occupationally exposed to benzene. Occup Environ Med 55(10):705-711.

Rothman N, Haas R, Hayes RB, et al. 1995. Benzene induces gene-duplicating but not gene-inactivating mutations at the glycophorin A locus in exposed humans. Proc Natl Acad Sci 92:4069-4073.

Rothman N, Li Gl, Dosemeci M, et al. 1996a. Hematotoxicity among Chinese workers heavily exposed to benzene. Am J Med 29(3):236-246.

Rothman N, Smith MT, Hayes RB, et al. 1996b. An epidemiological study of early biologic effects in Chinese workers. Environ Health Perspect 104(Suppl 6):1365-1370.

Rothman N, Smith MT, Hayes RB, et al. 1997. Benzene poisioning, a risk factor for hematological malignancy, is associated with the NQ01 609C-T mutation and rapid fractional excretion of chlozoxazone. Cancer Res 57(14):2839-2842.

Roush GJ, Ott MG. 1977. A study of benzene exposure versus urinary phenol levels. Am Ind Hyg Assoc J 38:67-75.

Rozen MG, Snyder CA. 1985. Protracted exposure of C57BL/6 mice to 300 ppm benzene depresses B- and T-lymphocyte numbers and mitogen responses. Evidence for thymic and bone marrow proliferation in response to the exposures. Toxicology 37:13-26.

Rozen MG, Snyder CA, Albert RE. 1984. Depression in B- and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene. Toxicol Lett 20:343-349.

Ruiz MA, Augusto LGS, Vassallo J, et al. 1994. Bone marrow morphology in patients with neutropenia due to chronic exposure to organic solvents (benzene): Early lesions. Path Res Pract 190:151-154.

*Ruiz MA, Vassallo J, De Souza CA. 1991. Morphologic study of the bone marrow of neutropenic patients exposed to benzene of the metallurgical industry of Cubatao, Sao Paulo, Brazil [letter]. J Occup Med 33(1):83.

Ruppert T, Scherer G, Tricker AR, et al. 1995. Determination of urinary *trans,trans*-muconic acid by gas chromatography-mass spectrometry. J Chromatogr B 666:71-76.

Ruppert T, Scherer G, Tricker AR, et al. 1997. *trans trans*-Muconic acid as a biomarker of non-occupational environmental exposure to benzene. Int Arch Occup Environ Health 69(4):247-251.

Rushmore T, Snyder R, Kalf G. 1984. Covalent binding of benzene and its metabolites to DNA in rabbit bone marrow mitochondria *in vitro*. Chem Biol Interact 49:133-154.

Rushton L, Romaniuk H. 1997. A case-control study to investigate the risk of leukaemia associated with exposure to benzene in petroleum marketing and distribution workers in the United Kingdom. Occup Environ Med 54(3):152-166.

Sabourin PJ, Bechtold WE, Birnbaum LS, et al. 1988. Differences in the metabolism and disposition of inhaled [^{3}H]benzene by F344/N rats and B6C3F$_1$ mice. Toxicol Appl Pharmacol 94:128-140.

Sabourin PJ, Bechtold WE, Griffith WC, et al. 1989a. Effect of exposure concentration, exposure rate, and route of administration on metabolism of benzene by F344 rats and B6C3F$_1$ mice. Toxicol Appl Pharmacol 99:421-444.

Sabourin PJ, Chen BT, Lucier G, et al. 1987. Effect of dose on the absorption and excretion of [^{14}C]benzene administered orally or by inhalation in rats and mice. Toxicol Appl Pharmacol 87:325-336.

Sabourin PJ, Muggenburg BA, Couch RC, et al. 1992. Metabolism of [^{14}C]benzene by cynomolgus monkeys and chimpanzees. Toxicol Appl Pharmacol 114(2):277-284.

Sabourin PJ, Sun JD, Birnbaum LS, et al. 1989b. Effect of repeated benzene inhalation exposures on subsequent metabolism of benzene. Exp Pathol 37:155-157.

Sabourin PJ, Sun JD, MacGregor JT, et al. 1990. Effect of repeated benzene inhalation exposures on benzene metabolism, binding to hemoglobin, and induction of micronuclei. Toxicol Appl Pharmacol 103:452-462.

Saito FU, Kocsis JJ, Snyder R. 1973. Effect of benzene on hepatic drug metabolism and ultrastructure. Toxicol Appl Pharmacol 26:209-217.

Salanitro JP. 1993. The role of bioattenuation in the management of aromatic hydrocarbon plumes in aquifers. Ground Water Monit Remed 13:150-161.

Sammett D, Lee EW, Kocsis JJ, et al. 1979. Partial hepatectomy reduces both the metabolism and toxicity of benzene. J Toxicol Environ Health 5:785-792.

Sandmeyer EE. 1981. Aromatic hydrocarbons. In: Clayton GD, Clayton FE, eds. Patty's industrial hygiene and toxicology. Vol. 2B. 3rd rev. ed. New York, NY: John Wiley & Sons, 3253-3283.

9. REFERENCES

Sanguinetti G, Accorsi A, Barbieri A, et al. 2001. Failure of urinary *trans trans*-muconic acid as a biomarker for indoor environmental benzene exposure at PPB levels. J Toxicol Environ Health A 63(8):599-604.

*Santesson CG. 1897. Chronic poisoning with benzene. Arch Hyg 31:336.

Sardas S, Karakaya AE, Furtun Y. 1994. Sister chromatid exchanges in workers employed in car painting workshops. Int Arch Occup Environ Health 66:33-35.

Sasiadek M. 1992. Nonrandom distribution of breakpoints in the karyotypes of workers occupationally exposed to benzene. Environ Health Perspect 97:255-257.

Sasiadek M, Jagielski J. 1990. Genotoxic effects observed in workers occupationally exposed to organic solvents. Pol J Occup Med 3(1):103-108.

Sasiadek M, Jagielski J, Smolik R. 1989. Localization of breakpoints in the karyotype of workers professionally exposed to benzene. Mutat Res 224:235-240.

Sato A, Nakajima T. 1979. Dose-dependent metabolic integration between benzene and toluene *in vivo* and *in vitro*. Toxicol Appl Pharmacol 48:249-256.

Sato A, Nakajima T, Fujiwara Y, et al. 1975. Kinetic studies on sex differences in susceptibility to chronic benzene intoxication - with special reference to body fat content. Br J Ind Med 32:321-328.

Sauer TC Jr. 1981. Volatile organic compounds in open ocean and coastal surface waters. Org Geochem 3:91-101.

Sawahata T, Neal RA. 1983. Biotransformation of phenol to hydroquinone and catechol by rat liver microsomes. Mol Pharmacol 23:453-460.

Saxton J, Narkus-Kramer M. 1975. EPA findings of solid waste from industrial chemicals. Chem Eng 82:107-112.

Schad H, Schafer F, Weber L, et al. 1992. Determination of benzene metabolites in urine of mice by solid-phase extraction and high-performance liquid chromatography. J Chromatog 593:147-151.

Schafer F, Schad H, Weber L. 1993. Determination of phenylmercapturic acid in urine of benzene-exposed BDF-1 mice. J Chromatog 620:239-242.

Scherer G, Ruppert T, Daube H, et al. 1995. Contribution of tobacco smoke to environmental benzene exposure in Germany. Environ Int 21(6):779-789.

Scheunert I, Topp E, Schmitzer J, et al. 1985. Formation and fate of bound residues on [^{14}C]benzene and [^{14}C]chlorobenzenes in soil and plants. Ecotox Environ Safety 9:159-170.

Schiestl RH, Aubrecht J, Khogali F. 1997. Carcinogens induce reversion of the mouse pink-eyed unstable mutation. Proc Natl Acad Sci USA 94(9):4576-4581.

Schlosser PM, Bond JA, Medinsky MA. 1993. Benzene and phenol metabolism by mouse and rat liver microsomes. Carcinogenesis 14:2477-2486.

Schnatter AR, Armstrong TW, Nicolich MJ, et al. 1996b. Lymphohaematopoietic malignancies and quantitative estimates of exposure to benzene in Canadian petroleum distribution workers. Occup Environ Med 53(11):773-781.

Schnatter AR, Armstrong TW, Thompson LS, et al. 1996c. The relationship between the low-level benzene exposure and leukemia in Canadian petroleum distribution workers. Environ Health Perspect Suppl 104:1375-1379.

Schnatter AR, Katz AM, Nicolich MJ, et al. 1993. A retrospective mortality study among Canadian petroleum marketing and distribution workers. Environ Health Perspect Suppl 101(Suppl 6):85-99.

Schnatter AR, Nicolich MJ, Bird MG. 1996a. Determination of leukemogenic benzene exposure concentrations: Refined analyses of the Pliofilm cohort. Risk Anal 16(6):833-840.

Schnatter AR, Rosamilia K, Wojcik NC. 2005. Review of the literature on benzene exposure and leukemia subtypes. Chem Biol Interact 153-154:9-21.

Schnier GG, Laethem CL, Koop DR. 1989. Identification and induction of cytochromes P450, P450IIE1 and P450IA1 in rabbit bone marrow. J Pharmacol Exp Ther 251:790-796.

Schreiber JS. 1993. Predicted infant exposure to tetrachloroethylene in human breastmilk. Risk Analysis 13:515-424.

Schrenk D, Bock KW. 1990. Metabolism of benzene in rat hepatocytes. Influence of inducers on phenol glucuronidation. Drug Metab Dispos 18(5):720-725.

Schrenk D, Ingelman-Sundberg M, Bock KW. 1992. Influence of P-4502E1 induction on benzene metabolism in rat hepatocytes and on biliary metabolite excretion. Drug Metab Dispos 20(2):137-141.

Schrenk HH, Yant WP, Pearce SJ, et al. 1941. Absorption, distribution and elimination of benzene by body tissues and fluids of dogs exposed to benzene vapor. J Ind Hyg Toxicol 23:20-34.

Schuberth J. 1994. Joint use of retention index and mass spectrum in post-mortem test for volatile organics by headspace capillary gas chromatography with ion-trap detection. J Chromatogr A 674:63-71.

Schultz B, Kjeldsen P. 1986. Screening of organic matter in leachates from sanitary landfills using gas chromatography combined with mass spectrometry. Water Res 20:965-970.

Schwartz CS, Snyder R, Kalf GF. 1985. The inhibition of mitochondrial DNA replication in vitro by the metabolites of benzene, hydroquinone and *p*-benzoquinone. Chem Biol Interact 53(3):327-350.

Seaton MJ, Schlosser PM, Bond JA, et al. 1994. Benzene metabolism by human liver microsomes in relation to cytochrome P450 2E1 activity. Carcinogenesis 15:1799-1806.

Seaton MJ, Schlosser PM, Medinsky MA. 1995. *In vitro* conjugation of benzene metabolites by human liver: Potential influence of interindividual variability on benzene toxicity. Carcinogenesis 16:1519-1527.

Seidel HJ, Bader R, Weber L, et al. 1990. The influence of ethanol on the stem cell toxicity of benzene in mice. Toxicol Appl Pharmacol 105(1):13-18.

Seidel HJ, Beyvers G, Pape M, et al. 1989. The influence of benzene on the erythroid cell system in mice. Exp Hematol 17:760-764.

Seidenberg JM, Anderson DG, Becker RA. 1986. Validation of an in vivo developmental toxicity screen in the mouse. Teratog Carcinog Mutagen 6:361-374.

Seiji K, Jin C, Watanabe T, et al. 1990. Sister chromatid exchanges in peripheral lymphocytes of workers exposed to benzene, trichloroethylene, or tetrachloroethylene, with reference to smoking habits. Int Arch Occup Environ Health 62:171-176.

Seixas GM, Andon BM, Hollingshead PG, et al. 1982. The aza-arenes as mutagens for *Salmonella typhimurium*. Mutat Res 102:201-212.

Selling L. 1916. Benzol as leucotoxin--Studies on the degeneration and regeneration of the blood hematopoietic organs. Johns Hopkins Hosp Rep 17:83-142.

Sellyei M, Kelemen E. 1971. Chromosome study in a case of granulocytic leukemia with "pelgerisation" 7 years after benzene pancytopenia. Eur J Cancer 7:83-85.

*Serraino D, Franceschi S, LeVecchio C, et al. 1992. Occupation and soft-tissue sarcoma in northeastern Italy. Cancer Causes Controls 3:25-30.

Setchell BP, Waites GMH. 1975. The blood-testis barrier. In: Creep RO, Astwood EB, Geiger SR, eds. Handbook of physiology: Endocrinology V. Washington, DC: American Physiological Society.

Shah JJ, Singh HB. 1988. Distribution of volatile organic chemicals in outdoor and indoor air. Environ Sci Technol 22:1381-1388.

Shamy MY, El Gazzar RME, El Sayed MA, et al. 1994. Study of some biochemical changes among workers occupationally exposed to phenol, alone or in combination with other organic solvents. Ind Health 32:207-214.

Shankar U, Kumar A, Rao GS, et al. 1993. Modulation of benzene induced toxicity by protein A. Biochem Pharmacol 46(3):517-524.

Sharma RK, Jacobsen-Kram D, Lemmon M, et al. 1985. Sister-chromatid exchange and cell replication kinetics in fetal and maternal cells after treatment with chemical teratogens. Mutat Res 158:217-231.

Shaw G, Lavey R, Jackson R, et al. 1984. Association of childhood leukemia with maternal age, birth order, and paternal occupation. A case-control study. Am J Epidemiol 119(5):788-795.

Sheets PL, Carlson GP. 2004. Kinetic factors involved in the metabolism of benzene in mouse lung and liver. J Toxicol Environ Health A 67(5):421-430.

Sheets PL, Yost GS, Carlson GP. 2004. Benzene metabolism in human lung cell lines BEAS-2B and A549 and cells overexpressing CYP2F1. J Biochem Mol Toxicol 18(2):92-99.

Shelby MD, Witt KL. 1995. Comparison of results from mouse bone marrow chromosome aberration and micronucleus test. Environ Mol Mutagen 25:302-313.

Shelby MD, Erexson GL, Hook GJ, et al. 1993. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. Environ Mol Mutagen 21(2):160-179.

Shell. 1980. A dominant-lethal inhalation study with benzene in rats. Shell Oil Co. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8E. OTS0539136.

Shell. 1992. Immunosuppression of $B6C3F_1$ female mice following subchronic exposure to benzene from drinking water. Submitted to the U.S Environmental Protection Agency under TSCA Section 8E. OTS0536214.

Sherwood RJ. 1972. Benzene: The interpretation of monitoring results. Ann Occup Hyg 15:409-421.

Sherwood RJ. 1988. Pharmacokinetics of benzene in a human after exposure at about the permissible limit. Ann NY Acad Sci 534:635-647.

Sherwood RJ, Carter FWG. 1970. The measurement of occupational exposure to benzene vapour. Ann Occup Hyg 13:125-146.

Shu XO, Gao YT, Brinton LA, et al. 1988. A population based case-control study of childhood leukemia in Shanghai. Cancer 62:635-644.

Sidhu KS, Chadzynski L. 1994. Risk communication: Health risks associated with environmentally contaminated private wells versus chloroform in a public water supply. J Environ Health 56(10):13-16.

Sinclair GC, Gray CN, Sherwood RJ. 1999. Structure and validation of a pharmacokinetic model for benzene. Am Ind Hyg Assoc J 60(2):249-258.

Singh GB, Salas LJ, Cantrell BK, et al. 1985. Distribution of aromatic hydrocarbons in ambient air. Atmos Environ 19:1911-1919.

Siou G, Conan L, el Haitem M. 1981. Evaluation of the clastogenic action of benzene by oral administration with 2 cytogenetic techniques in mouse and Chinese hamster. Mutat Res 90:273-278.

Skowronski GA, Turkall RM, Abdel-Rahman MS. 1988. Soil adsorption alters bioavailability of benzene in dermally exposed male rats. Am Ind Hyg Assoc J 49:506-511.

Smith MT. 1996a. Overview of benzene-induced aplastic anaemia. Eur J Haematol Suppl 60:107-110.

Smith MT. 1996b. The mechanism of benzene-induced leukemia: A hypothesis and speculations on the causes of leukemia. Environ Health Perspect 104:1219-1225.

Smith MT, Rothman N. 2000. Biomarkers in the molecular epidemiology of benzene-exposed workers. J Toxicol Environ Health A 61(5-6):439-445.

Smith MT, Zhang L. 1998. Biomarkers of leukemia risk: Benzene as a model. Environ Health Perspect Suppl 106:937-946.

Smith MT, Zhang L, Jeng M, et al. 2000. Hydroquinone, a benzene metabolite, increases the level of aneusomy of chromosomes 7 and 8 in human CD34-positive blood progenitor cells. Carcinogenesis 21(8):1485-1490.

Smith MT, Zhang L, Wang Y, et al. 1998. Increased translocations and aneusomy in chromosomes 8 and 21 among workers exposed to benzene. Cancer Res 58(10):2176-2181.

Smyth HF, Carpenter CP, Weil CS, et al. 1962. Range-finding toxicity data: List VI. Am Ind Hyg Assoc J 23:95-107.

Snyder R. 2000a. Overview of the toxicology of benzene. J Toxicol Environ Health A 61(5-6):339-346.

Snyder R. 2000b. Recent developments in the understanding of benzene toxicity and leukemogenesis. Drug Chem Toxicol 23(1):13-25.

Snyder R. 2002. Benzene and leukemia. Crit Rev Toxicol 32(3):155-210.

Snyder R, Hedli CC. 1996. An overview of benzene metabolism. Environ Health Perspect 104(Suppl 6):1165-1171.

Snyder R, Kalf GF. 1994. A perspective on benzene leukemogenesis. Crit Rev Toxicol 24(3):177-209.

Snyder CA, Goldstein BD, Sellakumar A. 1978a. Hematotoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. J Toxicol Environ Health 4:605-618.

Snyder CA, Goldstein BD, Sellakumar A, et al. 1982. Toxicity of chronic benzene inhalation: CD-1 mice exposed to 300 ppm. Bull Environ Contam Toxicol 29:385-391.

Snyder CA, Goldstein BD, Sellakumar AR. 1984. Evidence for hematotoxicity and tumorigenesis in rats exposed to 100 ppm benzene. Am J Ind Med 5:429-434.

Snyder CA, Goldstein BD, Sellakumar AR, et al. 1980. The inhalation toxicology of benzene: Incidence of hematopoietic neoplasms and hematotoxicity in AKR/J and C57BL/6J mice. Toxicol Appl Pharmacol 54:323-331.

Snyder CA, Green JD, LoBue J, et al. 1981. Protracted benzene exposure causes a proliferation of myeloblasts and/or promyelocytes in CD-1 mice. Bull Environ Contam Toxicol 27:17-22.

Snyder CA, Sellakumar AR, James DJ, et al. 1988. The carcinogenicity of discontinuous inhaled benzene exposures in CD-1 and C57BL/6 mice. Arch Toxicol 62:331-335.

Snyder R, Chepiga T, Yang CS, et al. 1993a. Benzene metabolism by reconstituted cytochromes P450, 2B1, and 2E1 and its modulation by cytochrome b5, microsomal epoxide hydrolase, and glutathione transferases: Evidence for an important role of microsomal epoxide hydrolase in the formation of hydroquinone. Toxicol Appl Pharmacol 122(2):172-181.

Snyder R, Dimitriadis E, Guy R, et al. 1989. Studies on the mechanism of benzene toxicity. Environ Health Perspect 82:31-35.

Snyder R, Jowa L, Witz G, et al. 1987. Formation of reactive metabolites from benzene. Arch Toxicol 60:61-64.

Snyder R, Lee EW, Kocsis JJ. 1978b. Binding of labeled metabolites to mouse liver and bone marrow. Res Commun Chem Pathol Pharmacol 20:191-194.

Snyder R, Witz G, Goldstein BD. 1993b. The toxicology of benzene. Environ Health Perspect 100:293-306.

*Sole F, Caballin MR, Coll MD, et al. 1990. Acute lymphoblastic leukemia with t(4;11) in a patient previously exposed to a carcinogen. Cancer Genet Cytogenet 49(1):133-136.

Songnian Y, Quilan L, Yuxiang L. 1982. Significance of leukocyte alkaline phosphates in the diagnosis of chronic benzene poisioning. Regul Toxicol Pharmacol 2:209-212.

Sonoda T, Nagata Y, Mori M, et al. 2001. Meta-analysis of multiple myeloma and benzene exposure. J Epidemiol 11(6):249-254.

Sporstol S, Urdal K, Drangsholt H, et al. 1985. Description of a method for automated determination of organic pollutants in water. Int J Environ Anal Chem 21:129-138.

Srbova J, Teisinger J, Skramovsky S. 1950. Absorption and elimination of inhaled benzene in man. Arch Ind Hyg Occup Med 2:1-8.

SRI. 1995. 1995 Directory of chemical producers. United States of America. Menlo Park, CA: Stanford Research Institute International, 466.

SRI. 2004. Benzene. 2004 Directory of chemical producers: United States. Menlo Park, CA: SRI Consulting, 460-461.

St-Germain F, Mamer O, Brunet J, et al. 1995. Volatile organic compound analysis by an inertial spray extraction interface coupled to an ion trap mass spectrometer. Anal Chem 67:4536-4541.

Staples CA, Werner AF, Hoogheem TJ. 1985. Assessment of priority pollutant concentrations in the United States using STORET database. Environ Toxicol Chem 4:131-142.

*Steineck G, Plato N, Gerhardsson M, et al. 1990. Increased risk of urothelial cancer in Stockholm during 1985-87 after exposure to benzene and exhausts. Int J Cancer 45:1012-1017.

Stetter JR, Jurs PC, Rose SL. 1986. Detection of hazardous gases and vapors: Pattern recognition analysis of data from an electrochemical sensor array. Anal Chem 58:860-866.

Stillman WS, Varella-Garcia M, Gruntmeir JJ, et al. 1997. The benzene metabolite, hydroquinone, induces dose-dependent hypoploidy in a human cell line. Leukemia 11(9):1540-1545.

Stoner RD, Drew RT, Bernstein DM. 1981. Benzene-inhalation effects upon tetanus antitoxin responses and leukemogenesis in mice. In: Mahlum DD, Gray RH, Felix WD, eds. Coal conversion and the environment. Oak Ridge, TN: U.S. Department of Energy, Technical Information Center, 445-461.

Strange RC, Fryer AA. 1999. The glutathione S-transferases: Influence of polymorphism on cancer susceptibility. IARC Sci Pub 148:231-249.

Stucker I, Mandereau L, Aubert-Berleur MP, et al. 1994. Occupational paternal exposure to benzene and risk of spontaneous abortion. Occup Environ Med 51:475-478.

Stutz DR, Janusz SJ. 1988. Hazardous materials injuries: A handbook for pre-hospital care. Beltsville, MD: Bradford Communications Corporation, 298-299.

Styles JA, Richardson CR. 1984. Cytogenetic effects of benzene: Dosimetric studies on rats exposed to benzene vapour. Mutat Res 135:203-209.

Subrahmanyam VV, Doane-Setzer P, Steinmetz KL, et al. 1990. Phenol-induced stimulation of hydroquinone bioactivation in mouse bone marrow in vivo: Possible implications in benzene myelotoxicity. Toxicology 62(1):107-116.

Subrahmanyam VV, Sadler A, Suba E, et al. 1989. Stimulation of *in vitro* bioactivation of hydroquinone by phenol in bone marrow cells. Drug Metab Dispos 17:348-350.

Sul D, Lee D, Im H, et al. 2002. Single strand DNA breaks in T- and B-lymphocytes and granulocytes in workers exposed to benzene. Toxicol Lett 134:87-95.

*Sun. 1971. Initial Submission. Subacute inhalation study of benzene on monkeys (final report) with attachment and cover letter dated 022892. Sun Company, Inc. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8E. OTS0539115.

Sun JD, Medinsky MA, Birnbaum LS, et al. 1990. Benzene hemoglobin adducts in mice and rats: Characterization of formation and physiological modeling. Fundam Appl Toxicol 15:468-475.

Surrallés J, Autio K, Nylund L, et al. 1997. Molecular cytogenetic analysis of buccal cells and lymphocytes from benzene-exposed workers. Carcinogenesis 18(4):817-823.

Susten A, Dames B, Burg J, et al. 1985. Percutaneous penetration of benzene in hairless mice: An estimate of dermal absorption during tire-building operations. Am J Ind Med 7:323-335.

Suzuki S, Atai H, Hatakeyama Y, et al. 1989. Administration-route-related differences in the micronucleus test with benzene. Mutat Res 223:407-410.

Swenberg JA, Petzold GL, Harbach PR. 1976. *In vitro* DNA damage/alkaline elution assay for predicting carcinogenic potential. Biochem Biophys Res Commun 72:732-738.

Sze CC, Shi CY, Ong CN. 1996. Cytotoxicity and DNA strand breaks induced by benzene and its metabolites in Chinese hamster ovary cells. J Appl Toxicol 16(3):259-264.

Tabak HH, Quave SA, Mashni CI, et al. 1981. Biodegradability studies with organic priority pollutant compounds. J Water Pollut Control Fed 53:1503-1518.

Taningher M, Perrotta A, Malacarne D, et al. 1995. Lack of significant promoting activity by benzene in the rat liver model of carcinogenesis. J Toxicol Environ Health 45:481-488.

Tannoka H. 1977. Development and application of *Bacillus subtilis* test systems for mutagens, involving DNA-repair deficiency and suppressible auxotrophic mutations. Mutat Res 42:19-32.

Tatrai E, Rodics K, Ungvary G. 1980a. Embryotoxic effects of simultaneously applied exposure of benzene and toluene. Folia Morphol (Praha) 28:286-289.

Tatrai E, Ungvary GY, Hudak A, et al. 1980b. Concentration dependence of the embryotoxic effects of benzene inhalation in CFY rats. J Hyg Epidemiol Microbiol Immunol 24:363-371.

9. REFERENCES

Tauber J. 1970. Instant benzol death. J Occup Med 12:91-92.

Taylor L, Morris SS. 1995. Final report on the operations and findings of the Anchorage volatile organic compounds (VOC) monitoring project. Proceedings of the 88th Annual Meeting and Exhibition, San Antonio, TX, June 1995. Pittsburgh, PA: Air and Waste Management Association, 1-15.

Teisinger J, Fišerová-Bergerová B. 1955. Valeus camparée de la détermination des sulfates et du phénol contenus dans l'urine pour l'évaluation de la concentration du benzène dans l'air. Arch Mal Prof 16:221-232.

Thienes H, Haley TJ. 1972. Clinical toxicology. 5th ed. Philadelphia, PA: Lea & Febiger, 124-127.

Thomas K, Colborn T. 1992. Organochlorine endocrine disruptors in human tissue. In: Colborn T, Clement C, eds. Chemically induced alterations in sexual and functional development: The wildlife/human connection. Princeton, NJ: Princeton Scientific Publishing, 365-394.

Thomas KW, Pellizzari ED, Clayton CA, et al. 1993. Temporal variability of benzene exposures for residents in several New Jersey homes with attached garages or tobacco smoke. J Expo Anal Environ Epidemiol 3(1):49-73.

Thomas KW, Pellizzari ED, Cooper SD. 1991. A canister-based method for collection and GC/MS analysis of volatile organic compounds in human breath. J Anal Toxicol 15:54–59.

Tice RR, Costa DL, Drew RT. 1980. Cytogenetic effects of inhaled benzene in murine bone marrow: Induction of sister chromatid exchanges, chromosomal aberrations, and cellular proliferation inhibition in DBA/2 mice. Proc Natl Acad Sci USA 77:2148-2152.

Tice RR, Vogt TF, Costa DL. 1982. Cytogenetic effects of inhaled benzene in murine bone marrow. Environ Sci Res 25:257-275.

Toft K, Olofsson T, Tunek A, et al. 1982. Toxic effects on mouse bone marrow caused by inhalation of benzene. Arch Toxicol 51:295-302.

Tompa A, Major J, Jakab MG. 1994. Monitoring of benzene-exposed workers for genotoxic effects of benzene: Improved-working-condition-related decrease in the frequencies of chromosomal aberrations in peripheral blood lymphocytes. Mutat Res 304(2):159-165.

Tondel M, Persson B, Carstensen J. 1995. Myelofibrosis and benzene exposure. Occup Med 45(1):51-52.

Topham JC. 1980. Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? Mutat Res 74:379-387.

Topp E, Scheunert I, Korte F. 1989. Kinetics of the uptake of ^{14}C-labeled chlorinated benzene from soil by plants. Ecotox Environ Safety 17:157-166.

Tough IM, Court Brown WM. 1965. Chromosome aberrations and exposure to ambient benzene. Lancet 1:684.

Tough IM, Smith PG, Brown WMC. 1970. Chromosome studies on workers exposed to atmospheric benzene. Eur J Cancer 6:49-55.

9. REFERENCES

Townsend JC, Ott MG, Fishbeck WA. 1978. Health exam findings among individuals occupationally exposed to benzene. J Occup Med 20:543-548.

Travis CC, Bowers JC. 1989. Protein binding of benzene under ambient exposure conditions. Toxicol Ind Health 5(6):1017-1024.

Travis CC, Quillen JL, Arms AD. 1990. Pharmacokinetics of benzene. Toxicol Appl Pharmacol 102:400-420.

Travis LB, Li CY, Zhang ZN, et al. 1994. Hematopoietic malignancies and related disorders among benzene-exposed workers in China. Leuk Lymphoma 14:91-102.

TRI90. 1992. Toxics Chemical Release Inventory. National Library of Medicine, National Toxicology Information Program, Bethesda, MD.

TRI92. 1994. Toxics Chemical Release Inventory. National Library of Medicine, National Toxicology Information Program, Bethesda, MD.

TRI02. 2005. TRI explorer: Providing access to EPA's toxics release inventory data. Washington, DC: Office of Information Analysis and Access. Office of Environmental Information. U.S. Environmental Protection Agency. Toxics Release Inventory. http://www.epa.gov/triexplorer/. April 11, 2005.

TRI04. 2006. TRI explorer: Providing access to EPA's toxics release inventory data. Washington, DC: Office of Information Analysis and Access. Office of Environmental Information. U.S. Environmental Protection Agency. Toxics Release Inventory. http://www.epa.gov/triexplorer/. January 29, 2007.

Tsai SP, Fox EE, Ransdell JD, et al. 2004. A hematology surveillance study of petrochemical workers exposed to benzene. Regul Toxicol Pharmacol 40:67-73.

Tsai SP, Wen CP, Weiss NS, et al. 1983. Retrospective mortality and medical surveillance studies of workers in benzene areas of refineries. J Occup Med 25:685-692.

Tsuruta H. 1989. Skin absorption of organic solvent vapors in nude mice *in vivo*. Ind Health 27:37-47.

Tsutsui T, Hayashi N, Maizumi H, et al. 1997. Benzene-, catechol-, hydroquinone- and phenol-induced cell transformation, gene mutations, chromosome aberrations, aneuploidy, sister chromatid exchanges and unscheduled DNA synthesis in Syrian hamster embryo cells. Mutat Res 373(1):113-123.

Tucker WA, Huang C, Bral JM. 1986. Validation of transport model. Benzene in Florida groundwater: An assessment of the significance to human health. Washington, DC: American Petroleum Council, Flordia Petroleum Council, 93-108.

Tuo J, Loft S, Thomsen MS, et al. 1996. Benzene-induced genotoxicity in mice in vivo detection by the alkaline comet assay: Reduction by CYP2E21 inhibition. Mutat Res 368(3-4):213-219.

*Turk R, Fuchs R, Momcilovic B. 1991. Granulocyte reserve in chronic experimental benzene poisoning in rats. Arh Hig Rada Toksikol 42(1):37-42.

Turkall RM, Skowronski G, Gerges S, et al. 1988. Soil absorption alters kinetics and bioavailability of benzene in orally exposed male rats. Arch Environ Contam Toxicol 17:159-164.

*Türkel B, Egeli U. 1994. Analysis of chromosomal aberrations in shoe workers exposed long term to benzene. Occup Environ Med 51:50-53.

Turtletaub KW, Mani C. 2003. Benzene metabolism in rodents at doses relevant to human exposure from urban air. Boston, MA: Health Effects Institute. Research Report 113.

U.S. Congress. 1986. Superfund Amendments and Reauthorization Act of 1986. 99th Congress, 2nd Session, October, 17, 1986.

Uchrin CG, Mangels G. 1987. Sorption equilibria of benzene and toluene on two New Jersey coastal plain ground water aquifer solids. J Environ Sci Health [A] 22:743-758.

Ugrekhelidze D, Korte F, Kvesitadze G. 1997. Uptake and transformation of benzene and toluene by plant leaves. Ecotoxicol Environ Saf 37:24-29.

Ungvary G, Tatrai E. 1985. On the embryotoxic effects of benzene and its alkyl derivatives in mice, rats and rabbits. Arch Toxicol Suppl 8:425-430.

USGS. 2005. Benzene. Group: VOC. Schedule: Volatile organic compounds. U.S. Geological Survey. http://infotrek.er.usgs.gov/docs/nawqa_www/nwq_paramx.htm. April 13, 2005.

USITC. 2005. Benzene. First unit of quantity by country name and first unit of quanity. United States International Trade Commission. http://dataweb.usitc.gov/scripts/REPORT.asp. March 03, 2005.

Utterback DF, Rinsky RA. 1995. Benzene exposure assessment in rubber hydrochloride workers: A critical evaluation of previous estimates. Am J Ind Med 27(5):661-676.

Vacha J, Znojil V, Seidel HJ, et al. 1990. Ferrokinetics and erythropoiesis in mice after long-term inhalation of benzene. Blut 60(1):41-47.

Vaishnav DD, Babeu L. 1987. Comparison of occurrence and rates of chemical biodegradation in natural waters. Bull Environ Contam Toxicol 39:237-244.

Valentine JL, Lee SS, Seaton MJ, et al. 1996a. Reduction of benzene metabolism and toxicity in mice that lack CYP2E1 expression. Toxicol Appl Pharmacol 141(1):205-213.

Valentine JL, Seaton MJ, Asgharian B. 1996b. Benzene metabolism and toxicity in transgenic CYP2E1 knockout mice. Toxicologist 30(1):73.

Van den Berghe H, Louwagie A, Broeckaert-Van Orshoven A, et al. 1979. Chromosome analysis in two unusual malignant blood disorders presumably induced by benzene. Blood 53:558-566.

Van Emon JM, Gerlach CL. 1995. A status report on field-portable immunoassay. Environ Sci Tech 29(7):312-317.

van Sittert NJ, Boogaard PJ, Beulink GD. 1993. Application of the urinary S-phenylmercapturic acid test as a biomarker for low levels of exposure to benzene in industry. Br J Ind Med 50(5):460-469.

van Wijnen JH, Verhoeff AP, Jans HWA, et al. 1995. The exposure of cyclists, car drivers and pedestrians to traffic-related air pollutants. Int Arch Occup Environ Health 67(3):187-193.

van Wijngaarden E, Stewart PA. 2003. Critical literature review of determinants and levels of occupational benzene exposure for United States community-based case-control studies. Appl Occup Environ Hyg 18(9):678-693.

Vara P, Kinnunen O. 1946. Benzene toxicity as a gynecologic problem. Acta Obstet Gynecol Scand 26:433-452.

Vieira I, Sonnier M, Cresteil T. 1996. Developmental expression of CYP2E1 in the human liver: Hypermethylation control of gene expression during the neonatal period. Eur J Biochem 238:476-483.

Vigliani EC, Forni A. 1976. Benzene and leukemia. Environ Res 11:122-127.

*Vigliani EC, Saita G. 1964. Benzene and leukemia. N Engl J Med 271(1):872-876.

Vogel E, Günther H. 1967. Benzene oxide-oxepin valence tautomerism. Angew Chem Int Ed Engl 6(5):385-476.

Vogel TM, Grbić-Galić D. 1986. Incorporation of oxygen from water into toluene and benzene during anaerobic fermentative transformation. Appl Environ Microbiol 52:200-202.

Von Oettingen WF. 1940. Toxicity and potential dangers of aliphatic and aromatic hydrocarbons: A critical review of the literature. Washington, DC: U.S. Public Health Service, Division of Industrial Hygiene, National Institute of Health, Public Health Bulletin No. 255, 66-97, 125-135.

Waidyanatha S, Rothman N, Fustinoni S, et al. 2001. Urinary benzene as a biomarker of exposure among occupationally exposed and unexposed subjects. Carcinogenesis 22(2):279-286.

Wallace LA. 1989a. Major sources of benzene exposure. Environ Health Perspect 82:165-169.

Wallace LA. 1989b. The exposure of the general population to benzene. Cell Biol Toxicol 5:297-314.

Wallace LA. 1995. Human exposure to environmental pollutants: A decade of experience. Clin Exp Allergy 25:4-9.

Wallace LA, Pellizzari ED. 1986. Personal air exposure and breath concentrations of benzene and other volatile hydrocarbons for smokers and nonsmokers. Toxicol Lett 35:113-116.

Wallace LA, Nelson WC, Ziegenfus R, et al. 1991. The Los Angeles team study: Personal exposures, indoor-outdoor air concentrations, and breath concentrations of 25 volatile organic compounds. J Exp Anal Environ Epidemiol 1(2):157-192.

Wallace LA, Pellizzari ED, Hartwell TD, et al. 1986. Total exposure assessment methodology (TEAM) study: Personal exposures, indoor-outdoor relationships, and breath levels of volatile organic compounds in New Jersey. Environ Int 12:369-387.

Wallace LA, Pellizzari ED, Hartwell TD, et al. 1985. Personal exposures, indoor-outdoor relationships, and breath levels of toxic air pollutants measured for 355 persons in New Jersey. Atmos Environ 19:1651-1661.

9. REFERENCES

Wallace LA, Pellizzari ED, Hartwell TD, et al. 1987. Exposures to benzene and other volatile compounds from active and passive smoking. Arch Environ Health 42:272-279.

Ward CO, Kuna RA, Snyder NK, et al. 1985. Subchronic inhalation toxicity of benzene in rats and mice. Am J Ind Med 7:457-473.

Ward E, Hornung R, Morris J, et al. 1996. Risk of low red or white blood cell count related to estimated benzene exposure in a rubberworker cohort (1940-1975). Am J Ind Med 29(3):247-257.

Ward JB Jr, Ammenheuser MM, Ramanujam VMS, et al. 1992. The mutagenic effects of low level sub-acute inhalation exposure to benzene in CD-1 mice. Mutat Res 268(1):49-57.

Weaver VM, Buckley T, Groopman JD. 2000. Lack of specificity of *trans,trans*-muconic acid as a benzene biomarker after ingestion of sorbic acid-preserved foods. Cancer Epidemiol Biomarkers Prev 9(7):749-755.

Wells MS, Nerland DE. 1991. Hematotoxicity and concentration-dependent conjugation of phenol in mice following inhalation exposure to benzene. Toxicol Lett 56(1-2):159-166.

West JR, Smith HW, Chasis H. 1948. Glomerular filtration rate, effective renal blood flow, and maximal tubular excretory capacity in infancy. J Pediatr 32:10-18.

Wester RC, Maibach HI, Gruenke LD, et al. 1986. Benzene levels in ambient air and breath of smokers and nonsmokers in urban and pristine environments. J Toxicol Environ Health 18:567-573.

White MC, Johnson CA, Ashley, et al. 1993. Exposure to methyl tertiary-butyl ether from oxygenated gasoline in Stamford, Connecticut. Arch Environ Health 50(3):183-189.

WHO. 2000. Air quality guidelines. 2nd ed. Geneva, Switzerland: World Health Organization. http://www.euro.who.int/air/Activities/20050104_1. February 15, 2005.

WHO. 2004. Guidelines for drinking-water quality. 3rd ed. Geneva, Switzerland: World Health Organization. http://www.who.int/water_sanitation_health/dwq/gdwq3/en/. February 15, 2005.

Widdowson EM, Dickerson JWT. 1964. Chemical composition of the body. In: Comar CL, Bronner F, eds. Mineral metabolism: An advanced treatise. Volume II: The elements Part A. New York: Academic Press, 1-247.

Wiester MJ, Winsett DW, Richards JH, et al. 2002. Partitioning of benzene in blood: Influence of hemoglobin type in humans and animals. Environ Health Perspect 110(3):255-261.

Williams JA. 2001. Single-nucleotide polymorphisms, metabolic activation and environmental carcinogenesis: Why molecular epidemiologists should think about enzyme expression. Carcinogenesis 22:209-214.

Williams GM, Tong C, Ved Brat S. 1985. Tests with the rat hepatocyte primary culture/DNA-repair test. Prog Mutat Res 5:341-345.

*Wilson RH. 1942. Benzene poisoning in industry. J Lab Clin Med 27:1517-1521.

9. REFERENCES

Wilson BH, Smith GB, Rees JF. 1986. Biotransformation of selected alkylbenzenes and halogenated hydrocarbons in methanogenic aquifer material: A microcosm study. Environ Sci Technol 20:997-1002.

Winek CL, Collom WD. 1971. Benzene and toluene fatalities. J Occup Med 13:259-261.

Winek CL, Collom WD, Wecht CH. 1967. Fatal benzene exposure by glue sniffing. Lancet (March 25):683.

Winn LM. 2003. Homologous recombination initiated by benzene metabolites: A potential role of oxidative stress. Toxicol Sci 72:143-149.

Witz G, Gad SC, Tice RR, et al. 1990a. Genetic toxicity of the benzene metabolite *trans,trans*-muconaldehyde in mammalian and bacterial cells. Mutat Res 240:295-306.

Witz G, Kirley TA, Maniara WM, et al. 1990b. The metabolism of benzene to muconic acid, a potential biological marker of benzene exposure. Biol React Intermed IV 283:613-618.

Witz G, Maniara W, Mylavarapu V, et al. 1990c. Comparative metabolism of benzene and *trans,trans*-muconaldehyde to *trans,trans*-muconic acid in DBA/2N and C57BL/6 mice. Biochem Pharmacol 40:1275-1280.

Witz G, Rao GS, Goldstein BD. 1985. Short-term toxicity of *trans,trans*-mucondialdehyde. Toxicol Appl Pharmacol 80:511-516.

Witz G, Zhang Z, Goldstein BD. 1996. Reactive ring-opened aldehyde metabolites in benzene toxicity. Environ Health Perspect 104(Suppl 6):1195-1199.

Wolf MA, Rowe VK, McCollister DD, et al. 1956. Toxicological studies of certain alkylated benzene and benzene. AMA Arch Ind Health 14:387-398.

*Wolff SP. 1992. Correlation between car ownership and leukaemia: Is non-occupational exposure to benzene from petrol and motor vehicle exhaust a causative factor in leukaemia and lymphoma? Experientia 48(3):301-304.

*Wong O. 1987. An industry wide mortality study of chemical workers occupationally exposed to benzene: II. Dose response analyses. Br J Ind Med 44:382-395.

Wong O. 1995. Risk of acute myeloid leukaemia and multiple myeloma in workers exposed to benzene. Occup Environ Med 52:380-384.

Wong O. 1999. A critique of the exposure assessment in the epidemiologic study of benzene-exposed workers in China conducted by the Chinese Academy of Preventive Medicine and the US National Cancer Institute. Regul Toxicol Pharmacol 30(3):259-267.

Wong O, Raabe GK. 1997. Multiple myeloma and benzene exposure in a multinational cohort of more than 250,000 petroleum workers. Regul Toxicol Pharmacol 26:188-199.

Wong O, Raabe GK. 2000. Non-Hodgkin's lymphoma and exposure to benzene in a multinational cohort of more then 308,000 petroleum workers, 1937-1996. J Occup Environ Med 42(5):554-568.

*Wong O, Morgan RW, Whorton MD. 1983. Comments on the NIOSH study of leukemia in benzene workers. Technical report submitted to Gulf Canada Ltd., by Environmental Health Associates, August 31.

Wood JA, Porter ML. 1987. Hazardous pollutants in Class II landfills. J Air Pollut Control Assoc 37:609-615.

Xia Z-L, Xi-Peng J, Pei-Lian L, et al. 1995. Ascertainment corrected prevalence rate (ACPR) of leukopenia in workers exposed to benzene in small-scale industries calculated wit capture-recapture methods. Biomed Environ Sci 8:30-34.

*Xing SG, Shi X, Wu ZL, et al. 1992. Transplacental genotoxicity of triethylenemelamine, benzene, and vinblastine in mice. Teratog Carcinog Mutagen 12(5):223-230.

Yardley-Jones A, Anderson D, Jenkinson PC, et al. 1988. Genotoxic effects in peripheral blood and urine of workers exposed to low level benzene. Br J Ind Med 45:694-700.

Yardley-Jones A, Anderson D, Lovell DP, et al. 1990. Analysis of chromosomal aberrations in workers exposed to low level benzene. Br J Ind Med 47(1):48-51.

Yeowell-O'Connell K, Rothman N, Smith MT, et al. 1998. Hemoglobin and albumin adducts of benzene oxide among workers exposed to high levels of benzene. Carcinogenesis 19(9):1565-1571.

Yeowell-O'Connor K, Rothman N, Waidyanatha S, et al. 2001. Protein adducts of 1,4-benzoquinone and benzene oxide among smokers and nonsmokers exposed to benzene in China. Cancer Epidemiol Biomarkers Prev 10(8):831-838.

Yin SN, Hayes RB, Linet MS, et al. 1996a. A cohort study of cancer among benzene-exposed workers in China: Overall results. Am J Med 29(3):227-235.

Yin S, Li G, Hu Y, et al. 1987b. Symptoms and signs of workers exposed to benzene, toluene or the combination. Ind Health 25:113-130.

Yin SN, Hayes RB, Linet MS, et al. 1996b. An expanded cohort study of cancer among benzene-exposed workers in China. Environ Health Perspect 104(Suppl 6):1339-1341.

Yin SN, Li GL, Tain FD, et al. 1987a. Leukemia in benzene workers: A retrospective cohort study. Br J Ind Med 44:124-128.

Yin SN, Li GL, Tain FD, et al. 1989. A retrospective cohort study of leukemia and other cancers in benzene workers. Environ Health Perspect 82:207-213.

Yin SN, Li Q, Liu Y, et al. 1987c. Occupational exposure to benzene in China. Br J Ind Med 44:192-195.

Yin SN, Linet MS, Hayes RB, et al. 1994. Cohort study among workers exposed to benzene in China: I. General methods and resources. Am J Ind Med 26(3):383-400.

Young RJ, Rinsky RA, Infante PF, et al. 1978. Benzene in consumer products. Science 199:248.

9. REFERENCES

Yu R, Weisel CP. 1996. Measurement of benzene in human breath associated with an environmental exposure. J Expo Anal Environ Epidemiol 6(3):261-277.

Zarani F, Papazafiri P, Kappas A. 1999. Induction of micronuclei in human lymphocytes by organic solvents in vitro. J Environ Pathol Toxicol Oncol 18(1):21-28.

Zhang L, Robertson ML, Kolachana P, et al. 1993. Benzene metabolite, 1,2,4-benzenetriol, induces micronuclei and oxidative DNA damage in human lymphocytes and HL60 cells. Environ Mol Mutagen 21:339-348.

Zhang L, Rothman N, Wang Y, et al. 1998a. Increased aneusomy and long arm delation of chromosomes 5 and 7 in the lymphocytes of Chinese workers exposed to benzene. Carcinogenesis 19(11):1955-1961.

Zhang L, Rothman N, Wang Y, et al. 1999. Benzene increases aneuploidy in the lymphocytes of exposed workers: A comparison of data obtained by fluorescence in situ hybridization in interphase and metaphase cells. Environ Mol Mutagen 34(4):260-268.

Zhang L, Wang Y, Shang N, et al. 1998b. Benzene metabolites induce the loss and long arm deletion of chromosomes 5 and 7 in human lymphocytes. Leuk Res 22(2):105-113.

Zhang Z, Cooper K, Goldstein BD, et al. 1997. Distribution studies in CD-1 mice administered [^{14}C]muconaldehyde. Arch Toxicol 71:703-708.

Zhang Z, Goldstein BD, Witz G. 1995a. Iron-stimulated ring-opening of benzene in a mouse liver microsomal system. Mechanistic studies and formation of a new metabolite. Biochem Pharmacol 50(10):1607-1617.

Zhang Z, Xiang Q, Glatt H, et al. 1995b. Studies of pathways of ring opening of benzene in a Fenton system. Free Radic Biol Med 18(3):411-419.

Zhu H, Li Y, Trush MA. 1995. Differences in xenobiotic detoxifying activities between bone marrow stromal cells from mice and rats: Implications for benzene-induced hematotoxicity. J Toxicol Environ Health 46(2):183-201.

Ziegler EE, Edwards BB, Jensen RL, et al. 1978. Absorption and retention of lead by infants. Pediatr Res 12:29-34.

9. REFERENCES

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10. GLOSSARY

Absorption—The taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD)—Usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a BMD_{10} would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

Benchmark Dose Model—A statistical dose-response model applied to either experimental toxicological or epidemiological data to calculate a BMD.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility.

Cancer Effect Level (CEL)—The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-controlled study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without outcome.

Case Report—Describes a single individual with a particular disease or exposure. These may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Describes the experience of a small number of individuals with the same disease or exposure. These may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure—Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome. At least one exposed group is compared to one unexposed group.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at one point in time.

Data Needs—Substance-specific informational needs that if met would reduce the uncertainties of human health assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the adverse effects.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurs. The terms, as used here, include malformations and variations, altered growth, and *in utero* death.

Environmental Protection Agency (EPA) Health Advisory—An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Epidemiology—Refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one half of a quantity of a chemical from the body or environmental media.

Immediately Dangerous to Life or Health (IDLH)—The maximum environmental concentration of a contaminant from which one could escape within 30 minutes without any escape-impairing symptoms or irreversible health effects.

Immunologic Toxicity—The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

Immunological Effects—Functional changes in the immune response.

Incidence—The ratio of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

In Vitro—Isolated from the living organism and artificially maintained, as in a test tube.

In Vivo—Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC$_{LO}$)—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC$_{50}$)—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD$_{Lo}$)—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD$_{50}$)—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT$_{50}$)—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

Mortality—Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a chemical.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An OR of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Organophosphate or Organophosphorus Compound—A phosphorus-containing organic compound and especially a pesticide that acts by inhibiting cholinesterase.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) allowable exposure level in workplace air averaged over an 8-hour shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests.

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic end points. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar

ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

q_1*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually µg/L for water, mg/kg/day for food, and µg/m^3 for air).

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the no-observed-adverse-effect level (NOAEL, from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a chemical.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—The American Conference of Governmental Industrial Hygienists (ACGIH) maximum concentration to which workers can be exposed for up to 15 minutes continually. No more than four excursions are allowed per day, and there must be at least 60 minutes between exposure periods. The daily Threshold Limit Value-Time Weighted Average (TLV-TWA) may not be exceeded.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

Time-Weighted Average (TWA)—An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose$_{(50)}$ (TD$_{50}$)—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Toxicokinetic—The absorption, distribution, and elimination of toxic compounds in the living organism.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis, 3 being the approximate logarithmic average of 10 and 1.

Xenobiotic—Any chemical that is foreign to the biological system.

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C.
9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–
499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with
the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most
commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological
profiles for each substance included on the priority list of hazardous substances; and assure the initiation
of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological
information and epidemiologic evaluations of a hazardous substance. During the development of
toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to
identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a
given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance
that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration
of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of
cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are
used by ATSDR health assessors to identify contaminants and potential health effects that may be of
concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or
action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor
approach. They are below levels that might cause adverse health effects in the people most sensitive to
such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and
chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently,
MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method
suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end
point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the
liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level
above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to
look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that

are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Environmental Medicine, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology and Environmental Medicine, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-32, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Benzene
CAS Numbers: 71-43-2
Date: August 2007
Profile Status: Post Public, Final Draft
Route: [x] Inhalation   [ ] Oral
Duration: [x] Acute   [ ] Intermediate   [ ] Chronic
Graph Key: 46
Species: Mouse

Minimal Risk Level: 0.009   [ ] mg/kg/day   [x] ppm

Reference: Rozen MG, Snyder CA, Albert RE. 1984. Depression in B- and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene. Toxicol Lett 20:343-349.

Experimental design: Male C57BL/6J mice (7–8/group) were exposed to benzene (0, 10.2, 31, 100, or 301 ppm) in whole-body dynamic inhalation chambers for 6 hours/day for 6 consecutive days. Control mice were exposed to filtered, conditioned air only. Erythrocyte counts were depressed in C57BL/6 mice only at 100 and 301 ppm. The 10.2 ppm exposure level resulted in significant depression of femoral lipopolysaccharide-induced B-colony-forming ability in the absence of a significant depression of total numbers of B cells. At 31 ppm, splenic phytohemagglutinin-induced blastogenesis was significantly depressed without a concomitant significant depression in numbers of T-lymphocytes. Peripheral lymphocyte counts were depressed at all exposure levels. These results demonstrate that short-term inhaled benzene even at low exposure concentrations can alter certain immune associated processes.

Effect noted in study and corresponding doses:

10.2 ppm =   No adverse effect on erythrocytes, depressed peripheral lymphocytes and mitogen-induced blastogenesis of femoral B-lymphocytes (less serious LOAEL).

31 ppm =   No adverse effect on erythrocytes, depression of mitogen-induced blastogenesis of splenic T-cells.

100 ppm =   Depressed erythrocyte counts.

Dose and end point used for MRL derivation:

[ ] NOAEL   [x] LOAEL

Uncertainty Factors used in MRL derivation: 300

[ ] 1 [ ] 3 [x] 10 (for use of a LOAEL)
[ ] 1 [x] 3 [ ] 10 (for extrapolation from animals to humans using dosimetric conversion)
[ ] 1 [ ] 3 [x] 10 (for human variability)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

<u>If an inhalation study in animals, list conversion factors used in determining human equivalent dose</u>: The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10.2 ppm) by 6/24 to correct for less than a full day of exposure. The resulting $LOAEL_{ADJ}$ is 2.55 ppm.

According to current EPA (1994b) methodology for calculating a human equivalent concentration (HEC) for extrarespiratory effects of a category 3 gas (such as benzene):

$$LOAEL_{HEC} = LOAEL_{ADJ} \times ([H_{b/g}]_A/[H_{b/g}]_H)$$

where:

$LOAEL_{HEC}$ = The LOAEL dosimetrically adjusted to a human equivalent concentration

$LOAEL_{ADJ}$ = The LOAEL adjusted from intermittent to continuous exposure

$[H_{b/g}]_A/[H_{b/g}]_H$ = The ratio of the blood:gas partition coefficient of the chemical for the laboratory animal species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a default value of 1 is used for the ratio. According to Wiester et al. (2002), benzene blood:gas partition coefficients for mice and humans are 17.44 and 8.12, respectively. Therefore the default value of 1 is applied, in which case, the $LOAEL_{HEC}$ is equivalent to the $LOAEL_{ADJ}$ = 2.55 ppm.

<u>Was a conversion used from intermittent to continuous exposure</u>? The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10.2 ppm) by 6/24 to correct for less than a full day of exposure. The resulting $LOAEL_{ADJ}$ is 2.55 ppm.

<u>Other additional studies or pertinent information that lend support to this MRL</u>: Increased number of micronucleated polychromatic erythrocytes (MN-PCEs), decreased numbers of granulopoietic stem cells (Toft et al. 1982), lymphopenia (Cronkite et al. 1985), lymphocyte depression, and increased susceptibility to bacterial infection (Rosenthal and Snyder 1985) are among the adverse hematological and immunological effects observed in several other acute-duration inhalation studies. The study by Rozen et al. (1984) shows benzene immunotoxicity (reduced mitogen-induced lymphocyte proliferation) at a slightly lower exposure level than these other studies. C57BI/6J mice were exposed to 0, 10.2, 31, 100, and 301 ppm benzene for 6 days at 6 hours/day. Lymphocyte counts were depressed at all exposure levels while erythrocyte counts were elevated at 10.2 ppm, equal to controls at 31 ppm, and depressed at 100 and 301 ppm. Femoral B-lymphocyte and splenic B-lymphocyte numbers were reduced at 100 ppm. Levels of circulating lymphocytes and mitogen-induced blastogenesis of femoral B-lymphocytes were depressed after exposure to 10.2 ppm benzene for 6 days. Mitogen-induced blastogeneses of splenic T-lymphocytes were depressed after exposure to 31 ppm of benzene for 6 days. In another study, mice exhibited a 50% decrease in the population of erythroid progenitor cells (CFU-E) after exposure to 10 ppm benzene for 5 days, 6 hours/day (Dempster and Snyder 1991). In a study by Wells and Nerland (1991), groups of 4–5 male Swiss-Webster mice were exposed to 0, 3, 25, 55, 105, 199, 303, 527, 1,150, or 2,290 ppm benzene for 6 hours/day for 5 days. The number of leukocytes in peripheral blood and spleen weights were significantly decreased compared with untreated controls at all concentrations ≥25 ppm. Therefore, 3 ppm was the NOAEL and 25 ppm was the LOAEL for these effects. Other end points were not monitored in this study. These data support the choice of Rozen et al. (1984) as a critical study.

<u>Agency Contacts (Chemical Managers)</u>: Sharon Wilbur, M.A., Sam Keith, M.S., C.H.P., Obaid Faroon, Ph.D.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Benzene
CAS Numbers: 71-43-2
Date: August 2007
Profile Status: Post Public, Final Draft
Route: [x] Inhalation   [ ] Oral
Duration: [ ] Acute   [x] Intermediate   [ ] Chronic
Graph Key: 126
Species: Mouse

Minimal Risk Level: 0.006   [ ] mg/kg/day   [x] ppm

Reference: Rosenthal GJ, Snyder CA. 1987. Inhaled benzene reduces aspects of cell-mediated tumor surveillance in mice. Toxicol Appl Pharmacol 88:35-43.

Experimental design: Male C57Bl/6 mice were exposed to 10, 30, or 100 ppm of benzene by inhalation 6 hours/day, 5 days/week for 20 exposure days. The number of lymphocytes and their functional capacities were evaluated in spleens of exposed mice. Following the 20 days of exposure, functional capacity of splenic lymphocytes was evaluated in two *in vitro* assays: mixed-lymphocyte culture (MLC) and 51Cr-release cytotoxicity assay. Measured mean daily benzene concentrations in the 10, 30, and 100 ppm groups were 11.1 (±1.5) ppm, 29.5 (±4.4) ppm, and 99.7 (±7.0) ppm, respectively. No changes were observed in the relative proportions of splenic leukocytes, in the percentage of T-cell subsets or in the ratio of T-helper and T-suppressor cells, even at the highest exposure level (100 ppm). Therefore, the functional assays could be normalized for particular lymphocyte populations by using equal numbers of splenic cells. MLC is an *in vitro* measure of alloreactivity (capacity to mount an immune response against foreign antigens). The MLC activity of spleen lymphocytes from 10- and 100-ppm mice was delayed on days 2–4 of culture (relative to air-exposed controls), indicating that benzene exposure causes impaired in vitro alloreactivity (data for the 30-ppm mice were not included in the reported results). This delayed alloreactivity was not due to spleen suppressor cells. The lymphocyte cytotoxic function evaluated in the 51Cr-release assay was also altered; splenic lymphocytes from 100-ppm mice had a significantly reduced lysing capacity. The results indicate that inhalation exposure of mice to benzene has an immunodepressive effect on *in vitro* alloreactivity and cytotoxicity of splenic lymphocytes.

Effect noted in study and corresponding doses:

10 ppm =   Significantly delayed splenic lymphocyte reaction to foreign antigens evaluated in *in vitro* mixed lymphocyte reaction (less serious LOAEL).

30 ppm =   Results not reported.

100 ppm =   Significantly delayed splenic lymphocyte reaction to foreign antigens evaluated in *in vitro* mixed lymphocyte reaction.

Dose and end point used for MRL derivation:

[ ] NOAEL   [x] LOAEL

APPENDIX A

Uncertainty Factors used in MRL derivation: 300

[ ] 1 [ ] 3 [x] 10 (for use of a LOAEL)
[ ] 1 [x] 3 [ ] 10 (for extrapolation from animals to humans using dosimetric conversion)
[ ] 1 [ ] 3 [x] 10 (for human variability)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose:
According to current EPA (1994b) methodology for calculating a human equivalent concentration (HEC)
for extrarespiratory effects of a category 3 gas (such as benzene):

$$LOAEL_{HEC} = LOAEL_{ADJ} \times ([H_{b/g}]_A / [H_{b/g}]_H)$$

where:

$LOAEL_{HEC}$ = The LOAEL dosimetrically adjusted to a human equivalent concentration

$LOAEL_{ADJ}$ = The LOAEL adjusted from intermittent to continuous exposure

$[H_{b/g}]_A / [H_{b/g}]_H$ = The ratio of the blood:gas partition coefficient of the chemical for the laboratory
                    animal species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a
default value of 1 is used for the ratio. According to Wiester et al. (2002), benzene blood:gas partition
coefficients for mice and humans are 17.44 and 8.12, respectively. Therefore, the default value of 1 is
applied, in which case, the $LOAEL_{HEC}$ is equivalent to the $LOAEL_{ADJ}$.

Was a conversion used from intermittent to continuous exposure? The concentration was adjusted for
intermittent exposure by multiplying the LOAEL (10 ppm) by 6 hours/24 hours to correct for less than a
full day of exposure and 5 days/7 days to correct for less than a full week of exposure. The resulting
$LOAEL_{ADJ}$ is 1.8 ppm.

Other additional studies or pertinent information that lend support to this MRL: Exposure of C57BL
mice to 10 ppm benzene for 6 hours/day, 5 days/week caused significant depressions in numbers of
lymphocytes (ca. 30% lower than controls) as early as exposure day 32; this effect was also noted at the
other scheduled periods of testing (exposure days 66 and 178) (Baarson et al. 1984). Splenic red blood
cells were significantly reduced (ca. 15% lower than controls) at exposure days 66 and 178. The failure
of the erythrons of benzene-exposed mice to support normal red cell mass was illustrated by the
significant reduction in peripheral red cell numbers in these animals at 66 and 178 days of benzene
exposure. Green et al. (1981a, 1981b) exposed male CD-1 mice to benzene vapors at concentrations of
0 or 9.6 ppm for 6 hours/day, 5 days/week for 50 days and assessed the effects of exposure on cellularity
in the spleen, bone marrow, and peripheral blood. Exposure-related effects included a 90% increase in
numbers of multipotential hematopoietic stem cells (CFU-S) (Green et al. 1981a), approximately 25%
increase in spleen weight and total splenic nucleated cellularity (Green et al. 1981b), and 80% increase in
nucleated RBCs (Green et al. 1981b). The results of Baarson et al. (1984) and Green et al. (1981a,
1981b) are limited for purposes of quantitative risk assessment because a single exposure level was
employed. However, they support the choice of Rosenthal and Snyder (1987) as the critical study, which
serves as the basis for the intermediate-duration inhalation MRL.

APPENDIX A

Agency Contacts (Chemical Managers): Sharon Wilbur, M.A., Sam Keith, M.S., C.H.P., Obaid Faroon, Ph.D.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Benzene
CAS Numbers: 71-43-2
Date: August 2007
Profile Status: Post Public, Final Draft
Route: [x] Inhalation   [ ] Oral
Duration: [ ] Acute   [ ] Intermediate   [x] Chronic
Graph Key: 161
Species: Human

<u>Minimal Risk Level</u>: 0.003   [ ] mg/kg/day   [x] ppm   [ ] mg/m^3

<u>Reference</u>: Lan Q, Zhang L, Li G, et al. 2004a. Hematotoxicity in workers exposed to low levels of benzene. Science 306:1774-1776.

<u>Experimental design</u>: A cross-sectional study was performed on 250 workers (approximately two-thirds female) exposed to benzene at two shoe manufacturing facilities in Tianjin, China, and 140 age- and gender-matched workers in clothing manufacturing facilities that did not use benzene. The benzene-exposed workers had been employed for an average of 6.1±2.9 years. Benzene exposure was monitored by individual organic vapor monitors (full shift) 5 or more times during 16 months prior to phlebotomy. Post-shift urine samples were collected from every worker. Urinary benzene concentrations were highly correlated with mean individual air levels. Benzene was not found (detection limit 0.04 ppm) in workplace and home air samples of control workers taken at three different time periods. Study subjects were categorized into four groups (140 controls, 109 at <1 ppm, 110 at 1–<10 ppm, and 31 at ≥10 ppm) according to mean benzene exposure levels measured twice during the month prior to phlebotomy. Of the 250 exposed workers, 109 were exposed to <1 ppm benzene. Each of these individuals worked at the larger of the two facilities included in the study. Exposure concentrations were generally higher at the smaller facility due to a less adequate ventilation system. Complete blood count (CBC) and differential were analyzed mechanically. Coefficients of variation for all cell counts were <10%.

Mean 1-month benzene exposure levels in the four groups (controls, <1 ppm, 1–<10 ppm, and ≥10 ppm) were <0.04, 0.57±0.24, 2.85±2.11, and 28.73±20.74 ppm, respectively (see Table A-1). An evaluation of potential confounding factors showed that age, gender, cigarette smoking, alcohol consumption, recent infection, and body mass index were associated with at least one hematological end point. The values in Table A-1 represent values that were adjusted to account for these variables. All types of white blood cells (WBCs) and platelets were significantly decreased in the lowest exposure group (<1 ppm), ranging in magnitude from approximately 8 to 15% lower than controls. Although similar statistical analyses for the mid- and high-exposure groups were not included in the study report, decreases in all types of WBCs and platelets were noted at these exposure levels as well; the decreases in the highest exposure group ranged in magnitude from 15 to 36%. Lymphocyte subset analysis revealed significantly decreased CD4+-T cells, CD4+/CD8+ ratio, and B cells. Hemoglobin concentrations were significantly decreased only within the highest (≥10 ppm) exposure group. Tests for a linear trend using benzene air level as a continuous variable were significant for platelets and all WBC measures except monocytes and CD8+-T cells. Upon restricting the linear trend analyses to workers exposed to <10 ppm benzene, excluding controls, inverse associations remained for total WBCs, granulocytes, lymphocytes, B cells, and platelets. In order to evaluate the effect of past benzene exposures on the hematological effects observed in this study, the authors compared findings for a group of workers who had been exposed to <1 ppm benzene over the previous year (n=60) and a subset who also had <40 ppm-years lifetime cumulative benzene exposure (n=50). The authors stated that the same cell types were significantly reduced in these groups, but did not provide further information of the magnitude (i.e., percent change) of

the hematological effects observed. These data suggest that the 1-month benzene exposure results could be used as an indicator of longer term low-level benzene hematotoxicity. To demonstrate that the observed effects were attributable to benzene, significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells were noted in a subgroup (n=30; mean 1-month exposure level of 0.29±0.15 ppm) of the <1 ppm group for which exposure to other solvents was negligible.

Table A-1. Significantly Reduced Blood Values in Workers Exposed to Benzene in Tianjin, China (Adapted from Lan et al. 2004a)

End point	Mean exposure level in ppm[a] (number of subjects)			
	<0.04 (140)	0.57±0.24 (109)	2.85±2.11 (110)	28.73±20.74 (31)
WBCs[b]	6,480±1,710	5,540±1,220[d]	5,660±1,500	4,770±892
Granulocytes[b]	4,110±1,410	3,360±948[d]	3,480±1,170	2,790±750
Monocytes[b]	241±92	217±97[d]	224±93	179±74
Lymphocytes[b]	2,130±577	1,960±541[d]	1,960±533	1,800±392
CD4+-T cells[b]	742±262	635±187[d]	623±177	576±188
CD4+/CD8+ ratio	1.46±0.58	1.26±0.41[d]	1.22±0.45	1.09±0.35
B cells[b]	218±94	186±95[d]	170±75	140±101
Platelets[c]	230±59.7	214±48.8[d]	200±53.4	172±44.8

[a]Arithmetic mean of an average of two measurements per subject collected during the month prior to phlebotomy
[b]Mean cell numbers per microliter blood±standard deviation
[c]Mean number of platelets (x10^3)
[d]Statistically significantly lower than controls (p<0.05) by linear regression on ln of each end point

Effect noted in study and corresponding doses: As shown in Table A-1, exposure-response relationships were noted for several blood factors. Benzene-induced decreased B cell count was selected as the critical effect for benchmark dose (BMD) modeling because it represented the highest magnitude of effect (i.e., B cell count in the highest exposure group was approximately 36% lower than that of controls). A BMD modeling approach was selected to identify the point of departure because the critical study (Lan et al. 2004a) identified a LOAEL in the absence of a NOAEL.

Dose and end point used for MRL derivation: 0.10 ppm (BMCL$_{0.25sd}$) for decreased B cell count.

All continuous variable models in the EPA Benchmark Dose Software (Version 1.3.2) were fit to the B cell count data shown in Table A-1. Visual inspection of the plots of observed versus expected values for B cell counts indicated that the Hill model provided the only adequate fit of the data set (see Figure A-1). A benchmark response (BMR) of 0.25 sd below the control mean B cell count was selected because it resulted in a BMC$_{0.25sd}$ of 0.42 ppm and its lower 95% confidence limit (BMCL$_{0.25sd}$) of 0.10 ppm (Figure A-1), which are below the mean exposure level of the lowest exposure group (0.57 ppm) for which a statistically significant decrease in mean B cell count (186 versus 218 in controls, see Table A-1) was observed. Although Lan et al. (2004a, 2004b) noted significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells in a subgroup (n=30; mean 1-month exposure level of 0.29±0.15 ppm) of the 0.57 ppm exposure group, this subgroup could not be included in the BMD analysis because the study authors did not include the means and standard deviations for the decreased blood factors, nor did they provide quantitative information regarding the remaining 70 subjects in the 0.57 ppm exposure group (n=109). Assuming that the 0.29 ppm exposure level may represent a minimally adverse exposure level, it seems reasonable to accept the BMCL$_{0.25sd}$ of 0.10 ppm as the point of departure for deriving a chronic-duration inhalation MRL for benzene.

APPENDIX A

Figure A-1. Observed and Predicted B Cell Counts in Human Subjects Occupationally Exposed to Benzene. BMD=BMC$_{0.25sd}$=0.42 ppm; BMDL=BMCL$_{0.25sd}$=0.10 ppm

Hill Model with 0.95 Confidence Level

08:53 04/11 2005
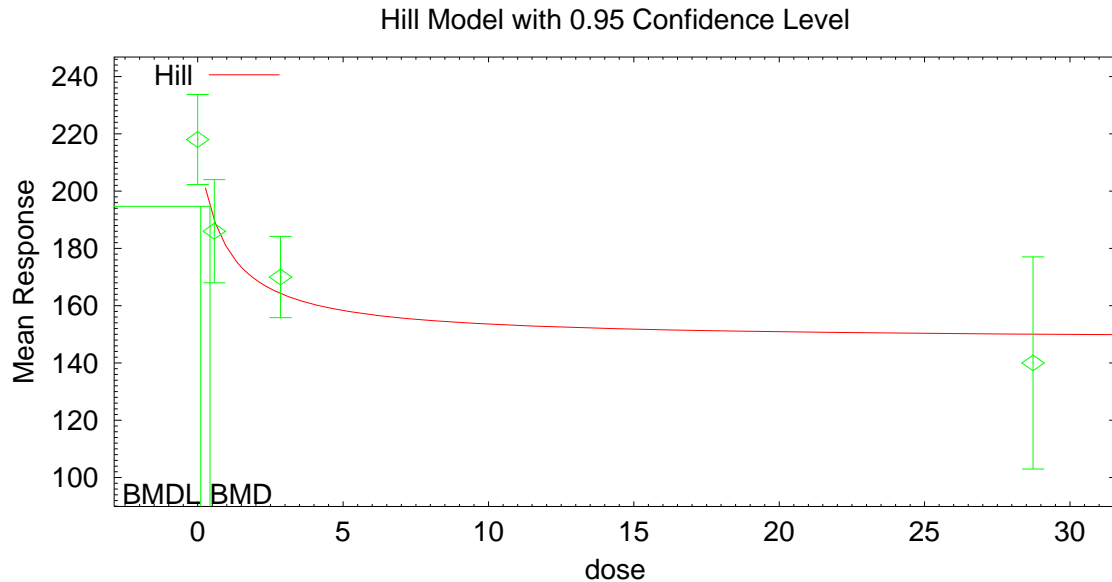
The computer output for fitting of the Hill model to B cell counts in human subjects occupationally exposed to benzene (Lan et al. 2004a) follows.

```
====================================================================
        Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
        Input Data File: C:\ATSDR\BENZENE\BMD FILES\BENZENELANBCELLS.(d)
        Gnuplot Plotting File:  C:\ATSDR\BENZENE\BMD
FILES\BENZENELANBCELLS.plt
                                        Mon Nov 20 09:27:13 2006
 ====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)

   Dependent variable = MEAN
   Independent variable = ppm
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

APPENDIX A


```
              Default Initial Parameter Values
                    alpha =        8224.64
                      rho =              0    Specified
                intercept =            218
                        v =            -78
                        n =        0.572459
                        k =         1.5675
```

Asymptotic Correlation Matrix of Parameter Estimates

```
        ( *** The model parameter(s)  -n  have been estimated at a
boundary point, or have been specified by the user, and do not appear in the
correlation matrix )
```

	alpha	rho	intercept	v	k
alpha	1	0	0	0	0
rho	0	1	0	0	0
intercept	0	0	1	0	0
v	0	0	0	1	0
k	0	0	0	0	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	8027.09	1
rho	0	1
intercept	217.113	1
v	-69.0144	1
n	1	NA
k	0.878186	1

```
NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.
```

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	140	218	94	217	89.6	0.0099
0.57	109	186	95	190	89.6	-0.0441
2.85	110	170	75	164	89.6	0.063
28.73	31	140	101	150	89.6	-0.113

Model Descriptions for likelihoods calculated

Model A1:   Yij = Mu(i) + e(ij)

APPENDIX A

```
          Var{e(ij)} = Sigma^2

 Model A2:          Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model  R:          Yi = Mu + e(i)
          Var{e(i)} = Sigma^2


Degrees of freedom for Test A1 vs fitted <= 0

                    Likelihoods of Interest

          Model      Log(likelihood)    DF        AIC
           A1        -1947.632025        5     3905.264050
           A2        -1943.411648        8     3902.823297
         fitted      -1948.162584        4     3904.325168
           R         -1962.157799        2     3928.315597

 Test 1:  Does response and/or variances differ among dose levels
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous (A1 vs A2)
 Test 3:  Does the Model for the Mean Fit (A1 vs. fitted)

                    Tests of Interest

   Test     -2*log(Likelihood Ratio)  Test df      p-value

   Test 1             37.4923            6          <.0001
   Test 2             8.44075            3          0.03773
   Test 3             1.06112            0            NA
```

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is less than .05. Consider running a
non-homogeneous variance model

NA - Degrees of freedom for Test 3 are less than or equal to 0. The Chi-
Square test for fit is not valid

```
Benchmark Dose Computation
Specified effect =          0.25

Risk Type         =      Estimated standard deviations from the control mean

Confidence level =          0.95

          BMC =        0.42196

          BMCL =       0.104163
```

Although Test 3 (mean fit) produced an invalid Chi-Square test (degrees of freedom ≤0), visual inspection of the observed vs expected B cell counts from the Hill model output (Figure A-1) resulted in the determination that the predicted B cell counts adequately reflect the observed values and that the

APPENDIX A

associated $BMCL_{0.25sd}$ of 0.104163 provides an appropriate point of departure for deriving a chronic-duration inhalation MRL for benzene.

[ ] NOAEL   [ ] LOAEL

Uncertainty Factors used in MRL derivation: 10

        [ ] 1 [ ] 3 [ ] 10 (for use of a LOAEL)
        [ ] 1 [ ] 3 [ ] 10 (for extrapolation from animals to humans)
        [ ] 1 [ ] 3 [x] 10 (for human variability)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? The $BMCL_{0.25sd}$ of 0.10 ppm was adjusted from the 8-hour TWA to a continuous exposure concentration ($BMCL_{0.25sdADJ}$) as follows:

$$BMCL_{0.25sdADJ} = BMCL_{0.25sd} \text{ x (8 hours/24 hours) x (6 days/7 days)}$$

Therefore:

$$BMCL_{0.25sdADJ} = 0.10 \text{ ppm x (8 hours/24 hours) x (6 days/7 days)}$$

$$BMCL_{0.25sdADJ} = 0.03 \text{ ppm}$$

Other additional studies or pertinent information that lend support to this MRL: Lan et al. (2004a, 2004b) was selected as the critical study for derivation of a chronic-duration inhalation MRL because it (1) was well designed, (2) provided adequate exposure-response information, (3) employed individual exposure monitoring data collected for up to 16 months prior to blood testing, (4) demonstrated effects that did not appear to be significantly influenced by previous high-level exposures, and (5) included larger numbers of subjects than previous studies (Qu et al. 2002, 2003; Rothman et al. 1996a, 1996b; Ward et al. 1996). In addition, Lan et al. (2004a, 2004b) measured lymphocyte subsets and colony formation from hematopoietic progenitor cells as measures of toxicity.

Previously conducted epidemiology studies provide support to the findings of Lan et al. (2004a). Qu et al. (2002, 2003) compared hematologic values among 105 healthy workers (51 men, 54 women) in industries with a history of benzene usage (Tianjin, China) and 26 age- and gender-matched workers in industries that did not use benzene. Benzene-exposed workers were chosen based on at least 3 years of exposure history. The mean duration of occupational exposure to benzene was 9.7 years (SD=6.2 years). At the time of the study, benzene exposure was monitored by individual organic vapor monitors at 1-week intervals for 4 weeks prior to collection of blood samples for analysis. Measured benzene levels were averaged for each individual to produce a 4-week mean exposure level. Exposure-response relationships were assessed according to ranges of benzene levels (unexposed, >0–5, >5–15, >15–30, and >30 ppm). Benzene hematotoxicity was assessed by mechanical counts of total WBCs, red blood cells (RBCs), and platelets. The WBC differential was hand-counted on a total of 900 cells. Calculations of the numbers of various WBC types were based on total WBCs and differential counts. The mean 4-week benzene level in the control group was 0.004±0.003 ppm. Among all the benzene-exposed workers, the mean 4-week benzene exposure level was 5.2±7.3 ppm. Within the >0–5, >5–15, >15–30, and >30 ppm exposure categories, mean 4-week benzene levels were 2.26±1.35, 8.67±2.44, 19.9±3.1, and

51.8±43.3 ppm, respectively. A significant exposure-related reduction in the numbers of neutrophils (ranging in magnitude from 12% in the 2.26 ppm exposure group to 31% in the 51.8 ppm exposure group) was observed in all four groups of benzene-exposed workers, relative to controls. Significantly reduced numbers of RBCs (approximately 11–16% lower than controls) were also noted in all benzene-exposed groups. Significantly reduced total WBCs were seen in the highest (>30 ppm) exposure group. The study authors identified a subgroup (within the >0–5 ppm exposure group) of 16 women with no measured exposure levels exceeding 0.5 ppm (4-week mean benzene exposure level of 0.14±0.04 ppm) and reported significantly reduced total WBCs, neutrophils, and RBCs in this subgroup as well. However, these results are based on a small number of workers within the larger group and the reduced counts of total WBCs, neutrophils, and RBCs within this subgroup are –much greater in magnitude than those reported for the main (>0–5 ppm) exposure group, rendering the results in this subgroup of questionable value for purposes of risk assessment. Qu et al. (2002, 2003) clearly identified a LOAEL of 2.26 ppm for significantly reduced total WBCs, neutrophils, and RBCs, and provided indication of benzene-induced changes in some hematological values at exposure levels lower than the current industry 8-hour TWA of 1 ppm.

Rothman et al. (1996a, 1996b) performed a cross-sectional study in 1992 on 44 healthy workers (23 males, 21 females) in Chinese (Shanghai) industries with a history of benzene usage and 44 age- and gender-matched workers in industries that did not use benzene. The mean duration of occupational exposure to benzene was 6.3 years (SD=4.4 years). At the time of the study, benzene exposure was monitored by individual organic passive dosimetry badges on 5 separate days during 1 to 2 weeks prior to the collection of blood and urine samples for analysis. Benzene hematotoxicity was assessed by mechanical counts of total WBCs, absolute lymphocytes (ALC), RBCs, and platelets, as well as hemoglobin value and mean corpuscular volume (MCV). The WBC differential was also hand-counted on 100 cells. Abnormal counts were reviewed by hand. Mean (geometric mean of the five exposure samples) 8-hour TWAs for the benzene-exposed workers ranged from 1 to 238 ppm (median 8-hour TWA of 31 ppm). Benzene-exposed workers exhibited statistically significantly reduced numbers of total WBCs, ALC, RBCs, and platelets (approximately 12, 21, 6, and 23% lower, respectively) and significantly increased MCV (approximately 3% higher), relative to unexposed workers. The results were comparable in both men and women. Among the benzene-exposed workers whose mean exposure levels were >31 ppm (median 8-hour TWA of 91.9 ppm; n=22), all measured blood parameters were significantly different from controls; only ALC, RBCs, and platelets were significantly lower in benzene workers with mean exposures of <31 ppm (median 8-hour TWA of 13.6; n=22), compared with controls. In a subgroup of benzene-exposed workers whose measured benzene exposure levels did not exceed 31 ppm on any of the five sampling days (median 8–hour TWA of 7.6 ppm; n=11), significantly reduced ALC (approximately 16% lower than controls) was noted.

In a nested case-control study of a cohort of workers in the Pliofilm production departments of a rubber products manufacturer in Ohio (Ward et al. 1996), incident cases were defined as the first occurrence of a low WBC or RBC count, and matched controls were chosen from those tested within approximately 6 months of the case's blood test date. Hematologic screening data were available for 657 of 1,037 individuals employed at the plant from 1939 through 1976. A total of 21,710 blood test records were identified; the number of blood tests per individual ranged from 1 to 354, but the majority of subjects had five or fewer blood tests. All blood tests were taken from 1940 through 1975, the majority of which were routine hematological screening tests. Benzene exposures were estimated using a job exposure matrix developed by Rinsky et al. (1987). The effects of benzene exposure in the 30, 90, and 180 days prior to the blood test date, as well as cumulative exposure up until the blood test date, were examined using conditional logistic regression. A total of 78 cases and 5,637 controls were included in the WBC analysis and 105 cases and 8,489 controls in the RBC analysis, all of whom had worked only within the rubber hydrochloride departments during the 180 days prior to the selected blood sample date. The maximum daily benzene exposure estimate was 34 ppm. A strong exposure-response relationship

was noted for WBCs, and all of the exposure metrics selected showed a significant relationship with low blood count. A weak positive exposure-response relationship was observed for RBCs, which was significant for the dose metric of cumulative exposure up until the blood test date. The study authors noted that there was no evidence for a threshold for hematologic effects and suggested that exposure to benzene levels <5 ppm may result in hematologic suppression.

Agency Contacts (Chemical Managers): Sharon Wilbur, M.A., Sam Keith, M.S., C.H.P., Obaid Faroon, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Benzene
CAS Numbers: 71-43-2
Date: August 2007
Profile Status: Post Public, Final Draft
Route: [ ] Inhalation   [x] Oral
Duration: [ ] Acute   [ ] Intermediate   [x] Chronic
Graph Key: 45
Species: Human

Minimal Risk Level: 0.0005   [x] mg/kg/day   [ ] ppm

Reference: Lan Q, Zhang L, Li G, et al. 2004a. Hematotoxicity in workers exposed to low levels of benzene. Science 306:1774-1776.

Experimental design: The chronic-duration oral MRL for benzene is based on route-to-route extrapolation of the results of benchmark dose analysis of a hematological endpoint (B cell count) assessed in 250 workers (approximately two-thirds female) exposed to benzene at two shoe manufacturing facilities in Tianjin, China, and 140 age- and gender-matched workers in clothing manufacturing facilities that did not use benzene. See the MRL worksheet for the chronic-duration inhalation MRL for details of study design.

Effect noted in study and corresponding doses: As described in the MRL worksheet for the chronic-duration inhalation MRL (see also Table A-1), exposure-response relationships were noted for several blood factors. Benzene-induced decreased B cell count was selected as the critical effect for benchmark dose (BMD) modeling because it represented the highest magnitude of effect (i.e., B cell count in the highest exposure group was approximately 36% lower than that of controls). A BMD modeling approach was selected to identify the point of departure because the critical study (Lan et al. 2004a) identified a LOAEL in the absence of a NOAEL.

Dose and end point used for MRL derivation: $BMCL_{0.25sdADJ}$ of 0.014 mg/kg/day for decreased B cell count, resulting from route-to-route extrapolation of the $BMCL_{0.25sdADJ}$ of 0.03 ppm described in the MRL worksheet for the chronic-duration inhalation MRL.

Results of toxicokinetic studies of inhaled benzene in humans (Nomiyama and Nomiyama 1974a; Pekari et al. 1992; Srbova et al. 1950) and inhaled and orally-administered benzene in rats and mice (Sabourin et al. 1987) indicate that absorption of benzene at relatively low levels of exposure is approximately 50% of an inhaled dose and essentially 100% of an oral dose. Based on these assumptions, inhalation data can be used to estimate equivalent oral doses that would be expected to similarly affect the critical targets of benzene toxicity. Therefore, the point of departure for the chronic-duration inhalation MRL for benzene, namely the $BMCL_{0.25sdADJ}$ of 0.03 ppm for decreased B cell counts in benzene-exposed workers (Lan et al. 2004a, 2004b), serves as the point of departure for deriving the chronic-duration oral MRL as well.

The point of departure (in ppm) was converted to mg/m^3 using the molecular weight of 78.11 for benzene and assuming 25 °C and 760 mm Hg:

$BMCL_{0.25sdADJ}$ of 0.03 ppm x 78.11/24.45 = 0.096 mg/m^3

The $BMCL_{0.25sdADJ}$ of 0.096 mg/m^3 for inhaled benzene was converted to an equivalent $BMDL_{0.25sdADJ}$ for ingested benzene using EPA (1988b) human reference values for inhalation rate (20 m^3/day) and body

weight (70 kg) and a factor of 0.5 to adjust for differences in absorption of benzene following inhalation versus oral exposure (50 versus 100%, respectively) as follows:

$BMDL_{0.25sdADJ} = BMCL_{0.25sdADJ}$ of 0.096 mg/m^3 x 20 m^3/day x 0.5 ÷ 70 kg = 0.014 mg/kg/day

[ ] NOAEL   [ ] LOAEL

Uncertainty Factors used in MRL derivation: 30

[ ] 1 [ ] 3 [ ] 10 (for use of a LOAEL)
[ ] 1 [ ] 3 [ ] 10 (for extrapolation from animals to humans)
[ ] 1 [ ] 3 [x] 10 (for human variability)
[ ] 1 [x] 3 [ ] 10 (for uncertainty in route-to-route extrapolation)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? The $BMCL_{0.25sd}$ of 0.10 ppm was adjusted from the 8-hour TWA to a continuous exposure concentration ($BMCL_{0.25sdADJ}$) as follows:

$BMCL_{0.25sdADJ} = BMCL_{0.25sd}$ x (8 hours/24 hours) x (6 days/7 days)

Therefore:

$BMCL_{0.25sdADJ}$ = 0.10 ppm x (8 hours/24 hours) x (6 days/7 days)

$BMCL_{0.25sdADJ}$ = 0.03 ppm

Other additional studies or pertinent information that lend support to this MRL: Results of toxicokinetic studies of inhaled benzene in humans (Nomiyama and Nomiyama 1974a; Pekari et al. 1992; Srbova et al. 1950) and inhaled and orally-administered benzene in rats and mice (Sabourin et al. 1987) indicate that absorption of benzene at relatively low levels of exposure is approximately 50% of an inhaled dose and essentially 100% of an oral dose. Based on these assumptions, inhalation data can be used to estimate equivalent oral doses that would be expected to similarly affect the critical targets of benzene toxicity. See the chronic-duration inhalation MRL worksheet for additional information that supports the selection of the principal study and critical effect for deriving the chronic-duration inhalation MRL.

Agency Contacts (Chemical Managers): Sharon Wilbur, M.A., Sam Keith, M.S., C.H.P., Obaid Faroon, Ph.D.

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APPENDIX B. USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public, especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order that they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, and dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal Risk Levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND
See Sample LSE Table 3-1 (page B-6)

(1)   Route of Exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.

(2)   Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3)   Health Effect. The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4)   Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).

(5)   Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6)   Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to "Chemical x" via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).

(7)   System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.

(8)   NOAEL. A NOAEL is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system, which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9)   LOAEL. A LOAEL is the lowest dose used in the study that caused a harmful health effect.
        LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help
        readers identify the levels of exposure at which adverse health effects first appear and the
        gradation of effects with increasing dose. A brief description of the specific end point used to
        quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key
        number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from
        Serious LOAELs.

(10)   Reference. The complete reference citation is given in Chapter 9 of the profile.

(11)   CEL. A CEL is the lowest exposure level associated with the onset of carcinogenesis in
        experimental or epidemiologic studies. CELs are always considered serious effects. The LSE
        tables and figures do not contain NOAELs for cancer, but the text may report doses not causing
        measurable cancer increases.

(12)   Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found
        in the footnotes. Footnote "b" indicates that the NOAEL of 3 ppm in key number 18 was used to
        derive an MRL of 0.005 ppm.

LEGEND
See Sample Figure 3-1 (page B-7)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the
reader quickly compare health effects according to exposure concentrations for particular exposure
periods.

(13)   Exposure Period. The same exposure periods appear as in the LSE table. In this example, health
        effects observed within the acute and intermediate exposure periods are illustrated.

(14)   Health Effect. These are the categories of health effects for which reliable quantitative data
        exists. The same health effects appear in the LSE table.

(15)   Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are
        graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log
        scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in
        mg/kg/day.

(16)   NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in
        the rat upon which an intermediate inhalation exposure MRL is based. The key number 18
        corresponds to the entry in the LSE table. The dashed descending arrow indicates the
        extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of
        0.005 ppm (see footnote "b" in the LSE table).

(17)   CEL. Key number 38m is one of three studies for which CELs were derived. The diamond
        symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the
        LSE table.

APPENDIX B

(18) <u>Estimated Upper-Bound Human Cancer Risk Levels</u>. This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) <u>Key to LSE Figure</u>. The Key explains the abbreviations and symbols used in the figure.

SAMPLE

1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

| Key to figure[a] | Species | Exposure frequency/ duration | System | NOAEL (ppm) | LOAEL (effect) | | Reference |
					Less serious (ppm)	Serious (ppm)	
2 → INTERMEDIATE EXPOSURE							
	[5]	[6]	[7]	[8]	[9]		[10]
3 → Systemic	↓	↓	↓	↓	↓		↓
18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
4 → CHRONIC EXPOSURE							
Cancer					[11]		
					↓		
38	Rat	18 mo 5 d/wk 7 hr/d			20	(CEL, multiple organs)	Wong et al. 1982
39	Rat	89–104 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79–103 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

12 → [a] The number corresponds to entries in Figure 3-1.
[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5×10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation

*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer end point.
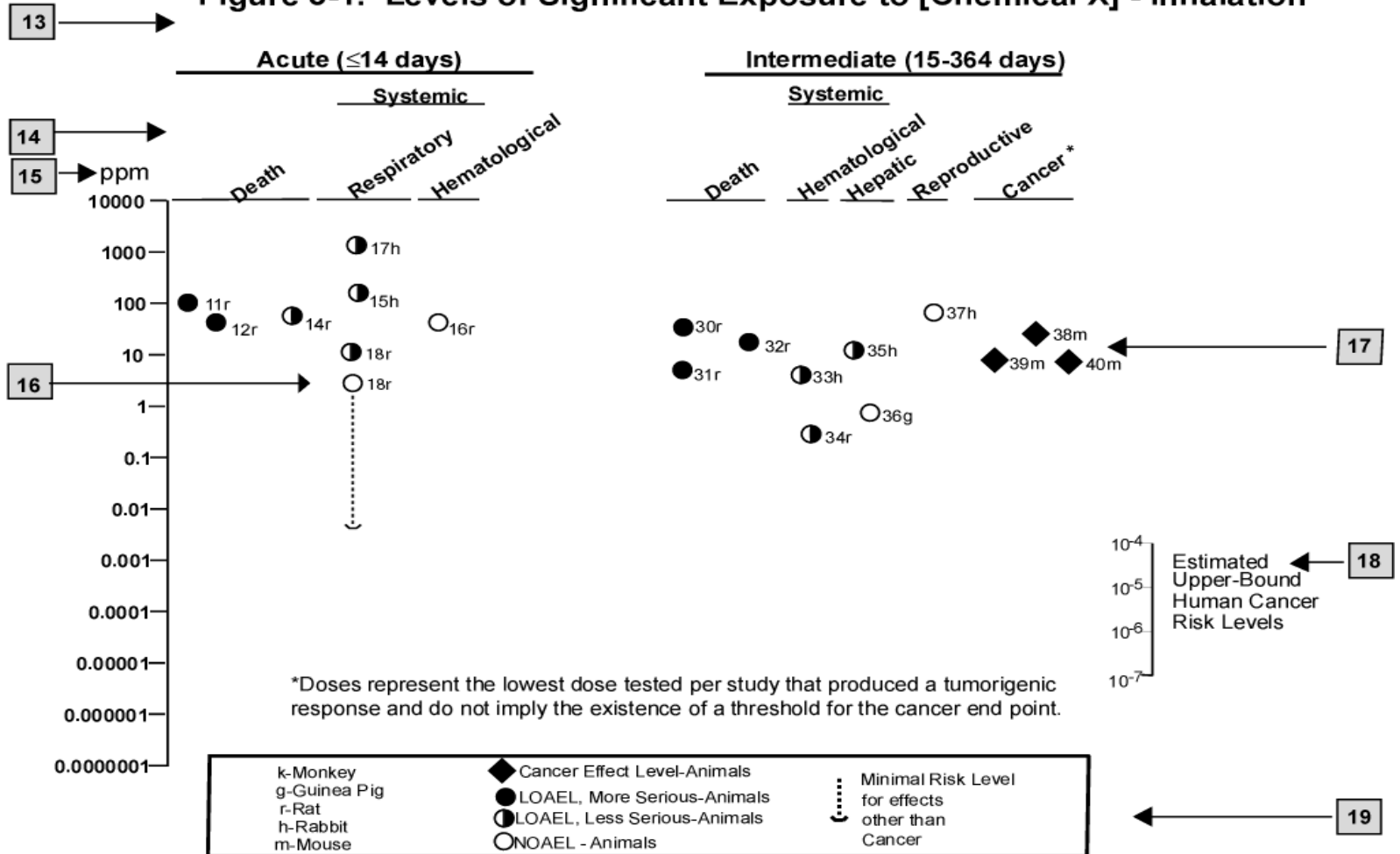
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APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH   American Conference of Governmental Industrial Hygienists
ACOEM   American College of Occupational and Environmental Medicine
ADI   acceptable daily intake
ADME   absorption, distribution, metabolism, and excretion
AED   atomic emission detection
AFID   alkali flame ionization detector
AFOSH   Air Force Office of Safety and Health
ALT   alanine aminotransferase
AML   acute myeloid leukemia
AOAC   Association of Official Analytical Chemists
AOEC   Association of Occupational and Environmental Clinics
AP   alkaline phosphatase
APHA   American Public Health Association
AST   aspartate aminotransferase
atm   atmosphere
ATSDR   Agency for Toxic Substances and Disease Registry
AWQC   Ambient Water Quality Criteria
BAT   best available technology
BCF   bioconcentration factor
BEI   Biological Exposure Index
BMD   benchmark dose
BMR   benchmark response
BSC   Board of Scientific Counselors
C   centigrade
CAA   Clean Air Act
CAG   Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS   Chemical Abstract Services
CDC   Centers for Disease Control and Prevention
CEL   cancer effect level
CELDS   Computer-Environmental Legislative Data System
CERCLA   Comprehensive Environmental Response, Compensation, and Liability Act
CFR   Code of Federal Regulations
Ci   curie
CI   confidence interval
CL   ceiling limit value
CLP   Contract Laboratory Program
cm   centimeter
CML   chronic myeloid leukemia
CPSC   Consumer Products Safety Commission
CWA   Clean Water Act
DHEW   Department of Health, Education, and Welfare
DHHS   Department of Health and Human Services
DNA   deoxyribonucleic acid
DOD   Department of Defense
DOE   Department of Energy
DOL   Department of Labor
DOT   Department of Transportation
DOT/UN/   Department of Transportation/United Nations/
  NA/IMCO   North America/Intergovernmental Maritime Dangerous Goods Code

APPENDIX C

DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
MA	*trans,trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor

APPENDIX C

MFO	mixed function oxidase
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
ND	not detected
NFPA	National Fire Protection Association
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OTS	Office of Toxic Substances
OW	Office of Water
OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon

APPENDIX C

PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
pg	picogram
PHS	Public Health Service
PID	photo ionization detector
pmol	picomole
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RQ	reportable quantity
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

APPENDIX C

>	greater than
$\geq$	greater than or equal to
=	equal to
<	less than
$\leq$	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

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APPENDIX D. INDEX

APPENDIX D

HISTORICAL OIL CONTAMINATION TRAVEL DISTANCES IN GROUND WATER AT SENSITIVE GEOLOGICAL SITES IN MAINE

BUREAU OF REMEDIATION & WASTE MANAGEMENT
MAINE DEPARTMENT OF ENVIRONMENTAL PROTECTION

APRIL 30, 2002

Introduction:

Private drinking water supply wells are an extremely important source of drinking water for many Mainers. Forty-one (41) percent of Maine's housing units[1] and approximately 50% of Maine residents[2] get their drinking water from private wells. Ensuring that these wells are adequately protected from contamination sources and continue to provide healthful drinking water is a fundamental public health and environmental protection policy of the State of Maine, as evidenced in the numerous Maine statutes and regulations with this common goal.

Based on findings that new UST facilities in Maine do have oil discharges despite the use of state-of-the-art technology (including secondary containment) and may subsequently pose a risk to groundwater and nearby receptors[3], the Maine Legislature in 2001 enacted a law governing the siting of new underground oil storage tank (UST) facilities (PL 2001, Chapter 302; An Act To Protect Sensitive Geologic Areas From Oil Contamination). One of the provisions of this law is to protect private drinking water supply wells from contamination. New UST facilities and their components were required as of October 1, 2001 to be located 300 or more feet from an existing private drinking water well. A variance from this standard is available for those cases where it can be demonstrated there is no hydrogeological connection between the proposed facility site and the wells within the 300 foot protective setback.

In response to a proposal during the 2002 legislative session to reduce the protection zone from 300 feet to 75 feet between existing private wells and proposed new UST facilities, the Department of Environmental Protection evaluated the technical merit of a 75 foot setback as a means of protecting drinking water wells from oil discharges that have been documented to occur at state-of-the-art facilities due to human error and equipment failure. The Department also took a broader look at oil

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[1] U.S. Census Bureau; Maine Housing Characteristics: 1990.
[2] State of Maine, Depts. Of Human Service, Environmental Protection and Conservation; The Presence of MTBE and Other Gasoline Compounds in Maine's Drinking Water; Oct. 13, 1998; p. 12.
[3] Maine DEP records indicate that 9% of new UST facilities installed between 1995 and 2000 suffered one or more discharges within their first five years of operation. Discharges ranged from one to 6,000 gallons.

contamination travel distances and their relationship to the numbers of private wells contaminated and associated remediation costs.

Methodology:

To evaluate the protectiveness of a 75 foot buffer, the Department studied travel distances of various oil compounds from documented discharge sources by way of groundwater to either drinking water supply wells or monitoring wells. A survey of sites in sensitive geological settings similar to those at the heart of this legislative policy debate was conducted. The survey was based on a 20 percent random sample of the 394 oil remediation sites on the Department's August 18, 2001 priority list of such sites. Bureau of Remediation & Waste Management project management staff, geologists and environmental engineers working on each of the 85 sites selected randomly, were surveyed as to the petroleum contaminants documented by laboratory analyses in the groundwater, the linear distance from the contaminant source to the farthest affected drinking or monitoring well, the number of private and/or public drinking water supply wells contaminated, and the investigation/remediation/third party damage claim costs incurred by the Department as of January 15, 2002. Groundwater contamination data was collected for Gasoline Range Organics (GRO)[4], Diesel Range Organics (DRO)[5], and MTBE or other gasoline oxygenates (e.g. TBA – tertiary butyl alcohol, TAME – tert-amyl methyl ether). For the purposes of this survey contamination was defined as any concentration above reporting limits for any one of the above analytes. For the State Health & Environmental Testing Laboratory (HETL) in the Maine Bureau of Health and most State certified private laboratories, the reporting limits for these parameters are 10 parts per billion (ppb) for GRO, 50 ppb for DRO, and 1 ppb for MTBE. This definition is consistent with that in the Department' s regulations, Chapter 691, Rules for Underground Oil Storage Facilities.

In the course of conducting this general analysis, the geology of the individual sites and wells were not differentiated (bedrock, sand and gravel aquifer vs. other geological settings). Based on the Department's overall experience investigating and remediating various oil discharge sites effecting private drinking water supplies, it would be reasonable to assume most of the contaminated private wells were bedrock wells. The survey also did not make any distinction between the various types of oil discharge sources (UST facilities, aboveground oil storage facilities – including home heating oil tanks and piping, or surface spills). Based on its over 15 years of field experience, the Department is comfortable with the assumption that once a discharge of oil has occurred at a sensitive site, the source is not a significant factor in determining how far contamination travels once reaching the soil and then groundwater. However, the Department did distinguish between different types of oil products and petroleum

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[4] Maine Health & Environmental Testing Laboratory (HETL) Method 4.1.17, Modified Method for Determining Gasoline Range Organics; Sept. 6, 1995
[5] Maine HETL Method 4.1.25, Modified Method for Determining Diesel Range Organics; Sept. 6, 1995.

analytes to test whether their different chemical and physical properties made a difference in the distances they traveled and, in particular, the frequency with which they traveled more than 75 feet.

Findings:

Of the 85 oil discharge sites selected for the survey, 38 sites lacked one or more of the needed data. The most common reasons for the unavailability of data for a particular site or survey parameter included the source of the oil contamination being unknown, multiple sources of contamination, and sites where a hydrogeological investigation has not yet been undertaken.

The findings show that the majority of petroleum discharges result in contamination traveling in groundwater further than 75 feet. The frequency with which the dissolved phase of petroleum contamination (for all product types and analytes) traveled more than 75 feet is summarized in Figure 1. Of the 47 sites for which adequate data was available, at seventy (70) percent of sites, the oil contamination traveled in excess of 75 feet. Breaking the findings down by the product type and analyte, travel distances greater than 75 feet were evident in 56% of heating oil/diesel fuel (DRO only) contamination cases, 80% of gasoline cases (GRO only), and 82% of MTBE cases. Travel distance findings for #2 heating oil/diesel fuel are consistent with those of an earlier study in Maine, where 50% of wells were contaminated above the Maine drinking water standard of 50 ppb beyond 75 feet from the contamination source.[6]

Taking a broader view of the survey findings when comparing the number of sites and travel distances, it was found that at three-quarters (76%) of sites, groundwater contamination traveled as far as 300 feet. As illustrated in Figure 2, at the remaining 24% of sites, one or more oil analytes were found to have traveled more than 300 feet. When analyzing the frequency with which individual analytes traveled distances greater than 300 feet from the contamination source, MTBE did so most frequently (32% of sites), followed by GRO (25%) and DRO (17%).

The average documented distance traveled for GRO and DRO were 295 and 140 feet, respectively. The average MTBE travel distance was 300 feet. The maximum distance documented between a discharge source and a contaminated well was 1670 feet. The analyte was GRO, more specifically benzene, found at concentrations above the Federal and State drinking water standard of 5 ppb in a residential bedrock well.

Oil discharge sites within the sample population contaminated a total of 123 private wells and 18 public drinking water supply wells. Most of the public wells were transient (e.g. stores, restaurants, motels) or non-transient non-community supplies

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[6] Rand, John B.; Mother Nature's Cure, Proceedings of Lessons Learned in the Remediation of Petroleum-Contaminated Sites in Maine; Consulting Engineers of Maine & the Maine Dept. of Environmental Protection; Augusta, Maine; April 28, 1994.

Figure 1. Documented Travel Distances in Groundwater of Petroleum Contamination in Comparison to a Proposed 75' Setback (January, 2002)

Figure 2. Documented Travel Distances in Groundwater of Petroleum Contamination (January, 2002)
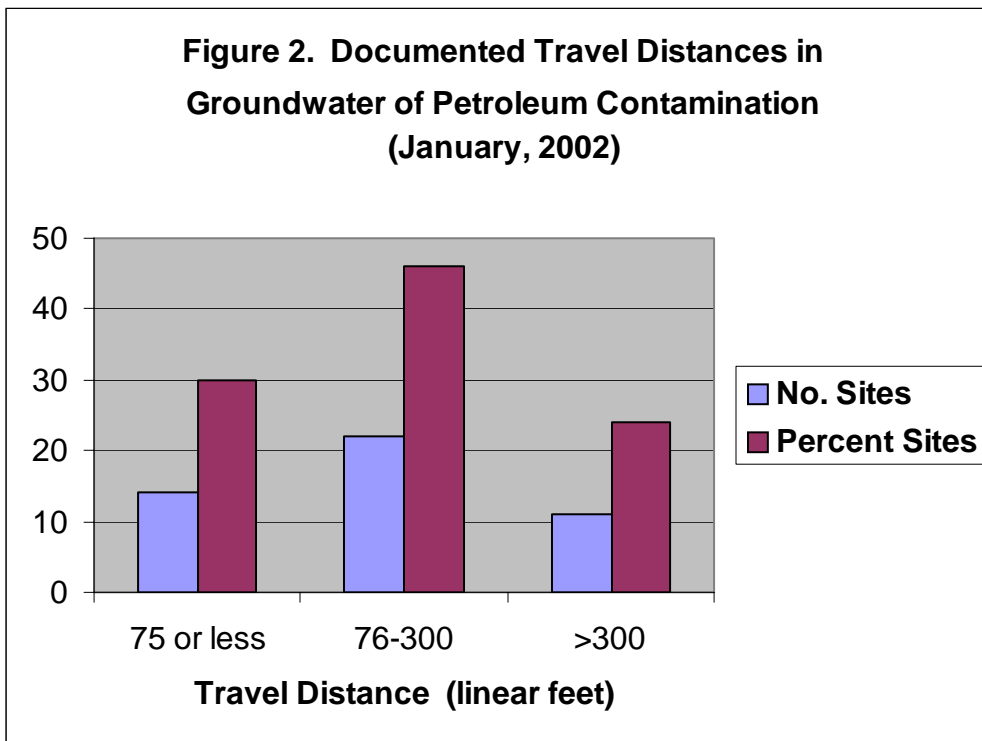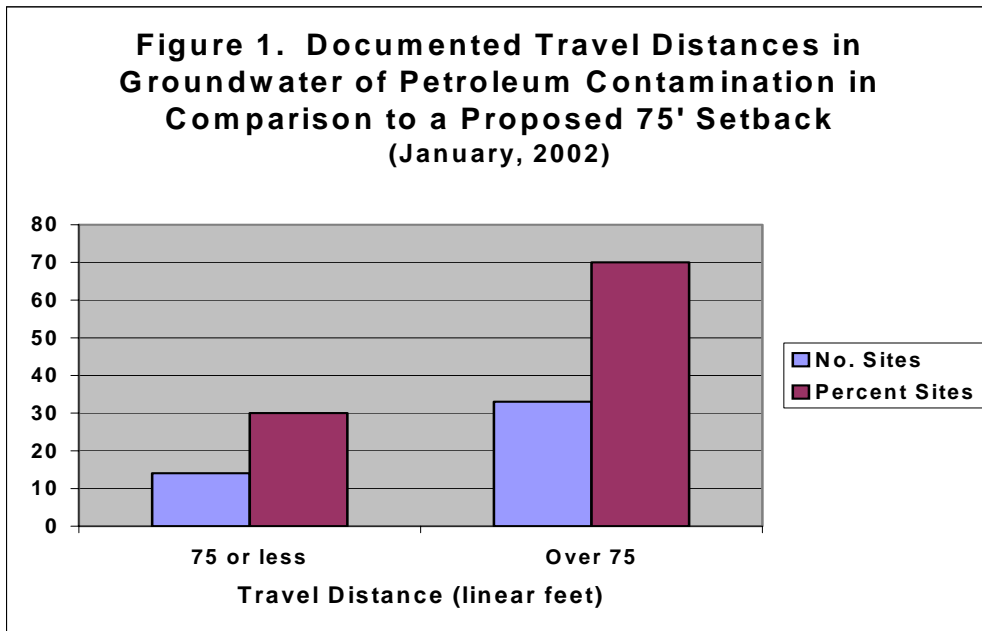
(e.g. schools and larger places of employment). Two community water supplies were impacted – one a trailer park and the second a multi-unit residential housing development.

The survey data presented in Table 1 below shows a clear relationship between oil (GRO, DRO and MTBE) contamination travel distance and remediation site costs. The smaller number of sites where petroleum contamination traveled over 300 feet, contaminated the most drinking water supply wells and resulted in a disproportionate percentage of the total remediation costs due to much higher average per site remediation costs. Of the total number of private wells contaminated, 58 percent were associated with sites where one or more petroleum analytes traveled in excess of 300 feet from its source. In comparison, sites where contamination traveled 75 feet or less, nine (9) percent of the total number of private wells contaminated were located.

Table 1
Oil Contamination Travel Distances and Remediation Costs[7]

Linear Distance (ft.)	Percent Sites	Percent of Total Remediation Costs	Average Per Site Remediation Cost
≤75	30	9	$45,000
76 – 300	46	43	$148,000
>300	24	48	$331,000

Survey Limitations:

The contaminant travel distance survey data have a number of limitations which are fairly obvious, but which should be highlighted in order to keep the survey's findings and conclusions in proper context. Foremost, no site geology information was collected which may effect travel distances. Instead the Department assumed that dissolved phase petroleum contamination traveled via bedrock fractures at most sites included in the survey sample. Although probably a very safe assumption in the vast majority of cases, it certainly prevents us the opportunity to document to what extent site specific geology was an important variable in determining linear travel distances.

Measuring travel distance from the contamination source in linear distance, although appropriate for the regulatory purposes of this survey, it does not reflect actual travel paths and distances in the field, especially in bedrock. Laws and regulations use linear protective setbacks, therefore it was necessary to look at linear travel distances in the survey. However, the actual travel distance of dissolved phase petroleum

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[7] Includes primarily emergency response, site investigation costs, and long-term soil and ground water remediation costs, including the treatment and replacement of contaminated drinking water supply wells.

contamination can be quite different. A remediation site in the municipality of South Thomaston in the mid-coast region of Maine and included in the survey, provides an excellent example of this difference. Loose piping joints resulted in a gasoline discharge from a small convenience store. Despite the removal of nearly 660 tons of contaminated soil, MTBE contaminated the bedrock groundwater. Forty-four (44) wells in the neighborhood provided monitoring locations, 18 of which were found contaminated. The linear distance to the farthest contaminated private drinking water well is approximately 935 feet. Based on well monitoring results and studying the local bedrock geology, it appears the MTBE traveled about 750 feet northwest from the source, then 560 feet back toward the southeast, and finally 740 feet to the southwest. In actuality, the MTBE contamination probably traveled in excess of 2000 feet in an indirect path dictated by bedrock fractures and other site-specific conditions.

It should also be noted that the travel distances presented are not the absolute maximum linear distances the analytes traveled from the source. They represent the distance between the source and the farthest, available groundwater monitoring point in which the analyte was documented above laboratory reporting levels. How much further contamination traveled beyond these monitoring locations is not known. The travel distances presented here are therefore somewhat conservative.

Conclusions:

1. There is no hydrogeological basis in Maine to support a 75 foot setback as adequate to protect private drinking water supplies from contamination resulting from petroleum discharges at new UST facilities. At most sites studied (70%), petroleum contamination in groundwater was found to travel more than 75 feet from its source.
2. Even in the case of the least mobile contaminant in groundwater studied, DRO (associated with heating oil and diesel fuel discharges), DRO traveled farther than 75 feet from the source of the petroleum at more than 50% of contamination sites. In so doing it contaminated wells at or above the State of Maine drinking water standard of 50 ppb.
3. For use in a statewide regulatory program, over a broad range of hydrogeological conditions and different product types, a 300 linear foot setback between UST facilities and private wells provides adequate protection at most sites (76 percent). The inclusion of a variance procedure as provided in Maine law based on site-specific hydrogeology provides the flexibility for that minority of sites where there is no hydrogeological connection between the UST facility and nearby wells.
4. A relatively small proportion of sites (24%) were found where petroleum contamination traveled in excess of 300 feet. However this minority of sites can have a disproportionately high fiscal impact (48% of total costs) due to significantly higher than average per site remediation costs. Average site remediation costs at such sites ranged from two to six times greater than the statewide average.

5. Despite Maine's UST siting standards, costly remediation cases effecting private drinking water supplies resulting from oil discharges at new UST facilities are likely to continue to occur, albeit hopefully in smaller numbers if Maine's siting standards prove effective.
6. The findings above are applicable to all potential petroleum discharge sources to the environment, including underground and aboveground oil storage tank facilities.

Questions may be directed to George Seel, Division of Technical Services, Bureau of Remediation & Waste Mgmt., MDEP at (207) 287-2651.

Getting Up to Speed
GROUND WATER CONTAMINATION

Ground water contamination is nearly always the result of human activity. In areas where population density is high and human use of the land is intensive, ground water is especially vulnerable. Virtually any activity whereby chemicals or wastes may be released to the environment, either intentionally or accidentally, has the potential to pollute ground water. When ground water becomes contaminated, it is difficult and expensive to clean up.

To begin to address pollution prevention or remediation, we must understand how surface waters and ground waters interrelate. Ground water and surface water are interconnected and can be fully understood and intelligently managed only when that fact is acknowledged. If there is a water supply well near a source of contamination, that well runs the risk of becoming contaminated. If there is a nearby river or stream, that water body may also become polluted by the ground water.

HOW DOES GROUND WATER BECOME CONTAMINATED?

Depending on its physical, chemical, and biological properties, a contaminant that has been released into the environment may move within an aquifer in the same manner that ground water moves. (Some contaminants, because of their phys-ical or chemical properties, do not always follow ground water flow.) It is possible to predict, to some degree, the transport within an aquifer of those substances that move along with ground water flow. For example, both water and certain contaminants flow in the direction of the topography from recharge areas to discharge areas. Soils that are porous and permeable tend to transmit water and certain types of contaminants with relative ease to an aquifer below.

Just as ground water generally moves slowly, so do contaminants in ground water. Because of this slow movement, contaminants tend to remain concentrated in the form of a **plume** (see Figure 1) that flows along the same path as the ground water. The size and speed of the plume depend on the amount and type of contaminant, its solubility and density, and the velocity of the surrounding ground water.

Figure 1   **CONTAMINANT PLUME**

Water Table

Direction of Ground Water Flow
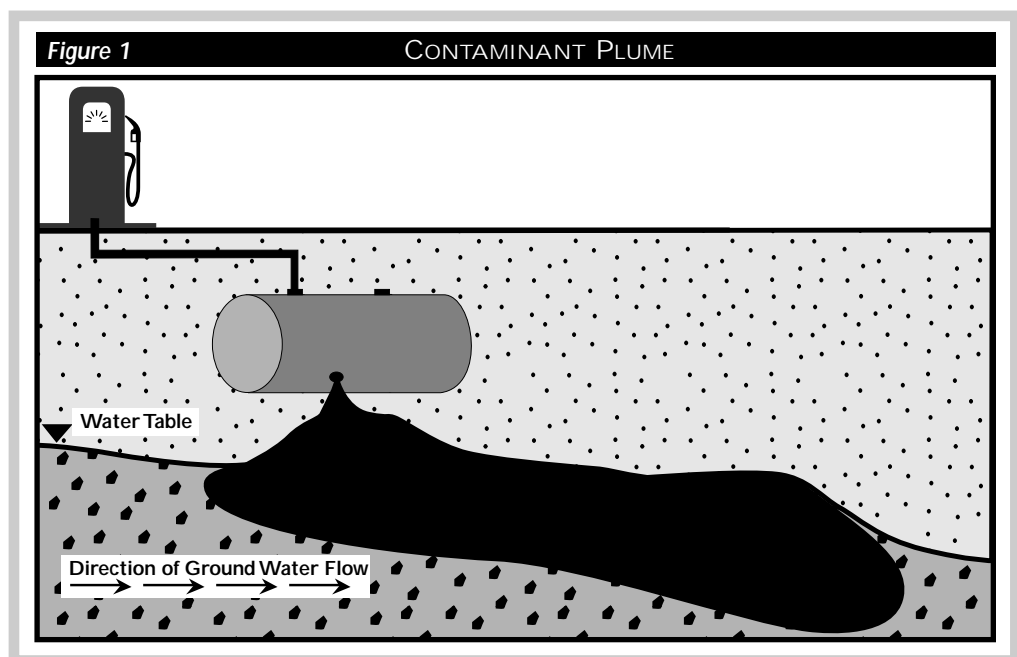
Ground water and contaminants can move rapidly through fractures in rocks. Fractured rock presents a unique problem in locating and controlling contaminants because the fractures are generally randomly spaced and do not follow the contours of the land surface or the hydraulic gradient. Contaminants can also move into the ground water system through macropores—root systems, animal burrows, abandoned wells, and other systems of holes and cracks that supply pathways for contaminants.

In areas surrounding pumping wells, the potential for contamination increases because water from the **zone of contribution**, a land area larger than the original recharge area, is drawn into the well and the surrounding aquifer. Some drinking water wells actually draw water from nearby streams, lakes, or rivers. Contaminants present in these surface waters can contribute contamination to the ground water system. Some wells rely on artificial recharge to increase the amount of water infiltrating an aquifer, often using water from storm runoff, irrigation, industrial processes, or treated sewage. In several cases, this practice has resulted in increased concentrations of nitrates, metals, microbes, or synthetic chemicals in the water.

Under certain conditions, pumping can also cause the ground water (and associated contaminants) from another aquifer to enter the one being pumped. This phenomenon is called **interaquifer leakage**. Thus, properly identifying and protecting the areas affected by well pumping is important to maintain ground water quality.

Generally, the greater the distance between a source of contamination and a ground water source, the more likely that natural processes will reduce the impacts of contamination. Processes such as oxidation, biological degradation (which sometimes renders contaminants less toxic), and adsorption (binding of materials to soil particles) may take place in the soil layers of the unsaturated zone and reduce the concentration of a contaminant before it reaches ground water. Even

contaminants that reach ground water directly, without passing through the unsaturated zone, can become less concentrated by dilution (mixing) with the ground water. However, because ground water usually moves slowly, contaminants generally undergo less dilution than when in surface water.

SOURCES OF GROUND WATER CONTAMINATION

Ground water can become contaminated from natural sources or numerous types of human activities. (See Tables 1 and 2 and Figure 1.) Residential, municipal, commercial, industrial, and agricultural activities can all affect ground water quality. Contaminants may reach ground water from activities on the land surface, such as releases or spills from stored industrial wastes; from sources below the land surface but above the water table, such as septic systems or leaking underground petroleum storage systems; from structures beneath the water table, such as wells; or from contaminated recharge water.

■ Natural Sources

Some substances found naturally in rocks or soils, such as iron, manganese, arsenic, chlorides, fluorides, sulfates, or radionuclides, can become dissolved in ground water. Other naturally occurring substances, such as decaying organic matter, can move in ground water as particles. Whether any of these substances appears in ground water depends on local conditions. Some substances may pose a health threat if consumed in excessive quantities; others may produce an undesirable odor, taste, or color. Ground water that contains unacceptable concentrations of these substances is not used for drinking water or other domestic water uses unless it is treated to remove these contaminants.

■ Septic Systems

One of the main causes of ground water contamination in the United States is the effluent (outflow) from septic tanks, cesspools, and privies.

Table 1	TYPICAL SOURCES OF POTENTIAL GROUND WATER CONTAMINATION BY LAND USE CATEGORY	
Category	**Contaminant Source**	
Agriculture	Animal burial areas	Irrigation sites
	Animal feedlots	Manure spreading areas/pits
	Fertilizer storage/use	Pesticide storage/use
Commercial	Airports	Jewelry/metal plating
	Auto repair shops	Laundromats
	Boat yards	Medical institutions
	Construction areas	Paint shops
	Car washes	Photography establishments
	Cemeteries	Railroad tracks and yards
	Dry cleaners	Research laboratories
	Gas stations	Scrap and junkyards
	Golf courses	Storage tanks
Industrial	Asphalt plants	Petroleum production/storage
	Chemical manufacture/storage	Pipelines
	Electronics manufacture	Septage lagoons and sludge sites
	Electroplaters	Storage tanks
	Foundries/metal fabricators	Toxic and hazardous spills
	Machine/metalworking shops	Wells (operating/abandoned)
	Mining and mine drainage	Wood preserving facilities
Residential	Fuel oil	Septic systems, cesspools
	Furniture stripping/refinishing	Sewer lines
	Household hazardous products	Swimming pools (chemical storage)
	Household lawns	
Other	Hazardous waste landfills	Recycling/reduction facilities
	Municipal incinerators	Road deicing operations
	Municipal landfills	Road maintenance depots
	Municipal sewer lines	Storm water drains/basins
	Open burning sites	Transfer stations

Source: U.S. EPA, 1991a.

Approximately one-fourth of all homes in the United States rely on septic systems to dispose of their human wastes. Although each individual system releases a relatively small amount of waste into the ground, the large number and widespread use of these systems makes them a serious contamination source. Septic systems that are improperly sited, designed, constructed, or maintained can contaminate ground water with bacteria, viruses, nitrates, detergents, oils, and chemicals. Along with these contaminants are the commercially available septic system cleaners containing synthetic organic chemicals (such as 1,1,1-trichloroethane or methylene chloride). These cleaners can contaminate water supply wells and interfere with natural decomposition processes in septic systems.

Most, if not all, state and local regulations require specific separation distances between septic systems and drinking water wells. In addition, computer models have been developed to calculate suitable distances and densities.

■ Improper Disposal of Hazardous Waste

Hazardous waste should always be disposed of properly, that is to say, by a licensed hazardous waste handler or through municipal hazardous waste collection days. Many chemicals should not be disposed of in household septic systems, including oils (e.g., cooking, motor), lawn and garden chemicals, paints and paint thinners, disinfectants, medicines, photographic chemicals, and swimming pool chemicals. Similarly, many substances used in industrial processes should not be disposed of in drains at the workplace because they could contaminate a drinking water source. Companies should train employees in the proper use and disposal of all chemicals used on site. The many different types and the large quantities of chemicals used at industrial locations make proper disposal of wastes especially important for ground water protection.

■ Releases and Spills from Stored Chemicals and Petroleum Products

Underground and aboveground storage tanks are commonly used to store petroleum products and other chemical substances. For example, many homes have underground heating oil tanks. Many businesses and municipal highway departments also store gasoline, diesel fuel, fuel oil, or chemicals in on-site tanks. Industries use storage tanks to hold chemicals used in industrial processes or to store hazardous wastes for pickup by a licensed hauler. Approximately 4 million underground storage tanks exist in the United States and, over the years, the contents of many of these tanks have leaked and spilled into the environment.

If an underground storage tank develops a leak, which commonly occurs as the tank ages and corrodes, its contents can migrate through the soil and reach the ground water. Tanks that meet federal/state standards for new and upgraded systems are less likely to fail, but they are not foolproof. Abandoned underground tanks pose another problem because their location is often unknown. Aboveground storage tanks can also pose a threat to ground water if a spill or leak occurs and adequate barriers are not in place.

Improper chemical storage, sloppy materials handling, and poor-quality containers can be major threats to ground water. Tanker trucks and train cars pose another chemical storage hazard. Each year, approximately 16,000 chemical spills occur from trucks, trains, and storage tanks, often when materials are being transferred. At the site of an accidental spill, the chemicals are often diluted with water and then washed into the soil, increasing the possibility of ground water contamination.

■ Landfills

Solid waste is disposed of in thousands of municipal and industrial landfills throughout the country. Chemicals that should be disposed of in hazardous waste landfills sometimes end up in municipal landfills. In addition, the disposal of many household wastes is not regulated.

Once in the landfill, chemicals can leach into the ground water by means of precipitation and surface runoff. New landfills are required to have clay or synthetic liners and leachate (liquid from a landfill containing contaminants) collection systems to protect ground water. Most older landfills, however, do not have these safeguards. Older landfills were often sited over aquifers or close to surface waters and in permeable soils with shallow water tables, enhancing the potential for leachate to contaminate ground water. Closed landfills can continue to pose a ground water contamination threat if they are not capped with an impermeable material (such as clay) before closure to prevent the leaching of contaminants by precipitation.

■ Surface Impoundments

Surface impoundments are relatively shallow ponds or lagoons used by industries and municipalities to store, treat, and dispose of liquid wastes. As many as 180,000 surface impoundments exist in the United States. Like landfills, new surface impoundment facilities are required to have liners, but even these liners sometimes leak.

Table 2	POTENTIAL HARMFUL COMPONENTS OF COMMON HOUSEHOLD PRODUCTS
Product	**Toxic or Hazardous Components**
Antifreeze (gasoline or coolants systems)	Methanol, ethylene glycol
Automatic transmission fluid	Petroleum distillates, xylene
Battery acid (electrolyte)	Sulfuric acid
Degreasers for driveways and garages	Petroleum solvents, alcohols, glycol ether
Degreasers for engines and metal	Chlorinated hydrocarbons, toluene, phenols, dichloroperchloroethylene
Engine and radiator flushes	Petroleum solvents, ketones, butanol, glycol ether
Hydraulic fluid (brake fluid)	Hydrocarbons, fluorocarbons
Motor oils and waste oils	Hydrocarbons
Gasoline and jet fuel	Hydrocarbons
Diesel fuel, kerosene, #2 heating oil	Hydrocarbons
Grease, lubes	Hydrocarbons
Rustproofers	Phenols, heavy metals
Car wash detergents	Alkyl benzene sulfonates
Car waxes and polishes	Petroleum distillates, hydrocarbons
Asphalt and roofing tar	Hydrocarbons
Paints, varnishes, stains, dyes	Heavy metals, toluene
Paint and lacquer thinner	Acetone, benzene, toluene, butyl acetate, methyl ketones
Paint and varnish removers, deglossers	Methylene chloride, toluene, acetone, xylene, ethanol, benzene, methanol
Paint brush cleaners	Hydrocarbons, toluene, acetone, methanol, glycol ethers, methyl ethyl ketones
Floor and furniture strippers	Xylene
Metal polishes	Petroleum distillates, isopropanol, petroleum naphtha
Laundry soil and stain removers	Hydrocarbons, benzene, trichloroethylene, 1,1,1-trichloroethane
Other solvents	Acetone, benzene
Rock salt	Sodium concentration
Refrigerants	1,1,2-trichloro-1,2,2-trifluoroethane
Bug and tar removers	Xylene, petroleum distillates
Household cleansers, oven cleaners	Xylenols, glycol ethers, isopropanol
Drain cleaners	1,1,1-trichloroethane
Toilet cleaners	Xylene, sulfonates, chlorinated phenols
Cesspool cleaners	Tetrachloroethylene, dichlorobenzene, methylene chloride
Disinfectants	Cresol, xylenols
Pesticides (all types)	Naphthalene, phosphorus, xylene, chloroform, heavy metals, chlorinated hydrocarbons
Photochemicals	Phenols, sodium sulfite, cyanide, silver halide, potassium bromide
Printing ink	Heavy metals, phenol-formaldehyde
Wood preservatives (creosote)	Pentachlorophenols
Swimming pool chlorine	Sodium hypochlorite
Lye or caustic soda	Sodium hydroxide
Jewelry cleaners	Sodium cyanide

Source: "Natural Resources Facts: Household Hazardous Wastes," Fact Sheet No. 88-3, Department of Natural Science, University of Rhode Island, August 1988.

■ Sewers and Other Pipelines

Sewer pipes carrying wastes sometimes leak fluids into the surrounding soil and ground water. Sewage consists of organic matter, inorganic salts, heavy metals, bacteria, viruses, and nitrogen. Other pipelines carrying industrial chemicals and oil brine have also been known to leak, especially when the materials transported through the pipes are corrosive.

■ Pesticide and Fertilizer Use

Millions of tons of fertilizers and pesticides (e.g., herbicides, insecticides, rodenticides, fungicides, avicides) are used annually in the United States for crop production. In addition to farmers, home-owners, businesses (e.g., golf courses), utilities, and municipalities use these chemicals. A number of these pesticides and fertilizers (some highly toxic) have entered and contaminated ground water following normal, registered use. Some pesticides remain in soil and water for many months to many years. Another potential source of ground water contamination is animal wastes that percolate into the ground from farm feedlots. Feedlots should be properly sited and wastes should be removed at regular intervals.

Between 1985 and 1992, EPA's Office of Pesticides and Toxic Substances and Office of Water conducted a National Pesticide Survey to determine the number of drinking water wells nationwide that contain pesticides and nitrates and the concentration of these substances. The survey also analyzed the factors associated with contamination of drinking water wells by pesti-cides and nitrates. The survey, which included samples from more than 1,300 public community and rural domestic water supply wells, found that approximately 3.6 percent of the wells contained concentrations of nitrates above the federal maxi-mum contaminant level, and that over half of the wells contained nitrates above the survey's mini-mum reporting limit for nitrate (0.15 mg/L).

The survey also reported that approximately 0.8 percent of the wells tested contained pesticides at

levels higher than federal maximum contaminant levels or health advisory levels. Only 10 percent of the wells classified as rural were actually located on farms. There is a higher incidence of contami-nation by agricultural chemicals in farm wells used for drinking water.

After further analysis, EPA estimated that for the wells that contain pesticides, a significant percent-age probably contain chemical concentrations that exceed the federal health-based limits (e.g., maxi-mum contaminant levels or health advisory levels). Approximately 14.6 percent of the wells tested contained levels of one or more pesticides above the minimum reporting limit set in the survey. The most common pesticides found were atrazine and metabolites (breakdown products) of dimethyl tetrachloroterephthalate (DCPA, commonly known as Dacthal), which is used in many utility easement weed-control programs and for lawn care.

■ Drainage Wells

Drainage wells are used in wet areas to help drain water and transport it to deeper soils. These wells may contain agricultural chemicals and bacteria.

■ Injection Wells/Floor Drains

Injection wells are used to collect storm water runoff, collect spilled liquids, dispose of waste-water, and dispose of industrial, commercial, and utility wastes. These wells are regulated by the U.S. EPA's Underground Injection Control Program. In New England, these wells may not be used to inject hazardous wastes from industrial, commercial, and utility operations. The injection wells used in this region are typically shallow and include sumps and dry wells used to handle storm water.

Floor drains were historically used by businesses to handle spills. Today, if a business operates or handles waste fluids that drain to a septic system, dry well, or floor drain, it is required to submit information regarding its operation to the U.S. EPA or its state environmental protection agency. Disposal wells that pose threats to drinking water supplies are prohibited and must be closed, con-

nected to a public sewage system, or connected to a storage tank.

■ Improperly Constructed Wells

Problems associated with improperly constructed wells can result in ground water contamination when contaminated surface or ground water is introduced into the well.

■ Improperly Abandoned Wells

These wells can act as a conduit through which contaminants can reach an aquifer if the well casing has been removed, as is often done, or if the casing is corroded. In addition, some people use abandoned wells to dispose of wastes such as used motor oil. These wells may reach into an aquifer that serves drinking supply wells. Abandoned exploratory wells (e.g., for gas, oil, or coal) or test hole wells are usually uncovered and are also a potential conduit for contaminants.

■ Active Drinking Water Supply Wells

Poorly constructed wells can result in ground water contamination. Construction problems, such as faulty casings, inadequate covers, or lack of concrete pads, allow outside water and any accompanying contaminants to flow into the well. Sources of such contaminants can be surface runoff or wastes from farm animals or septic systems. Contaminated fill packed around a well can also degrade well water quality. Well construction problems are more likely to occur in older wells that were in place prior to the establishment of well construction standards and in domestic and livestock wells.

■ Poorly Constructed Irrigation Wells

These wells can allow contaminants to enter ground water. Often pesticides and fertilizers are applied in the immediate vicinity of wells on agricultural land.

■ Mining Activities

Active and abandoned mines can contribute to ground water contamination. Precipitation can leach soluble minerals from the mine wastes (known as spoils or tailings) into the ground water below. These wastes often contain metals, acid, minerals, and sulfides. Abandoned mines are often used as wells and waste pits, sometimes simultaneously. In addition, mines are sometimes pumped to keep them dry; the pumping can cause an upward migration of contaminated ground water, which may be intercepted by a well.

EFFECTS OF GROUND WATER CONTAMINATION

Contamination of ground water can result in poor drinking water quality, loss of water supply, degraded surface water systems, high cleanup costs, high costs for alternative water supplies, and/or potential health problems.

The consequences of contaminated ground water or degraded surface water are often serious. For example, estuaries that have been impacted by high nitrogen from ground water sources have lost critical shellfish habitats. In terms of water supply, in some instances, ground water contamination is so severe that the water supply must be abandoned as a source of drinking water. In other cases, the ground water can be cleaned up and used again, if the contamination is not too severe and if the municipality is willing to spend a good deal of money. Follow-up water quality monitoring is often required for many years.

Because ground water generally moves slowly, contamination often remains undetected for long periods of time. This makes cleanup of a contaminated water supply difficult, if not impossible. If a cleanup is undertaken, it can cost thousands to millions of dollars.

Once the contaminant source has been controlled or removed, the contaminated ground water can be treated in one of several ways:

- Containing the contaminant to prevent migration.

- Pumping the water, treating it, and returning it to the aquifer.

- Leaving the ground water in place and treating either the water or the contaminant.

- Allowing the contaminant to attenuate (reduce) naturally (with monitoring), following the implementation of an appropriate source control.

Selection of the appropriate remedial technology is based on site-specific factors and often takes into account cleanup goals based on potential risk that are protective of human health and the environment. The technology selected is one that will achieve those cleanup goals. Different technologies are effective for different types of contaminants, and several technologies are often combined to achieve effective treatment. The effectiveness of treatment depends in part on local hydrogeological conditions, which must be evaluated prior to selecting a treatment option.

Given the difficulty and high costs of cleaning up a contaminated aquifer, some communities choose to abandon existing wells and use other water sources, if available. Using alternative supplies is probably more expensive than obtaining drinking water from the original source. A temporary and expensive solution is to purchase bottled water, but it is not a realistic long-term solution for a community's drinking water supply problem. A community might decide to install new wells in a different area of the aquifer. In this case, appropriate siting and monitoring of the new wells are critical to ensure that contaminants do not move into the new water supplies.

Potential Health Problems

A number of microorganisms and thousands of synthetic chemicals have the potential to contaminate ground water. Drinking water containing bacteria and viruses can result in illnesses such as hepatitis, cholera, or giardiasis. Methemoglobinemia or "blue baby syndrome," an illness affecting infants, can be caused by drinking water that is high in nitrates. Benzene, a component of

gasoline, is a known human carcinogen. The serious health effects of lead are well known—learning disabilities in children; nerve, kidney, and liver problems; and pregnancy risks. Concentrations in drinking water of these and other substances are regulated by federal and state laws. Hundreds of other chemicals, however, are not yet regulated, and many of their health effects are unknown or not well understood. Preventing contaminants from reaching the ground water is the best way to reduce the health risks associated with poor drinking water quality.

REGULATIONS TO PROTECT GROUND WATER

Several federal laws help protect ground water quality. The **Safe Drinking Water Act (SDWA)** established three drinking water source protection programs: the Wellhead Protection Program, Sole Source Aquifer Program, and the Source Water Assessment Program. It also called for regulation of the use of underground injection wells for waste disposal and provided EPA and the states with the authority to ensure that drinking water supplied by public water systems meets minimum health standards. The **Clean Water Act** regulates ground water that is shown to have a connection with surface water. It sets standards for allowable pollutant discharges to surface water. The **Resource Conservation and Recovery Act (RCRA)** regulates treatment, storage, and disposal of hazardous and nonhazardous wastes. The **Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA**, **or Superfund)** authorizes the government to clean up contamination or sources of potential contamination from hazardous waste sites or chemical spills, including those that threaten drinking water supplies. CERCLA includes a "community right-to-know" provision. The **Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)** regulates pesticide use. The **Toxic Substances Control Act (TSCA)** regulates manufactured chemicals.

KEY TERMS

- Clean Water Act

- Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, or Superfund)

- Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

- Interaquifer Leakage

- Plume

- Resource Conservation and Recovery Act (RCRA)

- Safe Drinking Water Act

- Toxic Substances Control Act (TSCA)

- Zone of Contribution

"Getting Up to Speed" for section C, "Ground Water Contamination" is adapted from US EPA Seminar Publication. *Wellhead Protection: A Guide for Small Communities.* Chapter 3. EPA/625/R-93/002.

Figure 2

SOME POTENTIAL SOURCES OF GROUND WATER CONTAMINATION
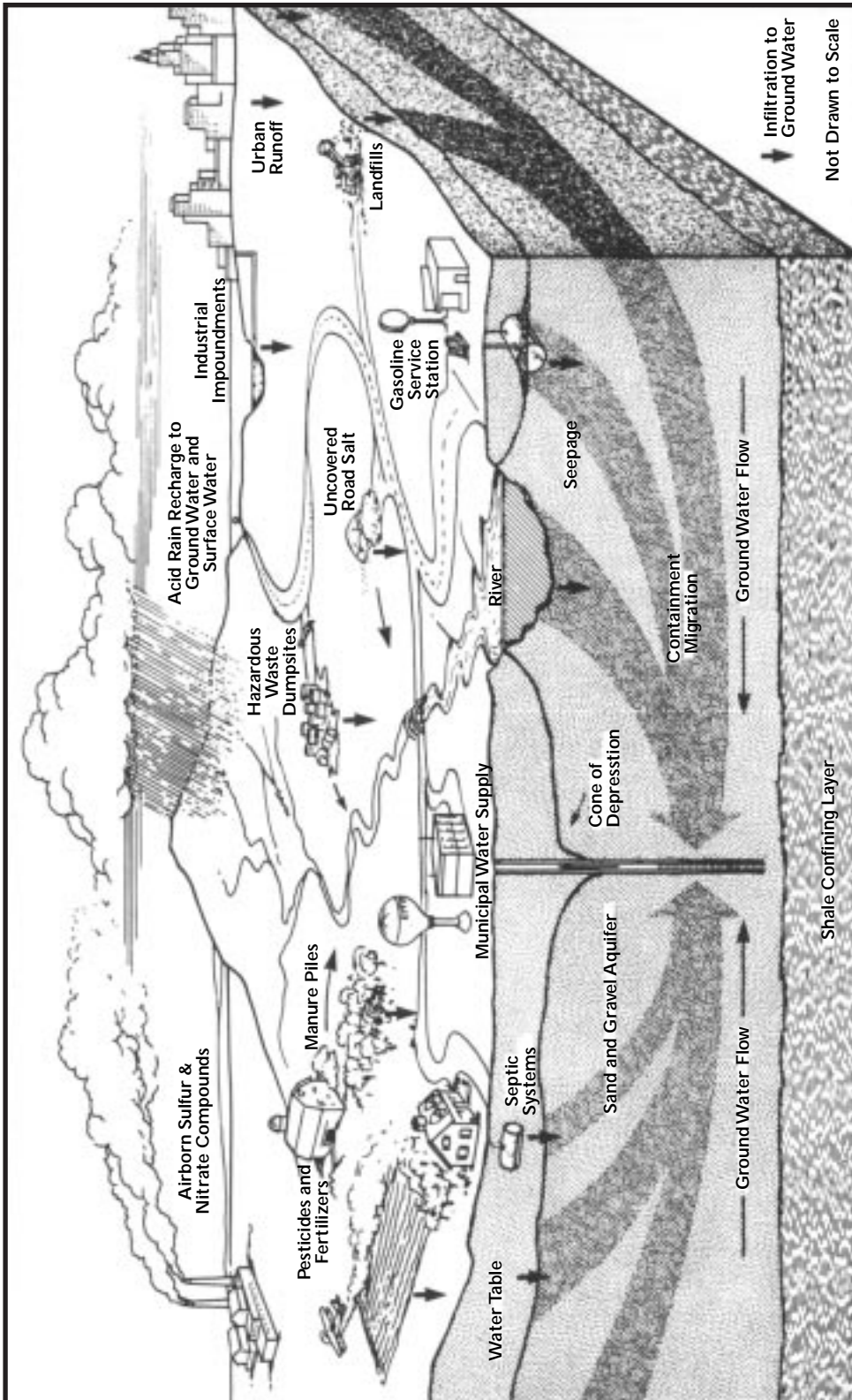
Source: Paly, Melissa and Lee Steppacher. The Power to Protect: Three Stories about Ground Water. U.S.E.P.A. Massachusetts Audubon Society and NEIWPCC.

Principles and Problems of Environmental Pollution of Groundwater Resources with Case Examples from Developing Countries

B. C. E. Egboka,* G. I. Nwankwor,† I. P. Orajaka,* and A. O. Ejiofor‡

The principles and problems of environmental pollution and contamination are outlined. Emphasis is given to case examples from developing countries of Africa, Asia, and Latin America with a comparative analysis to developed countries. The problems of pollution/contamination are widespread in developed countries but are gradually spreading from the urban to rural areas in the developing countries. Great efforts in research and control programs to check pollution-loading into the environment have been made in the industrialized countries, but only negligible actions have been taken in developing countries. Pollutants emanate from both point and distributed sources and have adversely affected both surface water and groundwaters. The influences of the geologic and hydrologic cycles that exacerbate the incidences of pollution/contamination have not been well understood by environmental planners and managers. Professionals in the different areas of pollution control projects, particularly in developing countries, lack the integrated multiobjective approaches and techniques in problem solving. Such countries as Nigeria, Kenya, Brazil, and India are now menaced by pollution hazards. Appropriate methods of control are hereby suggested.

Introduction

Environmental pollution and contamination are becoming a common occurrence in parts of the developing world. It is difficult to distinguish precisely between pollution and contamination. In modern hydrogeologic literature, pollution is regarded as occurring in such high dosages or concentrations that it renders the polluted medium very hazardous or highly deleterious to biota. Contamination may occur to a lesser magnitude when compared to pollution, but it also may render the contaminated medium unusable or make it slightly hazardous to life. Many urban and rural areas of the developed or industrialized world have been adversely affected by large-scale pollution and contamination, resulting in losses of human, material and financial resources. In many American, European, and Asiatic countries, huge amounts of money are spent annually

for research to combat and control widespread pollutants and contaminants. Volumes of these pollutants/contaminants are produced yearly through natural and anthropogenic activities such as industrial activities, agricultural practices, waste disposal systems, etc. High-level, medium-level, and low-level wastes in solid, liquid, or gaseous forms are released into the environment at discrete intervals or on a continuous basis. These pollutants may be physical, chemical, biochemical, biological, or microbiological in nature. They may have short or long half-lives in the environment. They have continued to damage many environments of the industrialized countries, having defied many painstaking control programs (1,2). Many urban centers of developing countries are now also similarly threatened. Unfortunately, these poor countries lack the necessary expertise and funds to wage any meaningful war against pollution, which continues to spread unabated.

Parts of the environment currently being polluted include the atmosphere, pedosphere, hydrosphere, lithosphere, and biosphere. This paper shall focus on pollution/contamination of the hydrosphere, with particular emphasis on the groundwater regime, and pollution incidences in developing countries. The scope shall embrace sources and types of pollution/contamination, processes generating them, implications of geology/hydrogeology, and pollution dynamics and mechanisms. Potentials of groundwater pollution in developing coun-

*Water Resources and Environmental Pollution Unit, Department of Geological Sciences, Anambra State University of Technology, P.M.B. 5025, Awka Campus, Awka, Nigeria.

†School of Applied and Natural Sciences, Federal University of Technology, P.M.B. 1526, Owerri, Nigeria.

‡Department of Applied Microbiology, Anambra State University of Technology, P.M.B. 5025, Awka Campus, Awka, Nigeria.

Address reprint requests to B. C. E. Egboka, Water Resources and Environmental Pollution Unit, Department of Geological Sciences, Anambra State University of Technology, P.M.B. 5025, Awka Campus, Awka, Nigeria.

tries *vis-a-vis* the developed ones shall be outlined, highlighting their health hazards. Relevant suggestions for combating pollution more effectively shall be made. The primary objective is to review the general incidences of environmental pollution/contamination in relation to the effects of pedology and geology in close association with the dynamics of the hydrologic cycle. Proper understanding of sources and types of pollutants/contaminants and their genesis and hydrodynamics would help determine the appropriate control measures to be considered for the situation. The goal is to contribute to better control methods. It is believed that present control methods in parts of the world lack the depth of understanding required. In addition, many of these control efforts seem to be uncoordinated. Developing nations still at the threshold of widespread pollution/contamination could learn from the costly mistakes of the industrialized nations and hence take the necessary actions to protect their environments.

The natural processes and anthropogenic activities that generate pollutants/contaminants are many and varied, and so are their sources. The natural processes include products of soil and gully erosion, physicochemical weathering and mass wasting, sediment transport, floods, volcanic eruptions, seawater intrusions, etc. The manmade ones include industrial, agricultural, sewage wastes and lagoons, garbage dumps and barnyards, mining wastes, etc.

These pollutants/contaminants in one way or the other via the hydrologic cycle reach the groundwater systems to pollute/contaminate them. Through the circulation of water within the hydrologic cycle, pollutants on the ground surface are transferred through the soil zone into the aquifer horizons where they damage potable water supplies. To reduce degradation of these water supplies, a comprehensive management strategy is required, as discussed later. The present control techniques with regard to pollution and contamination hazards, particularly in developing countries, need to be greatly improved. Priority and concern are not shown adequately by government authorities, and hence, appropriate planning and management strategies to check pollution are generally absent. The expertise or requisite manpower may be lacking. Funds for basic research may not be provided. Environmental protection laws or edicts may be nonexistent and where available are rarely enforced. These have exacerbated the spreading phenomenon of many pollutants/contaminants in many developing countries. In this review, necessary suggestions for improvement of this situation shall be given.

Sources and Types of Pollutants/ Contaminants

The two main sources of pollutants/contaminants are point sources (Table 1) and distributed sources (*2*) (Table 2). Pollutants/contaminants from the two sources may be released continuously (*3*) or at discrete intervals

Table 1. Point sources of pollution and contamination.

Type of pollution	Examples
Sewage disposal systems	Sewage lagoons
	Septic systems
	Cesspools
	Barnyards/feed lots
Surface waste disposal sites	Landfills/garbage dumps
	Surface waste dumps
Underground waste disposal sites	Storage tanks (low-, medium-, high-level wastes)
	Pit latrines, tunnels, trenches, caves
	Waste subsurface injections
Spills, washings, and intrusions	Oil/gas/waste spills
	Auto workshop washings
	Research/laboratory washings
	Seawater/saltwater intrusions
Mining sources	Acid mine drainages
	Gas explosions/seepages
	Mine dumps and gangue deposits
	Tunnels/excavations outflows
Natural mineral/ore deposits	Saline ponds/lakes
	Hot springs/mineralized waters
	Anhydrite/pyrite deposits/ evaporites

Table 2. Distributed sources of pollution and contamination (*1*).

Source	Examples
Agriculture	Cropland
	Pasture and rangeland
	Irrigated land
	Wood land
	Feed lots
Silviculture	Growing stock
	Logging
	Road building
Construction	Urban development
	Highway construction
Mining	Surface
	Underground
Terrestrial (many and scattered)	Landfills
	Dumps
Utility maintenance	Highways and streets
	Deicing
Urban run-off	Floods and snowmelt
Precipitation	Rainfall, snowfall, etc.
Background sources	Native forests
	Prairie land, etc.

(*4*). Point sources of pollution can be geometrically defined and the dimensions amenable to mathematical analysis in assessing pollution loads and rates of discharge determined. Point sources of pollution may assume any geometrical shape such as circular, triangular, spherical, etc. The areal sources of pollutants/contaminants or leachates are comparatively smaller, easily mappable, and readily distinguishable. However, where the input/output load functions from point sources into the hydrogeologic environment are continuous, the polluted/contaminated area may eventually become widespread. Distributed sources of pollutants/contaminants are much more widespread and can rarely be geometrically defined as precisely as a point source. Hence, it is more difficult to subject the input/output source to

precise mathematical analysis. Rather, a measured and intelligent assumption of the affected area is made for use in modeling and analysis. In heavily polluted/contaminated areas, both point sources and distributed sources may be occurring together or may be independent of one another. Successful control methods or mathematical modeling of the affected/polluted area must recognize this situation in order for the control program to be effective.

Point Sources of Pollution/Contamination

In the list of point sources given in Table 1, the pollutants or contaminants come from zones or areas of known and definable boundaries that are easily amenable to mathematical analysis and modeling. The pollution loads can be controlled at the point of input before they can spread into the surrounding environment in a time-discrete or continuous manner. Point sources include sewage lagoons (solid, gaseous, and liquid), industrial wastes, landfills/garbage dumps/barnyards, liquid/gaseous spills (oil, chemicals, etc.), mining (pits, holes, excavations, wastes, and gangue minerals), saline lakes and deposits, evaporite sequences, etc. Through the complex interplay of various soil and geologic factors and rain/water events of the hydrologic cycle, pollutant/contaminant substances reach the groundwater systems to pollute them. For example, buried refuse or garbage undergoes biodegradational decay in the pedologic/soil zone. The resulting leachates are released into the groundwater flow system where dissolved geochemical constituents are transported in various distances and directions. Piled up animal wastes in barnyards or liquid wastes in lagoons are similarly leached out and transported causing pollution of surface waters and groundwater areas. In many developing countries today, industrial and domestic wastes are indiscriminately dumped into rivers, lakes, streams, dry valleys, etc. This was the case in many developed countries, and such practices still persist in some of them today. These wastes damage surface waters and eventually destroy parts of the groundwater regime. Mining wastes and gangue products and other point sources of pollutants/contaminants produce similar havoc for the environment (4–9).

Distributed Sources of Pollution/Contamination

Distributed sources of pollutants/contaminants given in Table 2 are those in which the pollutants or contaminants are spread through a large area of hydrogeologic environment and in which they extend over the entire source area. A distributed source is very widespread, and the pollutants/contaminants may be introduced from various sources and directions. Spreading is enhanced by wind, rain, and snowfall activities through atmospheric circulation and precipitation. The areal extent or boundary conditions for the pollutants are difficult to define because of the regional nature of sources,

thereby posing problems for mathematical analysis. The sources include acid-alkaline rain, floods, erosion, agricultural fertilizer applications, and generated agricultural wastes, seasprays and intrusions, volcanoes, etc. Acid rain is a major distributed source of pollution in developed countries such as the United States, Canada, Germany, etc. Localized pollution of groundwater by acid rain in some developing countries such as Nigeria has been reported (10). Surface waters and shallow groundwater are polluted by atmospheric fallouts (3,11–13). In urban, suburban, and rural areas of many developing countries, particularly in the tropics, soil and gully erosion produce heavy sediment loads carried by floods that pollute surface water and groundwater systems (14–19). Waste products in urban areas are transported away by runoff. In mining areas, gangue materials dumped about recklessly on ground surface, decay and liquid wastes are leached from them becoming components of the hydrogeologic environment (20–22). In regions of intense geomorphic degradation and mass-wasting, physicochemical and biological weathering disintegrate pedologic and geologic materials to produce sediments that provide great quantities and varieties of pollutants. Fallouts from volcanic activities or atmospheric tests in one area may be spread into other regions of the world; wind, wave action, seaspray, or saltwater intrusion may drive contaminants inland and road salt application for de-icing during winter and widespread fertilizer usage, particularly in developed countries, are also major distributed sources of pollution. Similar events are now becoming prevalent in developing countries, particulary the industrializing ones.

Biological Pollutant/Contaminants in Groundwaters

Biological pollutants of groundwaters include dissolved organic constituents and microorganisms that seep or leach into groundwaters from polluted surface waters. Microorganisms may contribute to pollution in many ways, namely they may themselves be pathogenic; aesthetically they may produce undesirable biomass, or they may generate toxic metabolites in the groundwater. The microorganisms may be either pathogenic or nonpathogenic. In both cases, they produce undesirable effects in the groundwater itself and in the distribution network (where water may be distributed for domestic uses) and the populations using it.

Pathogenic Microorganisms. Pathogenic microorganisms are present in groundwaters, especially in the vicinity of facilities that are discharging sewage effluents or contaminated surface waters, and new septic tanks, agricultural wastes, and refuse tips. Microorganisms, however, must survive the tortuous task of passing through the soil cover, which constitutes an excellent natural process for water filtration and treatment. Even with this barrier, it follows that the nearer these sources of pollution are to groundwater sources, the greater the chance of successful seepage of these

microorganisms. Shallow wells and some deep boreholes are prone to contamination by these pathogens.

The isolation of pathogenic microorganisms from groundwaters is difficult but, when achieved, it serves as obvious proof of potential danger to the users, regardless of the number of pathogens present. Generally, however, the majority of waterborne pathogenic microorganisms enter water supplies as a result of fecal contamination. Therefore, the ability to detect fecal contamination at low levels is the main safeguard in preserving the potability of water supplies (23). Pathogenic microorganisms normally associated with water supplies are shown in Table 3 (24). All of these have been isolated from contaminated shallow wells and deep boreholes in Kaduna, Kano, Niger, and Plateau States of Nigeria (25). In addition, *Dracunculus medinesis* (Guineaworm) was reported from wells in parts of Nigeria such as in Kwara State (25). These parasites are widespread in many parts of Nigeria, sometimes occurring in epidemic proportions.

Fecal contamination in water is usually demonstrated by the detection of specific bacteria that are present in very large numbers in the intestines. The test normally employed is the presumptive Coliform test, which involves the most probable number (MPN) counts using liquid media. Coliform organisms include *Escherichia coli*, Citrobacter, Klebsiella, and Enterobacter spp., which are members of the family Enterobacteriaceae. They are gram-negative, oxidase-negative, nonspore-forming rods that can grow aerobically in a medium containing bile salts. They are able to ferment lactose within 48 hr, producing acid and gas at 37°C. A presumptive coliform test with a very high count is usually followed by a confirmatory test which is specific for *E. coli* (26).

Nonpathogenic Microorganisms. Many nonpathogenic bacteria are as important as the pathogenic ones in the pollution of surface water and groundwater supplies. These include the sulfur and iron bacteria. Among the sulfur bacteria are the sulfate reducers such as Desulfovibrio, Desulfomonas, and Desulfotomaculatum, which produce elemental sulfur from sulfates. On the other hand, some of the sulfur bacteria oxidize elemental sulfur to sulfates, all of which involve complex oxidation-reduction reactions. These include the ubiquitous chemolithotrophic Thiobacillus and the filamentous gliding bacteria Beggiatoa and Achromatium. The sulfur-oxidizing bacteria normally associated with groundwaters have been described by Trudinger (27), LeGall and Postgate (28), and Ehrlich (29) (Table 4).

Iron bacteria are frequently present in groundwaters and in particular those subject to a degree of organic pollution. They obtain energy for their metabolism by the oxidation of ferrous and/or manganous ions. These include the gliding bacteria Toxothrix; the sheathed bacteria, Spaethilus, Leptothrix, Crenothrix, and Clonothrix; the budding and/or appendage bacteria, Pedomicrobium, Gallionella, Metallogenium, and Kusnezovia and the gram-negative chemolithotrophic bacteria, Thiobacillus (*T. ferrooxidans*), Siderocapsa, Naumaniella, Ochrobium, and Siderococcus (30). Pathogenic and nonpathogenic microorganisms (bacteria, fungi, viruses) are thus hazardous environmental pollutants to the hydrogeologic environment. They enter this environment from waste disposal and treatment areas, sewage lagoons, barnyards, landfills, and mine areas (31).

Table 3. Pathogens associated with water supplies.

Pathogens	Diseases caused
Bacterial	
Salmonella typhis	Typhoid fever
Salmonella paratyphi A and B	Paratyphoid fever
Salmonella typhimurium	Salmonellosis
Shigella sonnei	
Shigella dysenteriae	Bacillary dysentery
Shigella flexneri	
Hycobacterium tuberculosis	Tuberculosis
Vibrio cholerae	Cholera
Francisella tularensis	Tularaemia
Enteropathogenic *Esherichia coli*	Enteritis
Leptospira icterohaemorrhagia	Leptospirosis
Viral	
Hepatitis A virus	Viral hepatitis Type A
Enteroviruses (polio, Coxsackie A and B and echo)	Respiratory tract infection, nonbacterial
Adenoviruses	enteritis
Parvoviruses	
Reoviruses	
Protozoan and metazoan	
Enteamoeba histolytica	Amoebic dysentery
Acanthamoeba spp.	Amoebic meningoencephalitis
Naegleria spp.	Amoebic meningoencephalitis
Giardia lamblia	Giardiasis
Ascaris lumbricoides	Helminthiasis
Trichuris trichura	
Taenia spp.	

Table 4. Sulfur oxidizing bacteria in groundwaters.

Genus or group	Habitat
Chemotrophs	
Sulfobacillus	Mine tips
Thiobacillus	Water, soil, marine
Sulfolobus	Geothermal springs
Thiobacferium	Water
Macromonas	Water
Thiovulum	Water
Thiospira	Water
Beggiatoa	Water, soil, marine
Thioploca	Water, soil
Thiothrix	Water, soil, marine
Achromatium	Water
Thiodendron	Water, soil
Phototrophs	
Chromatiaceae (purple S bacteria)	Water, marine
Chlorobiaceae (green S bacteria)	Water, marine
Chloroflexaceae	Geothermal springs
Oscillatoria (Blue-green algae)	Water

They occur in varying degrees in both oxidizing and reducing environments. In the process of complex redox activities that break down organic and inorganic materials to release energy for metabolic activities, poisonous substances are generated that may be fatal to the hosts that ingest them. These redox microbial activities may also degrade their habitats, rendering them unusable. Such degraded states may remain so for a long time.

Organic Pollutants in Groundwater

Organic pollutants that may be found in groundwater through shallow wells and deep boreholes include dissolved organic carbon (DOC) and particulate organic carbon (POC). They, in association with microorganisms, cause destructive pollution or contamination in hydrogeologic environments. They may serve as nutrient/energy sources for microorganisms. Where they are heavily loaded into groundwaters, DOC and POC enhance microbial multiplication and growth, thereby rendering the habitat anoxic. In such environments, denitrification, desulfurization, etc., are rampant, engendering an abundant growth of bacteria, fungi, and viruses that may be highly pathogenic. Hence, serious organic pollution signals a potential heavy microbial pollution of a groundwater system.

Thermal Pollution/Contamination

Thermal pollution/contamination may result from two main sources, namely, industrial and geothermal pollution. In industrialized countries and some developing ones, heat generated by industries is discharged through wastewater into the environment. High temperature waters eventually reach shallow aquifers and adversely affect groundwater. Hot waters discharged into lakes that are influent may form high temperature haloes that extend into the aquifers underlying the lake. Unchecked thermal pollution not only negatively affects the life in the lake but also that of the groundwater system associated with the lake. Other problems that can arise are the changing of physical, chemical, and biological characteristics of the hydrogeologic system, thereby rendering the surface water and groundwater unusable.

In many parts of the world, the locations of geothermal pollution hazards are known. Geothermal pollution is much more common in tectonically unstable environments where high temperature effluents and gases emanate from deep horizons or the core within the earth. They move up to the shallow hydrogeologic zones through fractures (joints, faults, shear zones) and heat up surrounding groundwaters to generate hot and warm springs. In addition to the hotness of such waters, they are also highly mineralized because the high temperatures enhance the dissolution of soil and geologic materials.

Geologic and Hydrologic Cycles

Pedologic, geologic, and hydrologic cycles have several components and characteristics that enhance or aggravate the incidences of pollutant/contaminant origin, transport, and spread through hydrodynamic dispersion (diffusion, advection and dispersion) into the hydrogeologic environments that embrace the atmosphere, pedosphere, lithosphere, hydrosphere, and biosphere.

Geologic Cycle

Geologic rock units may be fractured, faulted, and jointed during tectonic movements or may be layered during the deposition and consolidation of sediments. Weathering disaggregates rocks into soils and sediments that are transported away by wind, water, and/ or man. During these processes of the geologic cycle, pollutants and contaminants may be formed or released. Figure 1 shows significant parts of the complex geologic cycle that are relevant to groundwater pollution. Soil and geologic characteristics vary in horizontal, lateral, and vertical directions. Soil characteristics include grain size, porosity, permeability, stress-strength properties, cohesiveness, and other physical and chemical properties. The soil components of unconsolidated geologic units that form aquifers have similar physicochemical characteristics. Additionally, layering or stratification properties of both consolidated and unconsolidated geologic units are factors that affect pollutant/contaminant inputs, transport and dispersion. Stratigraphic properties of directional lithologic changes, facies changes, stratification and stratal thicknesses, degrees of sedimentation, cementation, and diagenetic changes affect the life of pollutants/contaminants in groundwater (Fig. 2) (14). The structural characteristics of fractures, faults, joints, and folds (Fig. 3) of igneous and metamorphic rocks and consolidated sedimentary rocks are also significant in groundwater pollution and contamination.

FIGURE 1.   Geologic cycle.

(a) Horizontal Stratification and Facies Changes

(b) Layering and Vertical Stratification

FIGURE 2.   Lithologic changes.

Subsequent discussions of the geologic and hydrogeologic settings shall explain further the obvious implications of these properties in the genesis, transport, and dispersion of pollutants/contaminants in groundwater flow systems. It will then be clear that any successful stoppage or control of groundwater pollutants and contaminants must take into serious consideration the implications of geologic and hydrogeologic characteristics of the particular polluted or threatened environment. Currently, pollution/contamination planners and managers do not seriously consider geologic prop-

FIGURE 3.   (a) Folds, (b) joints, (c) fault, (d) graben.

erties and characteristics in design and control programs, thereby creating situations that frequently produce failures in engineered structures.

Hydrologic Cycle

The several processes as briefly outlined below that occur within the hydrologic cycle (Fig. 4) are the driving forces and agents of groundwater pollution. The atmosphere serves as the gaseous envelope surrounding the earth. Precipitation through condensation of rain clouds falls down to earth as rain, snow, hail, etc. Atmospheric pollutants and contaminants may be washed out of the atmosphere as fallout. Runoff carries pollutants into surface waters for possible evaporation back into the atmosphere or storage in rivers, streams, lakes, and oceans, seas, etc. Some of the fallout or rainout may infiltrate into the soil zone to be evapotranspired to the atmosphere or percolate into the groundwater zone. Here moisture joins a complex hydrodynamic flow sys-

FIGURE 4. The hydrologic cycle.

tem possibly to be transported to the oceans or other surface waters where evaporation may return the water back to the atmosphere. In all these processes, pollutants and contaminants may be produced and cyclically dispersed from one point of the hydrologic cycle to another. This is graphically shown in the cyclic pollution/contamination of the hydrogeologic system otherwise called the hydrogeopollution cycle (Fig. 5). Pollutants and contaminants may be generated through natural or anthropogenic processes and circulated in the environment (atmosphere, pedosphere, lithosphere, biosphere,

FIGURE 5. The hydrogeopollution cycle.

and hydrosphere) through the activities of air, water, chemical, physical, and microbiological processes. These complex and cyclic processes may be continuous with respect to distance and time and may be localized or regional in areal spread. Thus, pollution at one source or an area may threaten nearby or distant places unless its spread is checked or controlled.

The implications of the geologic and hydrologic cycles are much more pronounced when one relates them to the long half-lives and transport of high-level radioactive wastes from industries. These materials are being stockpiled in different parts of the industrialized nations and some developing countries waiting to be safely disposed of in secure geologic rock units. It is now known that even deep-seated geologic hard rock formations have cracks, joints, and even faults through which moving groundwaters can transport pollutants/contaminants in many directions. It is also possible that the grain size and lattice structure of such rock units may permit widescale diffusion/dispersion of gaseous/liquid pollutants that may eventually threaten the biospheric environment with time. Hence, structural and stratigraphic characteristics of geologic units and the hydrodynamics of percolating or flowing groundwaters must prominently occupy the minds of planners, designers, and managers of waste disposal and management systems. Unfortunately, at present, this has not been the case, particularly in developing countries where pollution/contamination is becoming more commonplace. Even though those in developed countries now consider geologic and hydrogeologic effects, it has been late in coming, and pollution has ravaged many areas, precipitating devastating losses in financial, water, land, and human resources. Even with graphic examples that illustrate the consequences of poor planning, many plan-

ners and managers are still skeptical about the role of geology in environmental pollution. At the same time, the lack of consideration of geologic, pedologic, and hydrologic/hydrogeopollution cycles has not been fully appreciated in developing countries. There does not seem to be enough consciousness given to understanding the implications of these cycles. Because of this, no priority is given to examining the impact of pollution incidences and the consequences of spread in the hydrogeologic environments.
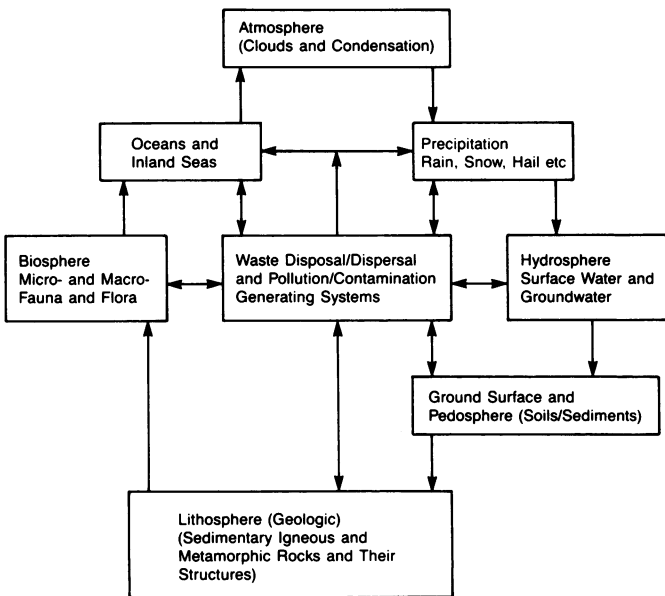
Processes and Activities Generating Pollutants/Contaminants

Various processes, some of which may be manmade or anthropogenic, generate pollutants and contaminants that enter groundwater flow systems. These processes include physicochemical weathering, mass wasting, erosion, sediment transport, and deposition; agricultural activities; mining, mine-waste disposal, and acid mine drainage problems; oil exploration, exploitation, and gas flaring; other industrial activities such as manufacturing, distribution of manufactured products, arms, and armaments, etc.; sewage treatment, disposal, and management; runoff, floods, and snowmelt; biological pollution of wetlands and impounded reservoirs; saline lakes, ponds, and evaporite deposits; geothermal springs and mineralized waters; atmospheric fallout and rainout; burial grounds, garbage dumps, landfills, etc. Some pollution sources in rural environments that are usually ignored, even though they may be hazardous, include pit latrines, open-space communal toilets, widescale and indiscriminate uses of the bush for defecation, personal hygienic uses of water for washings, etc., microbiological activities (bacteria, virus, fungi, worms, etc.), radioactive material, and thermal products, heavy metals, trace elements, ions, etc.

Chemical Pollutants/Contaminants of Groundwater

Many developing countries are witnessing a stage of development where groundwaters from shallow wells and boreholes are gradually supplementing the original source of drinking water (surface water). The preference for groundwater to surface water is borne out of the belief that when surface water has been distributed as tap water it must always be subjected to some purification prior to distribution. Although surface waters are easily accessible where they exist in lakes, rivers, streams, and springs, many people believe that water wells produce water of excellent quality. Thus, groundwater is not treated before use and is believed to be free from pollution.

One place where one can find groundwater about as pure as rainwater is under a bare dune made of pure quartz sand (32). The water under quartz sandstone is clean and pure because quartz is so insoluble in water that for practical purposes, it is inert and neither soil

nor vegetation contributes dissolved substances to the groundwater it contains. Areas of pure quartz sand dune are few. Most surface soils and water-bearing formations are not pure quartz sandstone. A sandstone is made up of soil particles of different mineralogy. These particles are bound together by cementing materials, which are generally calcite, hematite, or silica. In contrast to quartz sand, surface materials of soils, limestone, shale, and other lithologic types react with percolating and infiltrating water to produce dissolved materials that pollute groundwater (Figs. 4 and 5).

Any groundwater system may be naturally polluted or contaminated to a certain degree at all times. The concern of many water resource planners and managers is whether the amount of measured pollutants are within the acceptable limits of water quality. The number of chemical pollutants and the degree of chemical pollution/contamination of groundwater depend on the geology, pedology, and the mineral composition of the soil and rock through which the water flows before reaching the aquifers. Groundwaters may have pollutants that not only depend on the pedology, geology, and mineralogy of the formations it flows through but also on the constituent pollutants/contaminants in the water that recharges the groundwater.

Recharge water, on the other hand, may be contaminated by atmospheric fallout, industrial and domestic wastes, etc. The type of physical, economic, agricultural, and social activities of the people living in a groundwater recharge area (Fig. 6) may affect its water quality. Thus, urban planners and managers must be wary of human activities that occur in recharge areas of aquifers in urban and rural areas. This is one of the main avenues by which pollutants or contaminants enter the groundwater. Hydrogeologic maps of the areas are lacking or have not been produced, so the recharge areas are not identified. This problem affects both developed and developing countries. Massive pollution of groundwater systems is a common occurrence because wastes are recklessly deposited on top of recharge areas.

Groundwater pollution is an ever present risk in developing countries, particularly in areas of mining and extensive industrial activities. This must constantly be in the minds of those responsible for water supplies in these areas. Borehole waters must, as a rule, be analyzed for chemical contaminants before the water is distributed and supplied to households. Unsatisfactory color and taste are easily detected and are good indicators for groundwaters of poor quality. Some groundwaters taste of iron, others may have a disagreeable odor. Such groundwaters should be avoided and not used for domestic purposes. Water containing only several parts per million of sodium and chloride ions tastes slightly salty. Lead and sulfur are distasteful when present in appreciable quantities in water. Conversely, however, some toxic elements have no taste, but when present in very small quantities may be dangerous to health. Very small concentrations of poisonous trace elements such as lead, arsenic, mercury, cyanide, and boron must
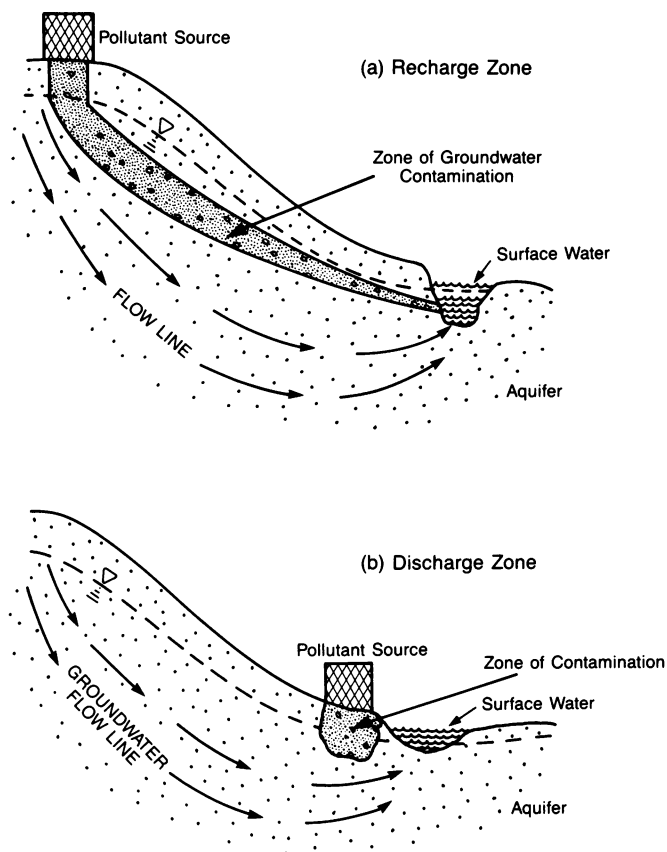
FIGURE 6. Schematic illustration of the extent of groundwater contamination for pollutants entering (a) recharge and (b) discharge zones.

be carefully documented. Water sampling and monitoring may reveal their presence.

The three components of water quality are bacteriological quality, physical quality, and chemical quality. Filtration and sedimentation processes take care of the physical quality. In practice, groundwaters are filtered by natural processes as they pass through columns of soils, sands, strata, or sedimentary layers of rocks. Groundwaters are usually clear of solid materials as they come from the aquifer, particulary if they are deep-seated ones. The intricate pore spaces or water passageways of the aquifer materials act as a fine filter and remove small particles of clay or any other fines. Organic materials decay or are destroyed in transit. Thus, the dirtiest and most polluted sewage water may become clear of suspended/particulate solid materials once it has gone through a thick bed of sand or geologic and pedologic units. As a result of this natural self-cleansing of polluted water by deep-seated aquifers, physical and some biological aspects of pollution may not pose serious problems in groundwaters.

Bacteriological quality of groundwater is taken care of by treatment with various chemicals that kill bacteria. Dissolved geochemical constituents, on the other hand, are difficult to remove entirely. They may be removed through filtration in charcoal, through cation exchange, precipitation, dissolution, degassing, etc. The treatments may be expensive and may result in the creation of new pollutants and contaminants and their introduction into the treated waters. In developed countries, waters supplied for public use are routinely analyzed to certify that the content of toxic elements or contaminants are below mandatory limits. In many developing countries, groundwaters and even surface waters are often distributed to various communities untreated. In general, it is wrongly believed that exploited groundwaters are free of pollution. Emphasis is usually placed on water supply and quantity, and minimal priority is given to water quality and treatment. This is the main cause of outbreaks of epidemics of waterborne diseases in many developing countries because untreated surface water and groundwater are consumed by the people, particularly in rural areas, without water treatment. Since there are no monitoring programs, it is not possible to detect incidences of pollution.

The most undesirable trace elements-pollutants in groundwater are mercury (Hg), lead (Pb), cadmium (Cd), arsenic (As), barium (Ba), boron (B), cyanide (CN) selenium (Se), chromium (Cr), uranium (U), sulfur (S), and nitrogen (N). The United States Food and Drug Administration has specified the lower mandatory limits of these elements in domestic water. In addition, there are internationally accepted water quality guidelines for concentrations of these elements in water for various uses. Most groundwaters may also contain many major inorganic elements, compounds, and ions in excess of acceptable standards, such as iron oxide (Fe_2O_3), manganese (Mn), calcium (Ca), magnesium (Mg), chloride (Cl), aluminum (Al) and silica (SiO_2). Anions and cations can be found in their dissolved states. Temperature, where high, affects the properties of groundwater, especially the solubility of minerals. Deep groundwaters contain more chemical elements dissolved in them than do shallow ones. Very deep, hot groundwaters may be objectionable because they contain very high concentrations of these mineralized elements dissolved out of rocks by the high temperatures. This is characteristic of hydrogeothermal regions.

Groundwaters in limestone or Karstic terrains may contain dissolved calcium and magnesium salts. These salts make water hard. Hardness in excess affects taste and soap consumption in laundry. Temporary hardness is caused by carbonates and bicarbonates of calcium and magnesium; permanent hardness is caused by sulfates, chlorides, nitrates, and silicates of calcium and magnesium. The combination of both of these groups of substances gives total hardness. Hard water may also have a slight taste that is caused by other ions in solution.

A few groundwaters naturally become softened as they pass through and react with geologic formations containing zeolite minerals, which remove calcium and other ions. Zeolites and hydrous silicates chemically exchange certain ions from water for other ions bound in the solids. If, for example, water carrying calcium ions travels through a zeolite formation that exchanges Na^+

for Ca^{2+}, the water comes out with sodium instead of calcium and the zeolite becomes richer in Ca and poorer in Na.

Iron in water gives a bitter taste and makes water reddish or brownish in color. It is found in the form of bicarbonates and sulfates. Iron in groundwater is due to the presence of hematite below the ground surface. Iron is soluble in water containing carbonic acid. Water containing high iron is unsuitable for laundries, paper mills, film industries, etc.

Manganese acts in a manner similar to iron. It produces an undesirable taste, and white clothes washed in water containing manganese or iron turn yellow or brown. In small concentrations, Mn affects odor. Iron and Mn in water precipitate and produce undesirable turbid yellow-brown water that stains laundry. These elements support growth of microorganisms in distribution systems. These growths can accumulate and reduce the carrying capacity of pipes and clog valves. Small concentrations of Fe and Mn impart a metallic taste to water. Groundwater supplies contain more Mn and Fe than surface waters.

Excess carbon dioxide in water makes water corrosive to metals. It can be present in the form of carbonic acid, bicarbonate, carbonate or as free carbon dioxide (CO_2). Groundwaters drawn from great depths are deficient in dissolved oxygen (DO). Shallow groundwaters are usually saturated with dissolved oxygen. Hydrogen sulfide (H_2S) makes water unpalatable because of bad odor and taste. It is found in groundwaters where sulfide minerals such as galena, pyrite, sphalerite, etc., are present. Other dissolved gases in addition to CO_2, DO, and H_2S that may cause pollution problems in groundwater include nitrogen dioxide (4,33), methane (31), and sulfur dioxide. They cause degrees of hazard in hydrogeologic systems that are functions of concentration but are hazards that can be removed during water treatment processes.

Geologic and Hydrogeologic Settings

Geologic Settings

Natural geological processes are primary contributors to groundwater pollution. In this regard, the rocks of the earth's crust are the major contributors of groundwater pollutants (Figs. 1 and 5). These contaminants are mostly minerals, gases, and the toxic elements they contain. A good understanding of the origin, occurrence, and distribution of these undesirable elements is essential for people concerned with the development of surface water and groundwater resources. Contaminants enter groundwaters from the recharge areas of aquifers. It is therefore very necessary to know the geology and mineral distribution in recharge areas of aquifers. Groundwater that is recharged from mining areas, fertilized agricultural farmlands, and industrial areas may not be safe for human consumption unless the recharge area is protected. Rain and surface water can leach pollutants from mine dumps, ore deposits, city dumps, and fertilizer applied to farm lands into the recharge areas for groundwater supplies (Fig. 6).

Groundwaters recharged from polluted areas must either be avoided or pollution sources checked and monitored very carefully. The geology of recharge areas of aquifers influences the quality of groundwater. The number of pollutants and the degree of pollution in groundwaters depend on the geology and the mineralogical compositions of the rocks through which the water flows. Groundwaters may also acquire pollutants and contaminants as they flow across difference geological and mineralogic zones or units of formations. Chemical reactions between the water and rock fragments in the soil and numerous chemical and physicochemical reactions between water and rock in the groundwater environment alter the composition of the groundwater. Surface waters recharged by surface water draining through limestone areas may be hard and need considerable softening treatment before being supplied for domestic and industrial use. Rainwater absorbs carbon dioxide in the air, forming carbonic acids, which react with limestone, liberating bicarbonates and carbonates which, when added to percolating groundwaters, make them hard. Surface materials of soils and shale react with rain to produce dissolved materials that contribute to the taste and color of groundwaters. The taste and color of such waters depend on the composition of the rocks through which the water passes. Surface waters draining metamorphic areas containing talc dissolve magnesium salts that contribute to the hardness of groundwater.

Surface water draining areas of sulfide mineralization introduce sulfur and many other metallic ions into groundwaters. The chemical characteristics of groundwater vary greatly as a result of the diversity of rock materials through which the water passes. Sedimentary rocks provide the largest aquifers and water passing through such rocks acquire considerable concentrations of chemical components of these rocks. Carbonate rocks, such as limestone and dolomite dissolve easily in acid water with the result that water in contact with these rocks are high in calcium and magnesium bicarbonates. In igneous and metamorphic terrains, groundwater occurs in weathered parts of the basement rocks or faults, joints, and fissures. Rainwater percolating through these fissures dissolve many soluble elements. The groundwater in basement areas may be contaminated by ions dissolved from the basement rocks (Fig. 3).

Weathering and oxidation of basement rocks create a favorable acid environment for mobilization of many metals. Weathering and fracturing of basement rocks also create porosity necessary for storing water. Consider a granite containing about 5 ppm of uranium in solution. During weathering uranium will be oxidized to the soluble uranyl oxide in the near surface weathering environment. The oxidized uranium is then mobilized and dissolved in shallow groundwater in the basement area. Data from Trenthan and Orajaka (34) and Orajaka (35) show that significant amounts of ura-

nium are leached and mobilized from felsic igneous rock by carbonic acid water. A significant quantity of uranium is leached from the albite-riebeckite-granite in Kaffo Valley, Northern Nigeria, during weathering of the roof of the granite mass (*36*). This example illustrates how abnormally high uranium and other major, minor, and trace elements are leached and introduced into shallow groundwaters in the veins, fissures, and porous parts of basement rocks.

It is thus important to test all shallow groundwaters in basement or hard rock areas for metal contamination before supplying such water to homes and industries. In many developing countries, rural dwellers obtain their water from shallow, hand-dug wells (*37*). In most cases where the water is tested, they are usually contaminated, particularly for shallow wells in urban centers situated in basement rock areas. Toxic metals, notably Pb from weathering products of ores in mineralized veins of faults and fissures, can enter natural water systems. The metal concentrations are abnormally high for water descending through fissures exposed during mining operations. These contaminated waters find their way into groundwaters, thereby polluting them. Surface water and floods in the Pb-Zn mineral belt of southeastern Nigeria contain high concentrations of Pb-Zn. These metal ions are also present in shallow groundwaters in the area. The high Pb content of the shallow groundwater have posed some serious health problems. Their implications and extent of hazards are yet to be fully investigated.

Structural Features and Sedimentary Characteristics

The structural and sedimentary properties of rocks that affect groundwater quality include layering, fractures, joints, faults, and shear zones (Figs. 2 and 3). These structural features act as hosts for mineralizations and also serve as channel ways that conduct surface waters to aquifers. Faults, shear zones, and joints are often mineralized. The most common veins and fissure-filling minerals are sulfides. Oxygen-laden surface waters and carbonic acid waters entering these fissures oxidize and dissolve cations and anions from the sulfide minerals and introduce them into shallow groundwaters. The toxic metallic elements introduced into the groundwater by this method include As, Cs, Hg, Pb, Ni, etc. Thus, in the surface or subsurface disposal of wastes, the structural, stratigraphic, and sedimentological natures of the area must be mapped, described, and known in order to produce a waste management design that would contain the wastes safely and keep these toxic elements from migrating into surrounding surface waters and groundwaters.

Hydrogeologic Settings

Because contaminants are transported in large part by the bulk motion of groundwater, the parameters of groundwater flow are of major importance in the understanding of contaminant processes. The various aspects of the groundwater environments, as well as stratigraphic factors that control or could influence groundwater motion, are also of major consideration. The hydrogeological environment is shown schematically in Figures 2 and 4. It consists mainly of the saturated and unsaturated zones. The unsaturated zone occurs above the capillary fringe where the soil pores are partially saturated with water. This zone is important in waste management because in most cases, it is the burial zones for wastes. Consequently, a thick unsaturated zone may sometimes be preferred for waste disposal since it would take a much longer time for contaminants to reach the water table. In the saturated zone, the pores are saturated with water. When this zone is capable of transmitting significant quantities of water for economic use it is referred to as an aquifer. In most field situations, two or more aquifers occur, separated by impermeable strata or aquitards. In the situation illustrated in Figure 2, the upper or unconfined aquifer is much more prone to pollution than the lower confined aquifer.

Fluid motion in saturated geological materials is dependent on the hydraulic gradient, porosity, and hydraulic conductivity. Average groundwater velocity $\bar{v}$ is obtained from the relation (*38*),

$$\bar{v} = -\frac{Ki}{n}; \quad i = \frac{dh}{dl} \tag{1}$$

where i(dh/dl) is the hydraulic gradient, n is the porosity, K is the hydraulic conductivity, and dh/dl is the change in hydraulic head (h) with respect to the change in distance (l). Porosity and hydraulic conductivity, in particular, are properties that are dependent on the geologic conditions of the waste disposal site. Differences in hydraulic conductivity values across a stratigraphic section could appreciably determine whether flow is upward, downward, or horizontal, as demonstrated by Freeze and Witherspoon (*39*). Thus, the ultimate fate of contaminants emanating from waste disposal sites can be strongly dependent on whether groundwater flow is upward or downward. Therefore, the direction of flow of groundwater is an important factor in the evaluation of sites for waste disposal. The actual magnitude of groundwater velocity is also an important factor. In low permeability geological materials, groundwater velocity can be as low as a few centimeters per year. For such conditions, contaminants would be transported over very short distances over a very long time span and hence may not pose hazards to the environment.

In contrast to the low velocity of groundwater that occurs in low permeability materials such as shales, the velocity in permeable deposits or fractured media can be quite large. High groundwater velocity zones provide a pathway through which water supply sources become quickly polluted. The search for and the evaluation of such high-velocity pathways is therefore an important task in the groundwater pollution studies. The ease and

extent to which contaminants can pollute an aquifer are also dependent on whether the contaminants are introduced into the groundwater system at the recharge or discharge areas. Figure 6 shows that a major portion of an aquifer may become contaminated if the contaminant is introduced into the subsurface from an upland recharge site. Hence in designing or planning a waste disposal site, the entire hydrogeological properties of the area must be well established to ensure a safe and long-lasting disposal network.

Contaminant Pathways and Processes in Groundwater Systems

Pathways of entry of contaminants into groundwater systems depend largely on patterns of waste disposal and human interferences with the environment. An understanding of the general methodologies of waste disposal is thus a prerequisite to any discussion of contaminant pathways into the subsurface environment. The various waste disposal options currently in use include sanitary landfills, open dumps, septic tanks and cesspools, and deep well injection systems.

Sanitary Landfills and Garbage Dumps

Much of the solid waste that is now disposed of on land is placed in sanitary landfills. In humid areas, in particular, buried waste in sanitary landfills and dumps is subject to leaching by percolating rainwater. The leaching process is accompanied by chemical reactions that tend to consume all available oxygen, while releasing carbon dioxide, methane, ammonium, bicarbonate, chloride, sulfate, and heavy metals. The liquid mix of these constituents is referred to as leachate. The total number and chemical concentrations of these constituents can be variable depending on the initial composition of the waste climatic conditions. Table 5 shows that leachates contain large numbers of inorganic contaminants and also have high total dissolved solids. Leachates also contain many organic contaminants. Robertson et al. (40), for example, identified over 40 organic compounds in leachate samples (contaminated groundwater in a sandy aquifer in Oklahoma). Leachates emanating from landfills contain contaminants and toxic constituents derived from solid wastes, as well as from liquid, industrial wastes placed in the landfill (41).

Rain water percolation through refuse in the landfill causes water table mounding, i.e., a rise in water table elevation within or below the landfill. The mounding process, according to Freeze and Cherry (38) causes leachate to flow downward and outward from the landfill, as illustrated in Figure 7. Thus, for shallow aquifers, in particular, water table mounding provides a pathway for the entry of contaminants into the groundwater system as a result of the buildup in hydraulic gradient and pressure head.

Septic Tanks and Cesspools

Septic tanks are designed to remove settleable solids, reduce biochemical oxygen demand, eliminate micro-

Table 5. Representative ranges for various inorganic constituents in leachate from sanitary landfills (36).

Parameter	Representative concentration range, mg/L
K^+	200–1000
Na^+	200–1200
Ca^{2+}	100–3000
Mg^{2+}	100–1500
Cl^-	300–3000
SO_4^{-2}	10–1000
Alkalinity	500–10,000
Fe (total)	1–1000
Mn	0.01–100
Cu	< 10
Ni	0.01–1
Zn	0.1–100
Pb	< 5
Hg	< 0.2
NO_3^-	0.1–10
NH^{4+}	10–1000
PO_4^{3-}	1–100
Organic N	10–1000
Total dissolved organic carbon	200–30,000
Chemical oxygen demand	1000–20,000
Total dissolved solids	5000–40,000
pH	4–8
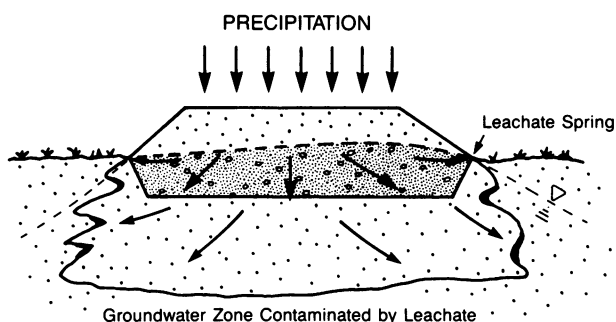
FIGURE 7. Water table mound beneath a landfill, causing migration of contaminants deeper into the groundwater zone (38).

organisms before (the treated) sewage is released through a drainfield into the ground. A generalized diagram illustrating the layout of a septic tank waste-disposal system is shown in Figure 8. The figure demonstrates that septic tank system effluents can quite easily reach and contaminate the groundwater system. According to the United States Environmental Protection Agency (42), septic tanks and cesspools are the largest contributors of wastewater to the ground and are the most frequently reported sources of groundwater contamination in the United States.

Apart from the effluent that is directly released into the ground, there are large volumes of solid residual materials known as sewage sludge. In many parts of the world, this sludge, which contains a large number of potential contaminants, is applied on agricultural lands to enhance crop nutrients such as nitrogen, phosphorous, and heavy metals that are needed for plant growth. Although this practice actually improves soil fertility, it has been observed that one of the potential
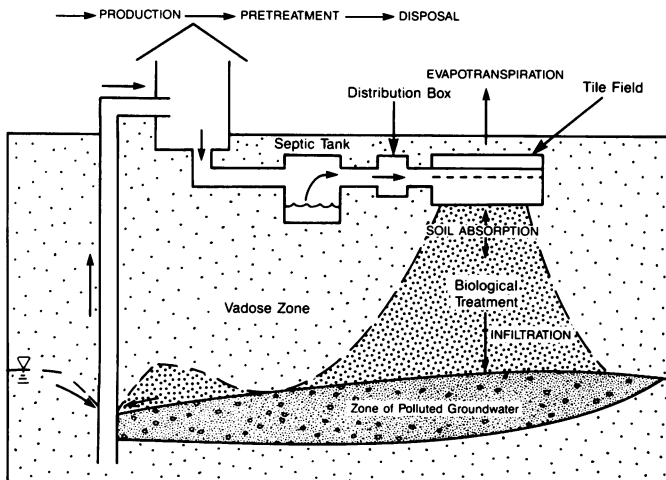
FIGURE 8. Contamination from a septic field (57).

negative impacts of this type of sewage disposal is degradation of groundwater quality (38). Contaminants in the sewage sludge/effluent reach and contaminate groundwater through infiltrating water from rain or snow.

Radioactive Waste Disposal

Radioactive wastes are generated at various stages in the nuclear industry. Mining and milling of radioactive ores result in the production of large volumes of waste rock and tailings. Nuclear plant operation generates radioactive fission products, reactor cooling waters, irradiated fuel rods, and other by-products.

Figure 9 illustrates several types of waste burial alternatives. In all cases, the wastes are stored in strong, engineered concrete containers. In the option illustrated by Figure 9a, the containers are placed on the surface of the ground and then covered with earth materials. Figures 9b and 9c illustrate the options in which the containers are placed a few meters below the surface of the ground either below or above the water table. The difference between these two options is that in the latter (Fig. 9c) the fill in the excavation is designed to provide enhanced containment capability for the system. A large number of burial options for radioactive wastes in Canada and the United States are in the category represented by Figure 9b (38). In Figures 9d and 9e, the containers are buried in large holes about 10 to 20 m deeper than in the previous examples.

Because of the highly lethal nature of radioactive wastes, particularly for those with long half-lives, it is necessary that these wastes are disposed of in systems that have high containment capability. Failure of the burial sites could result in the leakage of radioactive materials into the groundwater environment or into the biosphere. To avoid problems of subsurface radionuclide migration, numerous reported investigations including Cherry et al. (43) have suggested that burial sites should have the following characteristics: geomorphic and structural stability, isolation from fractured bedrock, absence of subsurface flowlines that lead directly to the biosphere or to subsurface zones of potable water, low measured or predicted radionuclide velocities, and water table conditions that are deep enough to permit waste burial to remain entirely in the unsaturated zone.

Deep-Well Disposal

Disposal of liquid wastes of industrial origin by injection into the deep underground is a widely accepted practice. The growing acceptance of this waste disposal option is mainly due to the numerous problems of pollution in near surface hydrologic environments (44). The growing acceptance of this option is suggested by the results of a survey conducted by Warner and Orcutt (45), which showed that waste injection wells increased from 30 in 1964 to at least 280 in 1973 in the United States. There are now more than 100,000 of these wells in North America. Although deep-well injection of liquid wastes is meant to minimize the problem of pollution in the near surface hydrologic environment as suggested by Piper (44), the potential for pollution of deep-seated aquifers is still obvious. If the pollution of deep-lying aquifers is to be avoided, the disposal option requires the isolation of formations receiving waste injections from permeable contact with other elements of the hydrologic environment.

Human Activities and Pollution/Contamination

Contaminants can be introduced into the groundwater system as a result of myriads of human activities. This category of contamination of groundwater systems differs from the ones described earlier in that such human activities do not ab initio introduce wastes into the subsurface. The major human activities that eventually end up polluting groundwater systems include agricultural activities, storage of gasoline tanks in the subsurface, pipe lines, road deicing, mining and pumpage of aquifers, etc.

Nitrate loading of shallow groundwater systems arising from fertilizer application occurs mainly through leaching. Where there is significant downward flow, deep-seated aquifers can become affected. According to Freeze and Cherry (38), widespread nitrate contamination of aquifers through fertilizer application is rare. Numerous investigators including Grisak (46) and Custer (47) have shown from case studies in various parts of the United States and Canada that nitrate derived by oxidation and leaching of natural organic nitrogen in the soil is more often responsible for extensive nitrate contamination of shallow groundwaters. The pathway down to the water table of those contaminants generated through mining activities and road salt application is similar to that for nitrates, i.e., they reach the water table through leaching and flushing through the unsaturated zone by infiltration of percolating water from rain and snowmelt.
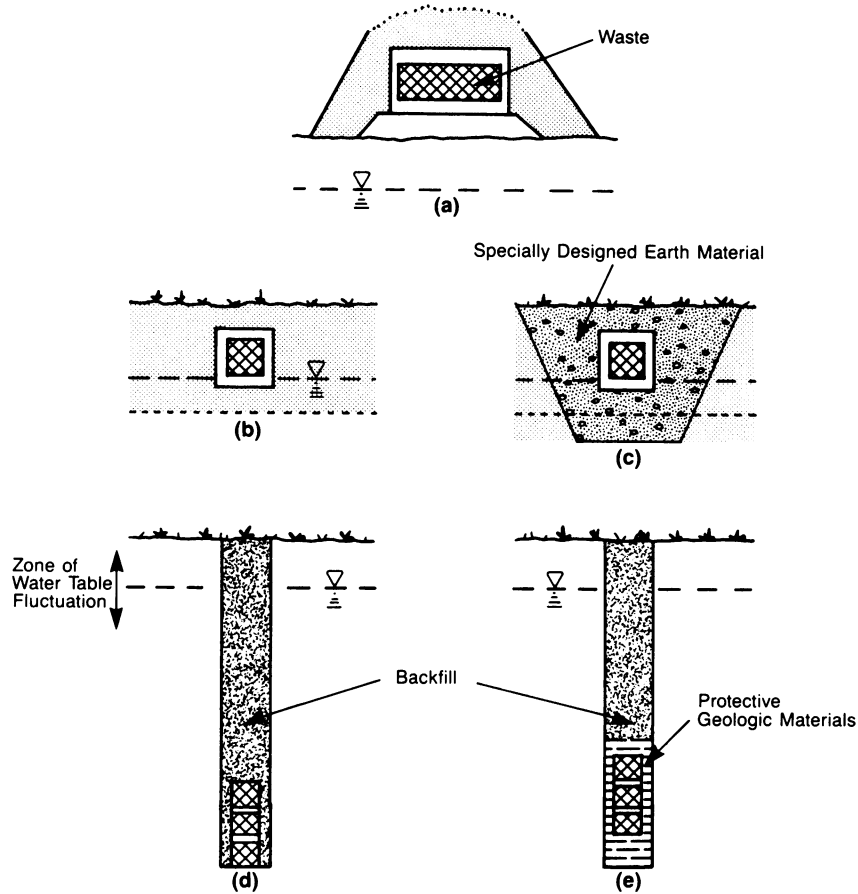
FIGURE 9.   Schematic diagrams illustrating methods of disposal of radioactive wastes (38).

Petroleum leakage from underground storage tanks and oil pipe lines, as well as spills from oil-producing wells, constitute an increasing threat to groundwater quality (Fig. 10). Petroleum contaminant pathways into the groundwater system are illustrated in Figure 10. A simple hydrogeologic condition is assumed. According to Freeze and Cherry (38), in the initial migration stage (seepage stage), the oil moves primarily in a downward
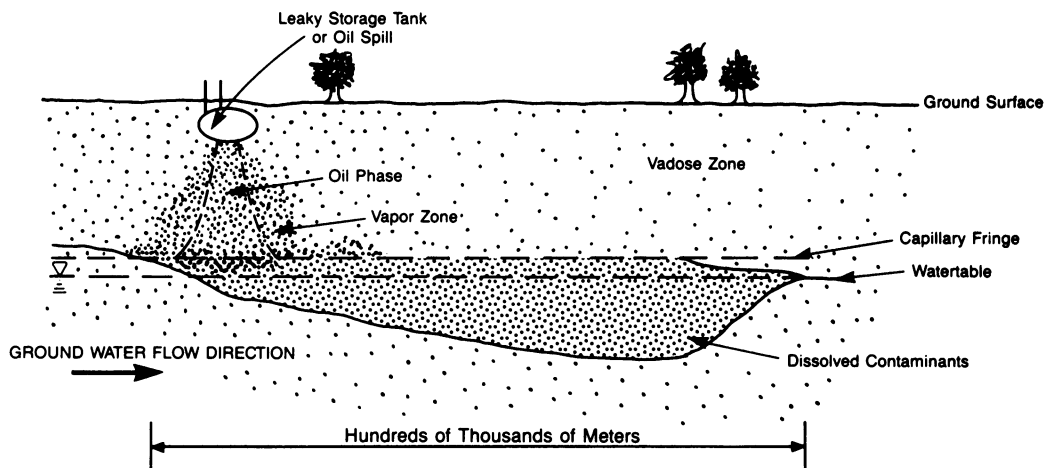
FIGURE 10.   Contamination from a leaky storage tank or oil spill (57).

direction under the influence of gravitational forces. On reaching the water table, the oil zone spreads laterally, first under the influence of gravity-related gradients and subsequently in response to capillary forces. Capillary spreading becomes very slow, and eventually a relatively stable condition is attained. Figure 11 summarizes the movement of mining wastes and pollutants within the total environment. At each state and position, metal-rich mine materials may get into the air, surface water, soils, and groundwater to possibly pollute them.

Figures 12a and 12b illustrate pathways by which contaminants in polluted surface waters and saline water can enter groundwater zones. In Figure 12a, contaminated surface water reaches the groundwater zone as induced by water recharge under the influence of the gradients set up by the well. Instances of polluted surface waters are common, particularly in industrial areas where effluents are discharged untreated or partially treated into streams and lagoons. Figure 12b shows a possible effect of overpumping that is often observed in coastal areas. Overpumping can cause contaminants from sea water to enter a freshwater aquifer.

Contaminant Transport: Hydrogeochemical Processes

The migration of contaminants in groundwater flow systems is due mainly to groundwater motion. Transport rates, however, are moderated by a variety of geochemical and biochemical processes that include complexation, acid-base reactions, oxidation-reduction processes, precipitation-desorption reactions, and mi-

(a)

(b)

FIGURE 12. Contaminant pathways into an aquifer (a) through induced infiltration from contaminated surface water and (b) through over pumping near saline water aquifers (89).

crobial reactions. According to Jackson and Inch (48), precipitation-dissolution, adsorption-desorption, and microbial reactions can lead to the removal of contaminants from solution, whereas the other processes affect the availability of the contaminant for adsorption or precipitation. Appropriate instrumentation, sampling, and monitoring would make these hydrogeochemical species veritable environmental tracers.

Complex-Ion Formation

Complex-ion formation is important in the study of groundwater pollution because the concentration and mobility of most contaminants are governed by the concentration and nature of the complexes they form. For example, hydroxide and carbonate complexes appreciably affect the mobility of uranium and the heavy metals in groundwater flow systems. It has also been observed by Jackson and Inch (48) that when heavy metals (e.g., Pb^{2+}, Cd^{2+}) are complexed by inorganic or organic ligands (e.g., Cl^-, EDTA), the contaminants may not be immediately available for adsorption or precipitation, and consequently the mobility of heavy metal contaminants may be increased in the groundwater flow system. On the other hand, contaminants may become associated with adsorbed complexing ligands such as

FIGURE 11. Movements of mining pollutants within the total environment. Modified from Press and Siever (32).

humic acids. In that case, their mobility will be reduced. It is thus concluded that adsorbing and nonadsorbing ligands may compete for contaminant ions and thus determine, on the basis of the relevant formation constants, the distribution of complexed contaminants between adsorbed and solution states.

Acid-Base Reactions

Acid-base reactions are those chemical reactions involving the transfer of protons. Proton activity, H^+, expressed as $-\log H^+$, is referred to as pH. The numerical value of pH gives an indication of the acidity of natural waters. The pH of natural waters is controlled by calcite ($CaCO_3$) dissolution and the CO_2 in the soil zone according to the following equations:

$$CO_2 + H_2O = H_2CO_3 \tag{2}$$

$$H_2CO_3 + CaCO_3 = Ca^{+2} + 2HCO_3^- \tag{3}$$
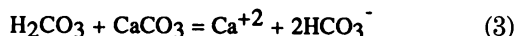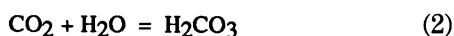
According to Stumm and Morgan (49), $CaCO_3$ is an efficient pH buffer only in the neutral and acid pH range. In the pH range of 9 and above, the incongruent dissolution of aluminosilicate minerals provides a greater buffer capacity. In pollution studies, it is important to know the pH of the groundwater and its buffering agents since the solubility of many minerals as potential contaminant sources and sinks are dependent on pH. Acid-base reactions become prominent in environmental pollution and degradation where situations create extremes of acidity or alkalinity. Thus, in acid mine drainage areas, acid rain, alkalinity, or alkaline rain situations, the hydrogeological processes such as oxidation-reduction, cation exchange, adsorption-desorption, etc., may result in the ultimate release of pollutant and contaminants into these systems to damage them.

Oxidation-Reduction Processes

Oxidation-reduction (or redox) processes are of a major importance in governing the geochemical behavior of those elements that may gain or lose electrons in groundwaters. By definition, oxidation is the loss of electrons and reduction is the gain in electrons, as shown in the following illustrated examples for the oxidation of iron:

$$O_2 + 4H^+ + 4e^- = 2H_2O \quad \text{(reduction)} \tag{4}$$

$$4Fe^{+2} = 4Fe^{+3} + 4e^- \quad \text{(oxidation)} \tag{5}$$
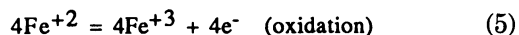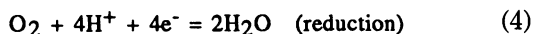
In reality, a reduction reaction is coupled to the corresponding oxidation reaction, so that the overall redox reaction for the oxidation of iron, for example, is of the form:

$$O_2 + 4Fe^{+2} + 4H^+ = 4Fe^{+3} + 2H_2O \tag{6}$$
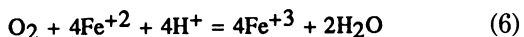
The redox state of groundwater is described by the redox potential pE (or Eh). Water infiltrating into shallow groundwaters has high redox potential due to its high dissolved oxygen content. Along the flow system, the tendency is toward oxygen depletion. The first stage in the oxygen depletion process is the oxidation of organic matter (CH_2O):

$$O_2 + CH_2O = CO_2 + H_2O \tag{7}$$
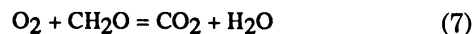
Reaction 7 is catalyzed by bacteria or isolated enzymes. They derive energy by facilitating the process of electron transfer. Organic matter oxidation of the type illustrated in Eq. (7) is a major redox reaction occurring in landfills and other similar waste disposal sites. Thus, leachates emanating from landfills have much lower redox potential. The leachates also have elevated concentrations of NH_4^+, H_2S, Fe^{2+}, Mn^{2+}, and FeS.

As the leachate enters the groundwater system, the following sequence of redox processes would occur (38):

(i)   Oxidation of Sulfide to Sulfate,

$$2O_2 + HS^- = SO_4^{-2} + H^+ \tag{8}$$

(ii) Oxidation of Ferrous Iron,

$$O_2 + 4Fe^{+2} + 4H^+ = 4Fe^{+3} + 2H_2O \tag{9}$$
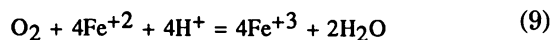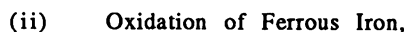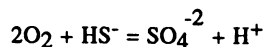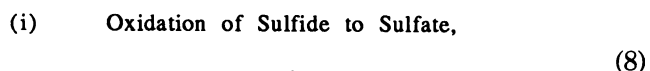
and the precipitation of Fe^{+3} as $Fe(OH)_3$

(iii) Nitrification

$$2O_2 + NH_4^+ = NO_3^- + 2H^+ + H_2O \tag{10}$$

(iv) Manganese Oxidation

$$O_2 + 2Mn^{+2} + 2H_2O = 2MnO_2 + 4H^+ \tag{11}$$
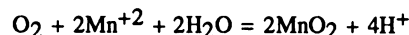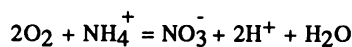
When all the dissolved oxygen in the groundwater is consumed, oxidation of organic matter can still occur as indicated in the following reaction equations (38):

(i)   Denitrification

$$5CH_2O + 4NO_3^- = 2N_2 + 5HCO_3^- + H^+ + 2H_2O \tag{12}$$

(ii) Manganese (iv) Reduction

$$CH_2O + 2MnO_2 + 4H^+ = 2Mn^{+2} + 3H_2O + CO_2 \tag{13}$$

(iii) Iron (iii) Reduction

$$CH_2O + 4Fe(OH)_3 + 8H^+ = 4Fe^{+2} + 11H_2O + CO_2 \tag{14}$$

(iv) Sulfate Reduction

$$2CH_2O + SO_4^{-2} = HS^- + 2CHO_3^- + H^+ \tag{15}$$

(v)   Methane Fermentation

$$2CH_2O + H_2O = \ \ \vdots H_4 + H^+ + HCO_3^- \tag{16}$$
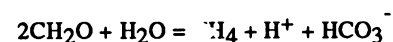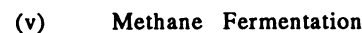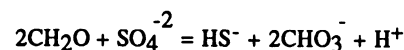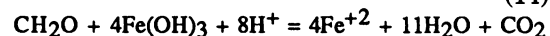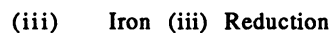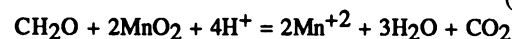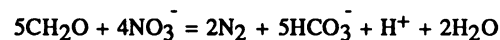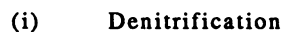
It is clear from these reaction sequences that adequate knowledge of the redox environment is needed for the purpose of predicting the mobility of those elements that have variable valences and which form low water-solubility oxides. For example, oxidized forms of iron [$Fe(OH)_3$] and manganese (MnO_2) are highly insoluble; the reduced forms (Fe^{2+}; Mn^{2+}), however, are soluble in water and thus move with the groundwater. Uranium, selenium, arsenic, and molybdenum are insoluble under reducing conditions and soluble under oxidizing conditions. Thus, depending on the physical, chemical, and biological conditions within the hydrogeochemical environment, contaminants may exist in polluted groundwater systems in various concentrations and forms. Detailed instrumentation and closely spaced monitoring programs easily delineate the geochemical zones (4,7,33,41).

Precipitation-Dissolution Reactions

Precipitation-dissolution reactions are a set of reactions by which contaminants may be removed from solution either by direct precipitation or by isomorphous substitution with an ion of similar atomic radius in a crystal that is forming or that has formed (48). The formation of metal carbonates, e.g., $Sr(CO_3)_2$, $Cd(CO_3)_2$, provide good examples of removal of contaminants from solution in groundwater by precipitation reactions. Saturation index calculations may be employed to determine whether a mineral species is likely to dissolve or precipitate in a groundwater flow system (38).

Adsorption-Desorption Reactions

Adsorption occurs when a dissolved ion becomes attached to the surface of a preexisting solid substrate (50). In porous media, contaminants can become adsorbed onto colloidal-size particles. The adsorption capacity of colloids is thought to be due to their ability to generate a charged solid-solution interface. The presence of a solid surface charge arises from imperfections or ionic substitutions within the crystal lattice of the colloids. The charge imbalance arising from the accumulation of charge on the colloid surface, however, is compensated for by a surface accumulation of ions of opposite charge known as counterions. Ion exchange occurs when the ions in the counterion layer become exchanged for other ions. Cation exchange capacity has been defined by Jackson and Inch (48) as the excess of counterions which can be exchanged for other cations in the bulk of the solution. It is usually expressed as the number of milliequivalents of cations that can be exchanged in a sample with a dry mass of 100 g. Cation exchange reactions are important in the predictive analysis of the mobility of contaminants in geological media. A measure of the mobility of contaminants that is used in predictive analysis is the distribution coefficient (K_d). It is defined as "the number of milliequivalents of an ion adsorbed per gram of exchanger divided

by the number of milliequivalents of that ion per milliliter remaining in solution at equilibrium" (48). The magnitude of the distribution coefficient is a measure of the extent of partitioning of a contaminant species between the solid and liquid phases along a groundwater flow system.

Microbial Reactions

Most of the geochemical reactions leading to the breakdown and transformation of complex molecules in groundwater systems are microbially mediated. These microorganisms derive energy and constituents needed for survival from these reactions. For bacteria to function and proliferate, it is also important for suitable temperatures and pH conditions to prevail in the medium (48). In the investigations carried out by Jackson and Inch (48), it was also observed that bacterially mediated chemical processes may have either beneficial or detrimental effects on particular pollutants. The beneficial effects include purification of contaminated water as organic pollutants are broken down into substances such as CO_2, H_2O, NO_3^-, and SO_4^{2-}. Elements such as N, S, C, and P are used in the synthesis of microbial protoplasm and are thereby removed from the groundwater system. Among the detrimental effects is the depletion of dissolved oxygen.

Two phases are involved in the infiltration of unpolluted groundwaters by polluted surface waters. These are associated with the oxygen-rich unsaturated zone and the oxygen-deficient zone which is usually saturated. Organic pollutants are usually removed by filtration in the unsaturated zone where an effective biological filter can be formed. By these processes some soils constitute an efficient filter for water treatment. Most microorganisms are not adapted to this tortuous and highly competitive environment, which limits their movement to no more than 3 m in depth (51). For this reason, any well or borehole not properly lined for its entire length stands a chance of being polluted by microorganisms from surface waters.

Adsorption is the main mechanism by which microorganisms are removed from the oxygen-depleted saturated zone. Under this condition, microorganisms may be carried passively in groundwaters up to a distance of 30 m horizontally (51). In view of this, a minimum protection zone of 30 m is essential in siting boreholes and wells if contamination from polluted surface water from septic tanks, agricultural wastes, and refuse tips is to be avoided. Fissured rock strata constitute an additional problem. Where they exist, the extent of passive travel by microorganisms is unlimited, as natural purification through soil is almost nonexistent (52).

During hydrogeomicrobiological processes, the activities of nonpathogenic bacteria in groundwaters could be beneficial because they are involved in the degradation of detergents, herbicides, pesticides, and general mineralization, including cycling of essential elements, nitrogen, phosphorus, and sulfur. When pollution is in excess, these beneficial processes could lead to problems

for groundwaters, such as depletion of dissolved oxygen, reduction of nitrate to nitrite or ammonia, reduction of sulfate to sulfide with attendant offensive odors and growth of filamentous bacteria, reaction of sulfide with iron to form an insoluble precipitate that can restrict groundwater flow, and mobilization of iron from soil under conditions of reduced oxygen tension only to be oxidized and precipitated in other regions of the aquifer either by chemical or microbiological means (24). Under anoxic conditions, gram-negative chemolithotrophic bacteria utilize nitrates, sulfates, and iron/manganese oxides as terminal hydrogen acceptors in respiration and other physiological processes.

Contaminant Transport: Physical Processes

An accurate description of the spatial and temporal distribution of contaminants in groundwater systems is of major importance in groundwater pollution studies. The model widely applied in the evaluation of contaminant migration is based on the advection-dispersion equation. It is derived from considerations of mass flux into and out of a fixed elemental volume within the flow domain. The physical processes that control these fluxes are advection (i.e., contaminant transport due to bulk movement of groundwater) and hydrodynamic dispersion that accounts for the mechanical mixing and molecular diffusion within the flow system. Loss or gain of contaminant mass in the elemental volume results from chemical or biochemical reactions, radioactive decay, or combinations of these. The exact form of the advection-dispersion equation depends on whether the contaminants under consideration are nonreactive or reactive.

For nonreactive constituents, the one-dimensional form of the advection-dispersion equation in saturated, homogeneous, isotropic materials under steady-state uniform flow is (38):

$$\frac{\partial C}{\partial t} = D_x \frac{\partial^2 C}{\partial x} - \bar{v} \frac{\partial C}{\partial x} \qquad (17)$$

where x is a curvilinear coordinate direction taken along the flowline, $\bar{v}$ is the average linear groundwater velocity, D_x is the coefficient of hydrodynamic dispersion along the x direction, C is the contaminant concentration, and t is time. The coefficient of hydrodynamic dispersion is of the form:

$$D_x = \alpha_x |\bar{v}| + D^* \qquad (18)$$

where α_x is the dispersivity, a characteristic property of the porous medium, and D^* is the coefficient of molecular diffusion. The term $\alpha_x|\bar{v}|$ expresses the mechanical mixing component of the dispersion process, which is the result of velocity variations within the porous medium.

The advection-dispersion equation is solved under prescribed boundary conditions. For the following boundary conditions:

$$C(x,0) = 0; \qquad x \geq 0 \qquad (19)$$

$$C(0,t) = C_o; \qquad t \geq 0 \qquad (20)$$

$$C(\infty,t) = 0; \qquad t \geq 0 \qquad (21)$$

the solution to Eq. (18) for a saturated homogeneous porous medium is given by Ogata (53) as

$$\frac{C}{C_o} = 0.5 \left[erfc\left(\frac{x - \bar{v}\,t}{2\sqrt{D_x t}}\right) \right.$$
$$\left. + exp\left(\frac{x\bar{v}}{D_x}\right) erfc\left(\frac{x + \bar{v}\,t}{2\sqrt{D_x t}}\right) \right] \qquad (22)$$

where erfc represents the complementary error function and all other terms are as previously defined.

Figure 13 illustrates the concentration profiles obtained with Eq. (22) and what is normally referred to as a breakthrough curve for contaminants migrating through a porous medium. The figure demonstrates the effect of mechanical dispersion and molecular diffusion, namely that of causing some of the contaminants to move faster and others to move slower than the average linear groundwater velocity. This causes a spreading out of the concentration profile along the direction of flow and to some extent in directions transverse to it. In the absence of dispersion and diffusion, the contaminant front will move as plug flow, and its position along a flow system will be entirely determined by the average linear groundwater velocity.

In the case of reactive contaminants, a sorption term is added to Eq. (22) to account for the transfer to or from the solids in the elemental volume. The advection-dispersion equation then takes the form (38):

$$\frac{\partial C}{\partial t} + \frac{\rho_b}{n}\frac{\partial s}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - \bar{v}\frac{\partial C}{\partial x} \qquad (23)$$

where ρ_b is the bulk density of the porous medium, n is the porosity, and s is the mass of the chemical constituent adsorbed on the solid part of the porous medium per unit mass of solids; $\partial s/\partial t$ represents the rate at which
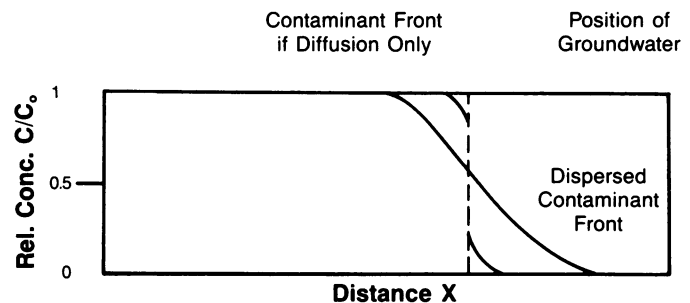

FIGURE 13. Schematic diagram showing the contribution of molecular diffusion and mechanical dispersion in causing spreading during contaminant migration (38).

the contaminant is adsorbed, and the term (ρ_b/n) $(\partial s/\partial t)$ represents the changes in concentration in the fluid phase caused by adsorption or desorption. If biochemical processes are ignored, the sorption term then depends only on ion exchange, precipitation and coprecipitation.

When a contaminant is adsorbed by a solid, it migrates at a rate slower than if there was no adsorption. Assuming a fast and reversible adsorption-desorption (cation-exchange) process, and if the concentration of the adsorbed contaminant is small compared with the total concentration of cations in solution, the rate of advance of the contaminant front is given by (50):

$$V_c = \frac{\bar{v}}{1 + \frac{\rho_b K_d}{n}} \tag{24}$$

where V_c is the velocity of the adsorbed contaminant and K_d is the distribution coefficient. The terms in the denominator are referred to as the retardation factor, which can be used to estimate how rapidly a contaminant would migrate through the flow system. The effect of retardation on contaminant migration is schematically shown in Figure 14. The contaminant front in the case of the adsorbed species, A^+, moves at a much slower rate than that of the nonadsorbed species, B^+. An important implication of this condition is that compared with a nonadsorbed species, it will take a much longer time for the adsorbed contaminant species to reach and pollute a groundwater supply source, and hence is less of a threat or hazard to the environment.
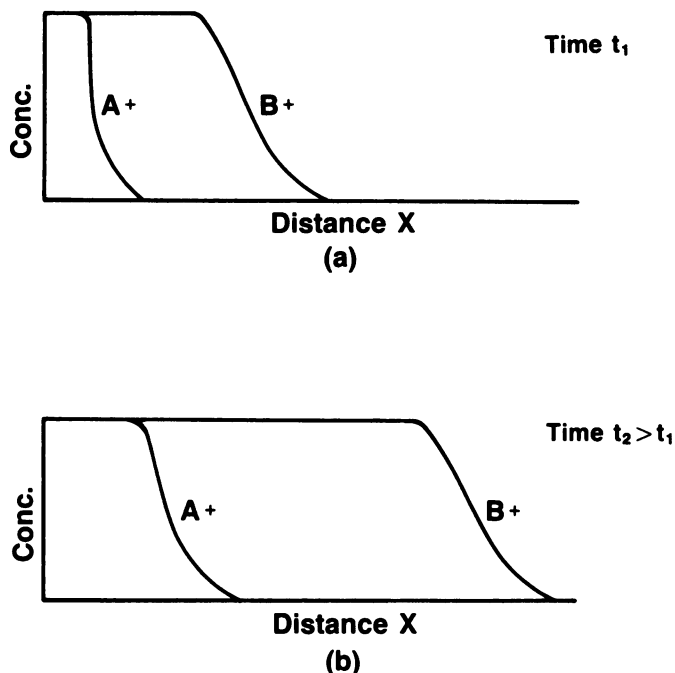
FIGURE 14. Schematic diagram showing the effect of retardation. The migration of an adsorbed species (A +); the position of non-adsorbed species (B +) (50).

Field Application of Advection-Dispersion Equation

An example of the application of the advection-dispersion equation in the solution of problems in contaminant migration is provided by a field study carried out by Egboka et al. (41). In the study, an analytical solution to the one-dimensional form of the advection-dispersion equation was used to simulate the distribution of tritium (a nonreactive species) produced during atmospheric testing and use of nuclear weapons along a contaminant plume under a landfill site in Borden, Ontario, Canada. Field values of radioactive bomb tritium were correctly simulated by using longitudinal dispersivity values of between 30 and 60 m and groundwater velocity values of about 10 m/year (Fig. 15). These aquifer parameters were used to predict the movement, spread, and dispersion of contaminants in the polluted groundwater such as sulfate (Fig. 16), chloride, etc. While Egboka et al. (41) investigated dispersion of contaminants introduced into the hydrogeologic system under natural conditions, Sudicky et al. (54) introduced artificial tracers into the same area and arrived at similar results usable for the planning and management of landfills and garbage dumps.
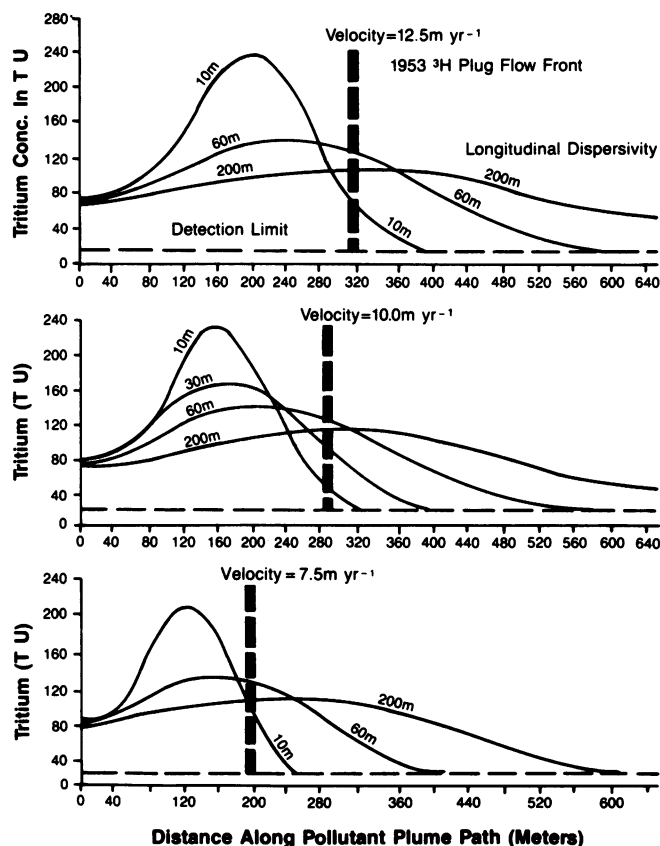
FIGURE 15. Simulated bomb ^{3}H versus distance in a landfill aquifer using varied velocities and longitudinal dispersivities (41).

Pollution Situation in Developed and Developing Countries

As a result of the ever-increasing industrial establishments and man's general activities, physical, chemical, and biological substances are being fed into the groundwater environment on a daily basis. This section summarizes the pollution situation in both developed and developing countries. Emphasis is focused on the developing countries.

Developed Countries

Groundwater literature is filled with incidences of groundwater pollution in many parts of the developed countries including the United States, Canada, USSR, and various parts of Europe. Table 6 presents a summary of groundwater contamination incidents in parts of the United States as reported by Lindorff (55). The number and percentage of incidents affecting or threatening groundwater supplies is shown in the second column. The third column shows the number and percentage of the cases that threatened or produced fires or explosions.

Table 6. Summary of groundwater contamination incidents (46).

Contaminant	No. of incidents	Water supplies, %	Fire or explosion, %
Industrial wastes	50	31 (62)	2 (4)
Landfill leachate	46	7 (15)	0
Petroleum products	27	18 (57)	10 (37)
Organic wastes	21	15 (71)	0
Chlorides	16	13 (81)	0
Radioactive wastes	7	2 (29)	0
Pesticides	4	2 (50)	0
Fertilizer	3	3 (100)	0
Mine drainage	3	1 (33)	0
	173	92 (53)	12 (7)

The ever-increasing use of organic pesticides and herbicides has constituted another source of groundwater contamination. Various investigators in the southwestern United States have observed that pollution by pesticides must be listed as an important potential hazard. Croll (56) arrived at a similar conclusion on the basis of a literature review and field studies in Kent, England.

In Canada, numerous cases of groundwater pollution have been reported. A comprehensive coverage of these cases has been presented by Cherry (57). Shallow groundwaters that have been contaminated by leachates from landfills include those that occur below three large landfills in the outskirts of the city of North Bay and near Alliston and Kitchenor-Waterloo in Ontario. Severe groundwater contamination from chlorophenols has been reported from Pentritton, British Columbia (57). Mine tailings are another major source of groundwater contamination in the mining districts of Canada. Extensive nitrate contamination of shallow aquifers have been observed in the Canadian prairies. This has been attributed to the use of agricultural fertilizers. Similar widespread occurrence of nitrate has been reported in a large regional carbonate-rock aquifer in England and the United States.

Leakages in nuclear power plants constitute another source of groundwater contamination. This is because the radiation would eventually be returned to the groundwater environment. Recent nuclear leakages include the Chernobyl incident in the Soviet Union. The impact of these leakages on the groundwater environment has, however, not been fully investigated. No doubt, a large number of cases of groundwater contamination have been reported. However, disposal sites that are known contamination sources probably account for only a small fraction of the total number of sites where groundwater contamination now occurs (57). It should, in addition, be expected that more severe cases
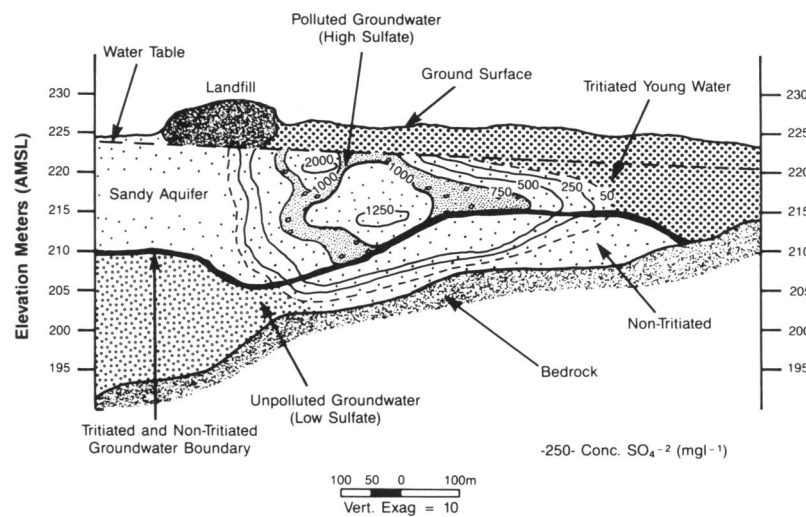
FIGURE 16.   Sulfate pollution of ground with tritiated tritium boundary (41).

of pollution could arise in the future as technology brings new and more hazardous chemical compounds into production and use. At the moment, great amounts of financial and material resources have been spent on the control of pollution. Health and environmental hazards posed by pollutants and contaminants are immense as many pollution-related diseases continue to emerge. Great losses in human and animal life and property have continued. As the developed countries are engrossed in these problems, it is unfortunate that the developing countries are becoming equally affected because no articulated control programs exist to any extent.

Developing Countries

Information on environmental pollution situations of developed countries such as the United States, Canada, and parts of Europe abound in the hydrogeologic literature (20,58–65). Acid rain has obliterated many ancient forests, acidified both surface water and shallow groundwaters, and defaced many buildings and monuments. Industrial wastes dumped as solids or discharged as liquids into surface waters have destroyed the fauna and flora of these waters. Many landfills and sewage lagoons dotted all over these industrialized nations have damaged the hydrogeologic environments. Many medium-level and high-level wastes from nuclear industries are stockpiled, waiting for safe disposal sites yet to be located in any part of the world. These pollutant/contaminant materials and their attendant problems have devoured huge funds for research and control activities. Despite the available manpower and expertise and the adequate financial resources in these countries, minimal successes in combating pollution have been achieved, so pollution threats so far seem to have defied man's efforts.

The fate of developed countries magnifies the helplessness of some developing countries that have now exposed parts of their hydrogeologic environments to pollution. Many of these countries in their race to become industrialized have accumulated waste products that now pollute the environment. Many of these countries have copied the developed countries in their science and technology, packaged and acquired the resulting technological outputs, and transplanted them into their countries without the necessary checks and balances such as an appropriate adaptation to the needs of their environments. These countries produce huge volumes of pollutants and contaminants from industries and urban centers and dispose of them into surface waters or dump them at the outskirts of their cities. They do not have enough pollution management experts and the necessary finances to control the spread of pollutants. Outbreaks of diseases that are pollution based occur from time to time. It is strongly believed that unless these developing countries do something to stop the present pollution trends that are fast growing, many of their environments shall be worse off than those now prevalent in parts of the developed world. Already some countries are closely approaching this stage.

The specter of widescale pollution of environments of developing countries is becoming more threatening for many other reasons. More and more urban, suburban, and rural communities are being polluted or exposed to pollution. Few of these countries have plans or management programs to combat pollution. Pollution problems are thus tolerated and given no priority. There is little or no public or government awareness because the dangers seem to be ignored or overlooked. There are no Environmental Protection Laws and where available, they are rarely enforced. When these poor countries soon reach the level of pollutant/contaminant generation as the rich industrialized countries, they shall be much worse off. Already various health hazards and polluted waters with waterborne diseases and epidemics ravage these countries from time to time. Even though it is known that many of these pollutants are produced through industrialization and urbanization activities, governments, groups, and individuals have not been doing anything to control their emission or production. Even in rural environments where agricultural, mining, and urbanizing programs continue to generate pollutants and contaminants, no one seems to show much concern.

Some developed countries, as a result of their stiff Environmental Protection Laws, indirectly encourage the export of waste products. This practice is either carried out directly because these materials cannot be disposed of economically or safely in their home countries or indirectly through establishment of industries in developing countries. These industries produce hazardous wastes that are carelessly dumped or discharged. Such exported industries lack adequate safety devices and efficient monitoring systems. Finally, in the drinking water supply program, emphasis is placed only on water quantity and little or none on quality. Because of this emphasis water potability is questionable most of the time. Typical pollution case examples from some developing countries shall be given below to provide more credence to these unfortunate observations.

Environmental/Health Hazards and Implications

Various environmental problems can arise as a result of groundwater pollution. A major consequence of groundwater pollution includes the potential contamination of surface waters. This can happen if the rivers, streams, or lakes in the area are recharged by a polluted aquifer. The converse becomes the case if contaminated surface waters recharge an aquifer. These cases are illustrated in Figures 17a and 17b, respectively. Water pollution can result in a reduction in economic and agricultural activities. For example, when surface waters are contaminated, they can result in higher fish mortality. In third world countries, in particular, where fishing on a subsistence level provides a means of livelihood, a significant drop in fish productivity due to pollution can have unpleasant consequences on the eco-
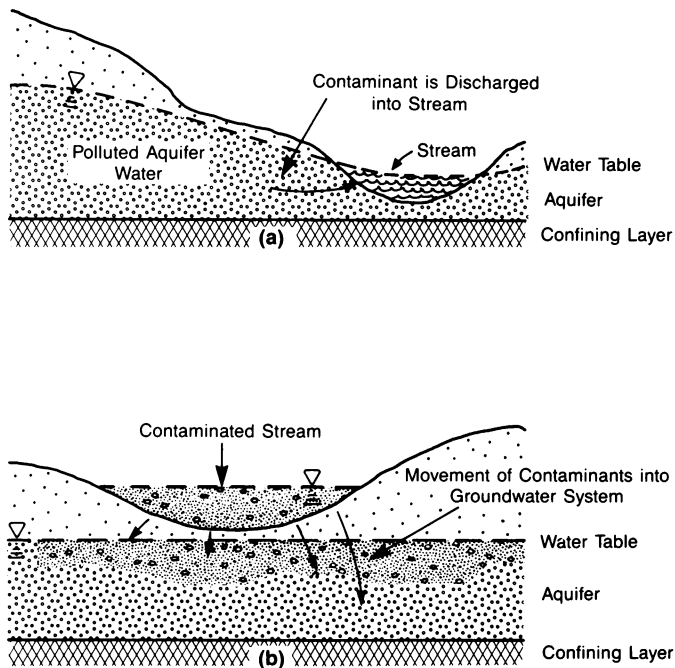
FIGURE 17. A schematic diagram showing the potential for contamination of (a) surface waters when an aquifer is polluted and (b) contamination of groundwaters when surface waters are polluted.

nomic life of the community. Polluted irrigation water poses health risks. It can also result in reduced crop productivity. Thus, water pollution can have serious negative effects on the agricultural sector too.

Other environmental hazards arising from water pollution include the presence of odor and color in the affected water. Pollution of surface waters (lakes, dams, rivers) may affect groundwater adversely. Water supplies are contaminated resulting in health risks and increased load on water treatment plants; increased costs on water treatment plants; fish kills or decline in productivity, quality and quantity; polluted irrigation water, posing health risks or inhibiting crop productivity; degradation of recreational and aesthetic characteristics of waters; etc. (1). Sometimes poisonous ions, dissolved gases, trace elements, heavy metals, and radioactive materials in water endanger the biospheric parts of the environments. Hence waterborne diseases are of epidemic occurrence in many developing countries where they form debilitating scourges to humans. Also major physiological ailments such as cancer, that may sometimes be caused by water or food have become rampant in both developed and developing countries. Many surface water and groundwater bodies are dead and remain anoxic as a result of heavy inputs of pollutants and contaminants. Many pollutants from one source can move for long distances through groundwater flow systems to polluted faraway areas, precipitating large-scale environmental destruction. Some high-level wastes have pollutants and contaminants

with long half-lives. Because of this property, they remain hazardous for long times within the hydrogeologic environment and are very difficult to remove.

In the hydrogeomicrobiologic literature, cases of microbial pollution and hazards abound that are a threat to both surface water and groundwater systems. Under certain circumstances, the pathogenic microorganisms listed in Table 3 escape the purification processes accompanying percolation of polluted surface waters into groundwaters where they constitute a dangerous health hazard. Salmonellosis, bacillary dysentery, schistosomiasis, helminthiasis, and viral infections are known to have been transmitted through drinking groundwaters polluted by surface waters and sewage in this way (66). Public interest in nitrogen oxides arises from the toxic effects of nitrite when nitrite ions enter the blood stream and react with hemoglobin, leading to an impairment of oxygen transport, particularly in infants. The disease is almost always attributable to high levels of nitrates in drinking water supplies including polluted groundwaters (23,31,35). Under certain conditions nitrate may be reduced to ammonia by some of the nitrate reducers. The ammonia can react with chlorine to produce chloramines, which can lead to undesirable tastes and odors. The presence of sulfides produced by sulfates reducers in groundwaters also impart unacceptable tastes and odors. Iron bacteria have caused problems in water supplies since the dawn of civilization, and there are many references in history to "red" water, undrinkable water covered with slime, and plugged wells (67).

In wells and boreholes, the major problems are a) growths that plug the screens; b) coatings on piping systems, impellers and motors, that reduce flow rates; c) reduced potability of water; and d) total plugging of the well. The iron and manganese bacteria that cause these problems are thought to be introduced into the wells and boreholes from their soil habitat during initial boring operations or by seepage into the aquifer feeding the well (68).

Groundwaters drawn from wells and boreholes constitute a major source of water supply in many African countries including Nigeria. In these circumstances, the water is usually untreated. The inadequate practices of waste disposal in these countries lend themselves as being a large source of pollution for groundwaters.

In Nigeria, feces are disposed of by one or more of the following ways, depending on the locality: disposal on ordinary dry ground, bucket latrines, the pit-latrine or pit-privy, and septic tank latrine (aqua privy). Domestic and industrial wastes are disposed of either by composting, sewage, or open drainage systems (66). The content of these waste products are usually organic and inorganic matter as well as microorganisms, some of which are pathogenic. Some of the wastes in refuse tips are washed into surface waters leading to eutrophication. In most circumstances in Nigeria, parts of Lagos, Ibadan, Benin, Enugu, Onitsha, Kaduna, Kano, Jos, Abakaliki, etc., adequate hydrological data are not sought on soil strata and the direction and rate of flow

of groundwaters before wells are sunk (*37*). The result is that sometimes wells are sunk less than 5 m away from obvious sources of pollution like pit latrines (*69,70*). Worse still, the wells are usually not lined at all, with the result that the groundwaters easily get contaminated by seepage from pollute surface waters.

Cases of guinea worm infestation in parts of Ilorin and Abakaliki have continually been linked to the drinking of groundwaters contaminated by heavily polluted surface waters. The outbreak of cholera in Ohaozara area of Nigeria in 1981–1984 was also linked to poorly sited wells in the area (*25*). It is obvious that, if systematically investigated, most outbreaks of waterborne diseases could be linked to pollution of groundwaters from surface waters, septic tanks, pit latrines, and compost heaps. It is in recognition of this danger that the Federal and State governments of Nigeria as well as many international organizations like the WHO and UNICEF are currently tackling health problems in Nigeria by the provision of properly sited boreholes in all the rural communities.

In 1977 the Food and Drug Administration Unit of the Federal Ministry of Health in Kaduna Nigeria (*25*) reported a widespread occurrence of iron bacteria in up to 60% of the boreholes in the Funtua, Bida, Malumfashi, Dutsi-ma, Daura, Katsina, and Kano areas (*23*). Except in the Kano areas, the genera of iron bacteria encountered were Siderocapse and Siderococcus. These microbial pollutants have caused many groundwater supplies problems, resulting in loss of well yields, water contamination, and increased costs of water supplies. These problems are worsened by the absence of continuous monitoring programs.

In the Kano area, the filamentous iron bacteria Leptothrix and Crenothrix were found abundantly in nearly all the boreholes in the Bompai area, on the outskirts of Kano municipality. The pollution was traced to the myriad of refuse tips made up of the waste products from the sugar, sweets, and biscuit factories in Kano. The global distribution of iron bacterial problems in groundwater was reported by Cullimore and McCann (*67*). Among the developing countries included in the draft are El Salvador, Guyana, India, Malaya, Nigeria, Singapore, and Sri Lanka. Crenothrix was found to be plugging water supply systems in Sri Lanka; Clonothrix reduced flow rates and potability of water in the Calcutta area of India. In all others, the offensive iron bacteria were not specified, but the damage they caused was observed.

The industrialized world has accumulated great amounts of pollutants and contaminants within their environments. Many of these pollutants have spread widely and in such a complex manner that their control will be a most difficult and costly venture. Many wastes are now piled up in storage tanks above and below the ground surface, while painstaking research efforts are being made to scout out possible safe geologic environments for their disposal. Unfortunately, so far, as a result of the structural, stratigraphic, sedimentological, and geotechnical properties of the pedologic and geo-logic units, no safe disposal environments have yet been found for waste products that have long half-lives. It seems that until a safe and more reliable disposal method is found, both the developed and developing countries, have no option but to reduce the volume of wastes both societies are now generating and abandoning or storing within hydrogeologic environments.

Pollution Case Examples from Some Developing Countries

Parts of urban and rural environments of many developing countries such as India, Kenya, Nigeria, Sudan, Egypt, Iraq, and Brazil are being polluted with a wide variety of hazardous substances. Such countries are struggling to become industrialized without adequate plans to contain the spread and hazards of pollution. There are many sources of pollution in developing countries. Some of those related to mining, mineralization, agricultural, domestic, municipal, human, and animal wastes shall be discussed. Typical case examples shall also be briefly described, and these shall be generally related and at some instances specific to some developing countries that are now industrializing at a rapid rate.

Mining Pollution

Contamination of groundwater due to mining activity is a major problem in many developing countries. Previous or present mining activities result in contamination from waste dumps, mine workings, fragments, and dust from ore and rock piles and smelter operations. Sulfides (usually pyrite, galena, and sphalerite) in mine dumps are especially susceptible to oxidation and produce acid mine waters that can be leached out in varying volumes and amounts. The ore minerals are not completely recovered during the beneficiation processes. The acid mine waters that also contain trace metals make their way into groundwater flow systems.

Acid mine waters from an abandoned mine in the Charcas District, San Luis Potosi, resulted in high metal values in drainage systems and groundwaters (*71*). The acid mine drainage problems in Enugu coal mines of Nigeria and their effects on groundwater pollution were highlighted by Egboka and Uma (*21*). Many coal beds may contain up to 10% sulfur, chiefly in the form of pyrite and marcasite. As the coal deposit is worked, air and water gain access to the seams that contain sulfur minerals, oxidizing the sulfide minerals. This results in the formation of enormous amounts of sulfuric acid. Groundwater recharged by water from the mine needs considerable treatment with lime before it can be used to supply domestic homes and industries. In the acid mine drainage problems in the Enugu coal mines of Nigeria, about 18.1 million liters of acid water with high iron content is pumped out daily into nearby rivers. Some of this acid water eventually enters groundwater flow systems. The acid waters also attack and corrode mining equipment causing great financial losses. Some

mine waters are colored brown by tannins from bark of trees. They have an objectionable taste and they render the water unfit for drinking. Sometimes the waters actually are sterile enough for use even though they are colored and may have a bad taste. Phenols are abundant in waters in coal swamps. Phenols are poisonous to many bacteria and are capable of making mine waters sterile if they are present in large quantities.

In most developing countries there is no legislation guiding the safe disposal of mine wastes and mine dumps or their proper management. Most of the mining companies involved are mostly foreign firms. The companies do not show much interest in tackling environmental pollution problems associated with the mining wastes they produce annually. Little or no money is spent on wastes research and management in many parts of Nigeria and other countries. The uneducated rural people often use polluted waters discharged from mines. Many people from Abakaliki Mining district of Anambra State, Nigeria, suffer from lead poisoning resulting from the contamination of their water sources by lead. The area is also ravaged by guinea worm and other waterborne diseases.

Domestic, Municipal, Human, and Animal Waste Contamination

Domestic wastes contribute a large number of elements to groundwater systems, all with unpleasant ramifications. The most common contaminants from household products include phosphates and boron in laundry detergents, copper and other elements as organometallic compounds in garbage; metals in urine and excreta; copper, lead, zinc, and asbestos from pipes; nickel from stainless-steel pipes and well casings. Municipalities that treat sewage and garbage reduce metal concentration in drinking waters, but the recent tendency to dispose of the treated material on land eventually yields metals to the drainage basin. Such metals may eventually reach the surface water and groundwater flow systems and pollute them. Similarly, metals are introduced into the hydrogeologic environment from the resultant ashes when garbage or solid wastes are composted as is commonly done in developing countries.

The tremendous increase in the use of septic tanks for home sewage disposal has contributed a great deal of dissolved polluting materials to groundwater (Fig. 8).

The septic tank waters seep into the soil and where water supply aquifers are shallow, will contaminate groundwater with phosphate and boron from detergents and a variety of other substances such as nitrates that are undesirable or harmful to health. In many rural communities in developing countries shallow pit latrines are used for disposal of human excreta. Other undesirable materials like expired drugs and unwanted chemicals are also dumped into pit latrines or shallow waterways. Human excreta collected in bucket toilets are also emptied into the pit latrines. Water infiltration into the ground through the pit latrines introduces a number of

undesirable compounds into groundwaters (18,69, 70,72).

In many cities all kinds of waste materials are strewn about on the outskirts of towns or are thrown into streams, lakes, and rivers as most of those cities originated and developed close to major rivers. Aerosol cans, drug containers, hospitals, and research laboratories, washings or wastewaters that may contain heavy metals such as mercury, lead, zinc, etc., may eventually decay or spread and become transported into groundwater flow domains. Underground and surface storage tanks, septic systems/fields, etc., washings from motor mechanic sheds and garages produce contaminating leachates (Fig. 7). These types of pollutants have threatened hydrogeologic systems in parts of Nigeria (69,70,72). In some urban areas of developing countries such as India in temperate climates, large quantities of road salt (NaCl and CaCl) are used for deicing the road in winter. Leachates from these activities will eventually contaminate aquifers. Egboka (15) briefly described the traditional habit of using the bush for toilet purposes in many rural areas and suburban centers. It is believed that defecation of this type contributes to widespread and prevalent waterborne diseases in such areas. It also accelerates large-scale incidences of eutrophication in lakes. Unfortunately, it is yet to be estimated the degree and extent of environmental pollution through defecation in the rural areas.

Agricultural Contamination

The use of pesticides, herbicides, fertilizers, and other materials to increase agricultural yields has some great negative effects on groundwater quality. Pesticides and herbicides applied to fields or orchards may find their way into groundwater when rain or irrigation water leaches the dissolved constituents downward into the soil. Nitrate from its fertilizer, one of the most widely used agricultural fertilizers, is harmful in drinking waters even in relatively small quantities. The nitrate is very soluble and although some may be used by plants, much of the dissolved nitrate escapes unused into deeper parts of the soil and into groundwater. Sewage and fertilizer can increase nitrate levels in some aquifers (4). Nitrate is toxic to humans even in amounts as small as 10 to 15 ppm.

Uranium and fluorine in phosphate fertilizers and probably rubidium in potash fertilizer are soluble under most conditions and will eventually find their way into the groundwater regimes. The use of lime for the production of fertilizer may result in lead and zinc contamination, if the lime is produced from metal-containing limestones. Mississippi-type lead-zinc deposits are common in limestones. Some limestone deposit used for production of lime may contain appreciable quantities of lead-zinc minerals.

In developing countries, the people and governments place their priorities on food production in enough quantities to stem the tide of hunger and mass deprivation and little or no consideration is given to the pollution

implications. The poor farmers, most of whom practice subsistence agriculture, are highly encouraged to apply fertilizer and use insecticides/herbicides for maximum crop yields. The prices for these chemicals are very low and affordable as the governments have subsidized the costs. Thus, the chemicals may be used indiscriminately. A large amount is released to the environment to pollute surface water and shallow groundwaters. This practice is common in many developing countries.

Radioactive Contamination of Groundwater

Another source of groundwater contamination is radioactive wastes from power plants and mine dumps. One of the serious long-range problems associated with the use of nuclear power plants is the disposal of highly radioactive nuclear wastes. These highly toxic wastes are by-products of nuclear power plants and the manufacture of nuclear weapons. These radioactive wastes are temporarily stored as liquids in tanks. Despite the fact that the waste must be isolated from humans and other organisms for many centuries before it is safe, it has not been possible to store the wastes for even a few decades without mishap. Several thousand gallons of waste do seep into groundwater from storage tanks before anyone realizes there is a leak. Radioactive materials in water even in very small amounts are harmful to all forms of life. Some developing countries are believed to have nuclear power.

The problem of disposal of radioactive wastes is especially common in industrialized countries, but the disposal of radioactive wastes in uranium mines is a problem that occurs more in developing countries. Some developing countries are producers of uranium and other raw materials needed for nuclear power plants. In most of these countries, there are no regulations governing the disposal of mine wastes. They are dumped around and abandoned by the operators in the mine environment.

Uranium is usually present in the tetravalent state (U^{4+}). In this valence state, uranium is not soluble, and is immobile. When exposed during mining and dumped in mine waste, uranium is oxidized to the hexavalent (U^{6+}) state occurring as uranyl ion (UO_2^{2+}). Uranium then moves from an oxygen-rich surface environment, in which uranium is in the hexavalent state or complexed with carbonate into the subsurface groundwater environment. The problem of contamination of groundwater in uranium mining areas by uranium and its daughter products in active and abandoned mines is as serious as those associated with nuclear wastes. In nuclear power plants, adequate precautionary measures are always taken in handling the radioactive wastes. In uranium mining areas no such precautions are taken and the danger of contamination of groundwater by radioactive materials leached from mine dumps is great. The problem is most serious in those developing countries where the inhabitants of mining areas are not aware of the problems. When it is realized that some

developing countries have nuclear capabilities and hence are generating high-level radioactive wastes, it becomes a matter of great concern to conjecture how these wastes are being isolated from the biospheric environment. So far, it is a top secret matter and those few developing countries that have nuclear capability hardly provide any information to the public.

Pollution/Contamination from Natural Sources

Pollution and contamination may come from natural sources such as during physicochemical weathering and mass wasting, soil and gully erosion, flooding, snowfall, wind activities, and seawater intrusion through wave action, volcanic or gas eruptions, geochemical evolution through groundwater infiltration, and percolation. A large quantity of pollutants and contaminants are released from these sources but it is very difficult to quantify and control release by natural processes. During weathering, geologic units are corroded, weathered, disintegrated, and disaggregated, thereby releasing dissolved geochemical constituents into the hydrogeologic systems. During sediment transport and deposition, geochemical reactions (38,49) may result in the release of more ions and dissolved gases that may become concentrated enough to be hazardous. In addition, soil and gully erosion (16,17) may remove volumes of sediments with potential pollutants and contaminants that may threaten the environment. In some situations deep gullying that intersects the watertable and shaley terrains may result in hydrogeomicrobiological reactions that may release deleterious pollutants into groundwater (17).

Snowmelt and rainfall and anthropogenic activities such as farming, excavation, and mining accelerate the transport of sediments and enhance their pollution potentials. Thus, in parts of the humid tropics, floodwaters are densely brownish in color, reflecting high sediment loads. Wind activities in areas at the fringes of deserts also transport sediments that pollute the environment. Nigeria, Chad, Sudan, Niger, and other countries that are close to the Sahara Desert suffer from these problems. These types of pollutants are very serious, particularly in developing countries because there does not exist any plan to combat them. Some of these countries may not even recognize their existence or just ignore them. Meanwhile the pollutants continue to ravage their environments.

Case Examples: Review of Pollution/ Contamination in Some Developing Countries

India has emerged as an industrial nation and a major producer of manufactured and agricultural products within the last 20 years. Because the population is large and industrial activities are intense, large volumes of gaseous, liquid, and solid wastes are continuously re-

leased into the environment. Surface waters and shallow and deep groundwaters have been polluted in urban and rural areas (73–75). Neighboring countries of Pakistan and Bangladesh are equally polluted or threatened. The excessive withdrawal of groundwater in the Saurashtra area of India has resulted in sea-water intrusion. Parts of the groundwater in Gujarat State are mineralized and polluted by high temperature waters. The groundwaters from the Khetri copper mines in Rajasthan, Mahakali coal field area of Maharashtra, and the Panandhro lignite field pose geotechnical and pollution problems. Currently, as a result of tourist activities, many ancient forest lands, hills, valleys, and even mountains are being strewn with garbage thrown away by tourists thereby polluting the environments.

Kenya is a typical industrializing East African country producing varying degrees of pollution. Other neighboring countries of Uganda, Zimbabwe, Tanzania, Malawi, and Botswana are not spared. In a water resources quality survey by Nair et al. (76) from 1286 boreholes from parts of Kenya, the majority of the samples (61.4%) have fluoride values above 1.0 ppm while 19.5% had above 5.0 ppm and sometimes in even greater amounts (76). Table 7 lists the summary of maximum fluoride levels taken from each province and in different locations in Kenya. The high fluoride areas coincided with volcanic rock areas. The high fluoride water caused extensive public health hazards such as deformity in children. In Malawi, localized pollution of groundwater affects the quality adversely. Waters of up to 4000 to 7000 μmhos/cm of electrical conductivity occur. High sulfate iron and magnesium concentrations are common (77). Foster et al. (78) reported serious nitrate and fecal pollution of shallow groundwaters in parts of Botswana through pit latrines.

Many hydrogeologic environments in Nigeria are polluted (10,15,18,19,22,69,70). Saline lakes and hot springs occur (79,80). Coastal towns such as Lagos and Port Harcourt suffer from saltwater intrusions from the Atlantic ocean. Inland waters such as rivers (Kaduna, Niger, Anambra) and lakes (Chad, Agulu) have received pollutants in varying degrees. Industrial wastes are indiscriminately disposed of on land or into surface waters. Sewage is similarly disposed of. Mineralized waters attack and destroy borehole networks in the Maiduguri areas of northern Nigeria. The rural communities are equally not spared as present attempts to develop rural areas have introduced many pollutants and contaminants into the environment. Soil and gully erosion and flooding have become rampant and pollute surface waters and groundwater. Outbreaks of waterborne disease such as cholera, yellow fever, dysentery, diarrhea, and Guineaworm occur periodically, resulting in fatalities. Mining companies in Jos, Abakaliki, Enugu, Nkalagu, and the Port Harcourt areas pollute the environments without restraint. Flaring of gases and oil spills have contaminated surface waters and groundwaters (10). There is yet no effective legislation to check these problems.

In a geochemical study of the Otamiri and Aba river watershed in southeastern Nigeria by Nwankwor and Okpala (81), nitrate concentrations in the order of 100 mg/L were found in the groundwater and surface water systems of the Otamiri watershed. Nitrate loading of the waters in the Otamiri watershed were attributed to intensive use of fertilizer by various government-sponsored agricultural establishments in the basin. Results from the Aba River, which drains the largely industrial city of Aba, showed abnormally high concentrations of Co^{2+} and gave values for pH that varied between 4.0 and 6.5 (81).

Egypt and the neighboring countries of Sudan and Libya have polluted surface waters and groundwaters. Primary sources of pollution are industrial/sewage wastes, agricultural/irrigation activities, and salinization processes. Volumes of waste products are drained into the River Nile and eventually into the sea through a complex network of canals. Some of the irrigation waters react with soil water/groundwater and with soil materials dissolving the soluble salts, salinizing the soil, and increasing the salt concentration in irrigation canals and groundwaters (82).

Alexandria, with a population of about 3 million people, is the main industrial center and is burdened with pollutants and contaminants from many sources. Parts of Meryut Lake have been destroyed by sewage, thereby contaminating fish. According to Preul (82), groundwater levels in lower Egypt rose considerably with the building of the Aswan dam in 1965. The regional rise in water levels of shallow aquifers compounded the problems of pollution spread through subsurface disposal of wastewaters and irrigation water. Villages "are experiencing considerable difficulties with wastewater disposal due to subsurface saturation, high groundwater, emerging surface pools of septic waters, gross groundwater pollution, deterioration of buildings and structures due to moisture absorption, and other related problems. A further complication in certain areas is the existence of a large irrigation canal which usually carries a level of flow above the general elevation of the village and therefore creates a hydraulic gradient of seepage through the dykes towards the village" (82).

Even though the government of Egypt has environmental protection laws, their enforcement, particularly in the rural areas, needs to be encouraged. These rural

Table 7. Maximum fluoride concentrations in Kenyan water samples taken from each province (76).

Province	District	Flouride concentration, ppm
Nairobi		30.2
Central	Nurang'a	22.0
Coast	Taita Taveta	15.0
Eastern	Machakos	19.3
Northeastern	Wajir	38.2
Nyanza	Kisumu	10.4
Rift Valley	Nakuru	57.0
Western	Bungoma	7.1
Nationally		57.0

communities continue to be exposed to increasing soil, surface water and groundwater pollution.

Al-Jabari and Al-Ansari (83) described the dissolution of geological outcrops and soils in Iraq. These are rich with calcium carbonate and gypsiferous deposits. Water inputs from flood plains and valleys with organic matter contents reaching up to 20%, high suspended sediments, inputs from springs (Table 8), and human activities pollute the environment. Their pollution potentials are enhanced by erosion. Tremendous amounts of dust generate fallouts from sediments in central Iraq at the rate of 2.1 cm/yr (83–85). The sediments contain pollutants and contaminants in the form of carbonates (calcite, dolomite grains) quartz, feldspars, gypsum, chert, muscovite, heavy minerals (pyroxenes, zircon, biotite, hornblende, epidote, rutile, garnet, chlorite, staurolite and Kyanite), and pyrites (83–85). The heavy minerals assemblage in the outfalls have been correlated with the heavy mineral content of the Tigris and Euphrates Rivers flood plains and older dune deposits (Table 8). Salman et al. (84), in their investigation of bacterial density in Tigris River within Bagdad, measured a high density of coliform bacteria and Escherchia coli, suggestive of fecal pollution from sewage disposal. The bacterial density correlated well with high suspended sediment loads, which are believed to transport the bacteria in water. The high sediment concentration is accelerated by anthropogenic activities such as dredging, swimming, and solid/liquid disposal on land and water.

Pollution of surface water and groundwater in the developing countries of South America have also been reported. Argentina, Brazil, Chile, Cuba, Nicaragua, etc., have been equally exposed. Brinkman (86) discussed the hydrogeochemistry of groundwater resources in the central Amazonian area of Brazil. Ducloux (87) also treated the central zone of La Pampa Province of Argentina. Other countries in Africa such as Ghana, Zaire, Sudan, Mauritania, Ivory Coast, etc., have been unduly exposed to the destructive hazards of environmental pollution (88,89).

Summary and Suggestions

According to Fano et al. (1), "it may be expected that over the next decade the management of water quality problems will be one of the outstanding issues relating to the protection and conservation of the national stock of water in each country... The rapid aggregation of population in major urban centers, the polarization of industries, and the heavy dependence of chemical products, particularly in the agricultural sector, are leading to a serious deterioration of water quality in developing countries." Already parts of the environments of many industrialized nations are highly polluted. The problems are being tackled with available manpower, expertise, and financial resources in these countries. Encouraging successes are yet to be achieved, as the pollutants continue to diffuse and disperse into the hydrogeologic environment, and several tons of high-level wastes are piling up in storage tanks while desperate efforts are being made to locate geologic formations for safe waste disposal.

Unfortunately, in an obvious attempt by many developing countries to industrialize and compete with the developed nations, waste products are being generated in large quantities. These countries have neither the manpower, expertise, nor the financial resources to control or safely dispose of these deleterious wastes. As a result, their environments are becoming heavily polluted at an alarming rate. The leadership of these countries seem to lack the will or the serious understanding to recognize and mount a control program. Because of this, pollution continues to spread unabated with its attendant hazards and problems. In the next 10 years, unless something is done quickly, pollution levels in many developing countries' hydrogeologic environments would have reached such destructive levels that they may become uncontrollable. Destruction of plants, animals, and humans through pollution-caused epidemics/diseases would have become commonplace as requisite funds and materials for their control may not be available.

Developing countries must now learn from the mistakes of the developed nations vis-a-vis pollutants and contaminants, to save their environments from pollution damages for future generations. Some of the following pollution-control programs being pursued or implemented in many industrialized countries should be of worldwide application. The present consciousness about the hazards of pollutants and contaminants in developed nations must be highly encouraged. Every effort must be made to reduce pollutant/contaminant loads into the environment through improvements in manufacturing techniques that could recycle waste products. More efficient techniques for the destruction of high-level pollutants and contaminants before they can reach the hydrospheric zones should be found through more research. Through more intense investigations, safe pedologic, and geologic formations for disposal of wastes can be located. The present careless dumping of wastes into surface waters or the poorly engineered subsurface burial of wastes must be stopped. These practices have damaged many hydrogeologic environments as these materials spread locally and regionally. The effects of geologic and pedologic structures and characteristics on

Table 8. Water discharge and solute concentration of springs on River Euphrates (83).

Spring no.	Average discharge, L/sec	Average TDS, ppm
1	—	3110
2	24	3272
3	—	3378
4	3700	3927
5	4	3002
6	100	3880
7	26	27910
8	360	3662
9	—	—
10	100	3445
11	—	3662
12	4	2690
13	29	3080

the dispersion of pollution must be recognized in order to be able to apply the correct engineered control methods.

At the moment, there is a loose/poorly coordinated, nonintegrated approach in the control of pollutants and contaminants by various professionals involved in pollution research and control. Hydrogeologists, chemical engineers, civil engineers, soil scientists, etc., do not seem to work together. The multidisciplinary and multiobjective techniques are not appreciated by pollution control planners and managers, particularly in developing countries. This unfortunate behavioral tendency may result from poor training or professional pride and hence must be discarded. Professionals working in pollution control must appreciate the contributions of others to maximize their successes. This could be achieved by proper training through improved curricula in higher education that exposes all trainees to the origin, life, spread, and hazards of pollution in the environment and the need to develop appropriate coordinated integrated control methods. Seminars, workshops, symposia, and short courses should be organized to educate all professionals, planners, and managers of pollution problems. Countries and international aid agencies should give these the desired priority.

The developed countries should assist developing ones in controlling pollution. Manpower, expertise, and some financial resources should be made available to these poor countries to aid them in planning and management of waste disposal programs. Industrial concerns in developing countries must now discard their polluting tendencies in many developing countries and cooperate in pollution control. Most of their industrial activities exacerbate incidences of pollution. Environmental Pollution Control laws must be enacted by developing countries to protect their environments. Such laws, when made, must also be effectively enforced. Egypt has established such laws and has been fairly successful in enforcing them and achieving beneficial results (1). Strong emphasis should be placed on public health and education programs and enlightenment. Even though pollution is becoming widespread in developing countries, there is still a paucity of data and records, poor documentation and information exchanges. Research centers and institutes devoted primarily to teaching and research on pollutants and contaminants are still not given any priority. Such institutions should be established urgently to work on the various aspects of pollution with particular emphasis on its genesis, spread, hazards, and control in relation to the geologic, hydrologic and hydrogeopollution cycles (Figs. 1, 4, and 5) (91).

Coordinated sampling and monitoring programs are required by zones, nations, and regions to check widespread/regional pollution. Hence, local, international, and regional pollution events should be traced and monitored on a continuous basis and warning signals against hazards issued to areas affected. Information exchanges should be encouraged between nations, among experts/professionals, governments, and aid-giving agencies.

Research programs in both developed and developing countries must give attention to sources and types of pollution, modes of occurrence and spread, dynamics of transport and dispersion, pollutants-life-expectancy, and means of disposal of wastes. Development of effective control technology should be continuously and adequately funded.

REFERENCES

1. Fano, E., Brewster, M., and Thompson, T. Water quality management in developing countries, Part 1. In: Proceedings of the 5th World Congress on Water Resources, Brussels, Belgium, 1985, pp. 641–654.
2. Fano, E., and Brewster, M. Industrial water pollution control in developing countries. In: Water Resources Management in Industrial Areas (L. R. Beard and W. H. C. Maxwell, Eds.), Tycooly International Publishing Ltd., Dublin, 1982.
3. Egboka, B. C. E., Cherry, J. A., and Farvolden, R. N. Estimation of the percentage of annual groundwater recharge with bomb tritium using a cumulative mass balance method. J. Pure Appl. Geophys. 120: 330–347 (1982).
4. Egboka, B. C. E. Nitrate contamination of shallow groundwaters in Ontario, Canada. Sci. Total Environ. 35: 53–69 (1984).
5. Feenstra, S. Subsurface contamination from spills of dense nonaqueous phase liquid (DNAPL) chemicals. In: Proc. Second Annual Tech. Seminar on Chemical Spills. Sponsored by Environment Canada, Montreal, P.Q., Feb. 5–7, 1982.
6. Edgett, J., and Coon, D. Petroleum on tap: the legacy of leaking underground storage tanks. Conservation Council of New Brunswich, Fredericton, New Brunswich, 1986.
7. Cherry, J. A. Occurrence and migration of contaminants in groundwater at municipal landfills on sand aquifers. In: Environment and Solid Wastes (C. W. Francis, S. I. Auerbach, and V. A. Jacobs, Eds), Butterworths, Boston, 1983, pp. 127–147.
8. Canter, L. W., and Knox, R. C. Septic tank system effects on groundwater quality. Lewis Publishers, Inc., Chelsea, MI, 1985.
9. Roberts, J. R., Cherry, J. A., and Schwartz, F. W. A case study of a chemical spill: polychlorinated biphenyls (PCBS). 1. History, distribution, and surface translocation. Water Resources Res. 18: 525–534 (1982).
10. Uma, K. O. Analysis of transmissivity and hydraulic conductivity values of sandy aquifers of the Imo River Basin, Nigeria. Unpublished Ph.D. Thesis, University of Nigeria, Nsukka, 1986.
11. Carter, M. W., and Moghissi, A. A. Three decades of nuclear testing. Health Phys. 33: 55–71 (1977).
12. Davis, G. H., Payne, B. R., Dincer, T., Florkowki, T., and Gattinger, T. Seasonal variations in the tritium content of groundwaters of the Vienna Basin, Austria. Isotopes in Hydrology, International Atomic Energy Agency, Vienna, 1967.
13. Thatcher, L. L., and Payne, B. R. The distribution of tritium in precipitation over continents and its significance to groundwater dating. In: Proceedings of the 6th International Conference on Radiocarbon and Tritium Dating, Pullman, Washington, 1965.
14. Egboka, B. C. E., and Orajaka, I. P. Models of soil and gully erosion dynamics. J. Water Resources (Iraq) 5(1): 279–299 (1986).
15. Egboka, B. C. E. Analysis of groundwater resources of Nsukka area and environs, Anambra State, Nigeria. Nig. J. Min. Geol. 1 and 2: 1–16 (1983).
16. Egboka, B. C. E., and Nwankwor, G. I. The hydrogeological and geotechnical parameters as agents for the expansion of Agulu-Nanka gully, Anambra State, Nigeria. J. Afr. Earth Sci. 3(4): 417–425 (1985).

17. Egboka, B. C. E., Orajaka, I. P., and Nwosu, V. C. Redox activities as additional causative factors in generation of gully erosion in Agulu-Nanka area of Nigeria. Sci. Total Environ. 53(3): 217-232 (1986).

18. Okpoko, E. I. The Water Resources of Awka Town and Environs. Unpublished M.Sc. Thesis, Anambra State University of Technology, Nigeria, 1986.

19. Mbanugoh, R. E. The Water Resources of Ogidi Area. Unpublished M.Sc. Thesis, Anambra State University of Technology, Nigeria, 1987.

20. Dubrovsky, N. M., Morin, K. A., Cherry, S. A., and Smyth, D. J. Uranium tailings acidification and subsurface contaminant migration in a sand aquifer. Water Pollut. Res. J. 19: 59-89 (1985).

21. Egboka, B. C. E., and Uma, K. O. Acid mine drainage problems of Enugu coal mines of Anambra State, Nigeria. Mine Water (Grenada, Spain) 1: 1-11 (1985).

22. Egboka, B. C. E. Water resources problems of Enugu area, Anambra State, Nigeria. In: IAHS Publ. No. 153, 1985, pp. 95-106.

23. Grant, W. D., and Long, P. E. Environmental Microbiology. Blackie and Sons Ltd., Glasgow, 1981.

24. Hutchinson, M., and Ridgeway, J. W. Microbiological aspects of drinking water supplies. In: Aquatic Microbiology (F. A. Skinner and J. M. Shewan, Eds.), Academic Press, London, 1977.

25. Anonymous. Annual Report of the Food and Drug Administration. Federal Ministry of Health, Kaduna, Nigeria, 1977.

26. Mitchell, R. Microorganisms and pollution. Water Pollut. Microbiol. 2: 179-192 (1978).

27. Trudinger, P. A. Assimilatory and dissimilatory metabolism of inorganic sulphur compounds. Adv. Microb. Physiol. 3: 111-158 (1969).

28. LeGall, J., and Postgate, J. R. The physiology of sulphate-reducing bacteria. Adv. Microb. Physiol. 10: 81-128 (1973).

29. Ehrlich, H. L. Inorganic energy sources for chemolithotropic and mixotrophic bacteria. Geomicrobiol. J. 1: 65-83 (1979).

30. Buchanan, R. E., and Gibbons, N. E., Eds. Bergy's Manual of Determinative Bacteriology, 8th edition. Williams and Wilkins, Baltimore, MD. 1974.

31. Egboka, B. C. E. Field investigations of denitrification in groundwater. Unpublished M.Sc. Thesis, University of Waterloo, Ontario, Canada, 1978.

32. Press, F., and Siever, R. Earth. W. H. Freeman and Company, San Francisco, 1982.

33. Gillham, R. W., and Cherry, J. A. Field evidence of denitrification in shallow groundwater flow systems. Water Pollut. Res. (Canada) 13: 53-71 (1978).

34. Trentham, R. C., and Orajaka, I. P. Leaching of uranium from felsic volcanic rocks. Experimental studies. Uranium 3: 55-67 (1986).

35. Orajaka, I. P. Behaviour of uranium during the formation and diagenetic alteration of silicic volcanoclastic sediments—a critical review. Uranium 4 (1): 1-23 (1987).

36. Orajaka, I. P. Geochemistry of Kaffo Valley albite—riebeckite granite, Liruei granite ring complex, northern Nigeria. Chem. Geol. 56: 85-92 (1986).

37. Egboka, B. C. E., Mbanugoh, R. E., Nwogute, N. S., and Uma, K. O. Implications of handdug wells in planning and management of water resources in a developing economy such as Nigeria. Water Int. (U.S.) 13(2): 98-105 (1988).

38. Freeze, R. A., and Cherry, J. A. Groundwater. Prentice-Hall, Englewood Cliffs, NJ, 1979.

39. Freeze, R. A., and Witherspoon, P. A. Theoretical analysis of regional groundwater flow 2. Effect of watertable configuration and subsurface permeability variation. Water Resources Res. 3: 623-634 (1967).

40. Robertson, J. M., Teussaint, C. R., and Jorque, M. A. Organic Compounds Entering Groundwater from a Landfill. Env. Protect. Technol. Serv. US EPA Report No. 660/2-74-077, Washington, DC, 1974.

41. Egboka, B. C. E., Cherry, J. A., Farvolden, R. N., and Frind, E. O. Migration of contaminants in groundwater: a case study. 3. Tritium as an indicator of dispersion and recharge. J. Hydrol. 63: 51-80 (1983).

42. U. S. Environmental Protection Agency. The Report to Con-gress: Waste Disposal Practices and Their Effects on Groundwater. U.S. EPA Office of Water Supply, Office of Solid Waste Management Programs, Washington, DC, 1977.

43. Cherry, J. A., Grisak, G. E., and Jackson, R. E. Hydrogeological factors in shallow subsurface radioactive waste management in Canada. In: Proceedings of International Conference on Land for Waste Management (J. Thomlinson, Ed.), National Research Council, Ottawa, Canada, 1974, pp. 131-146.

44. Piper, A. M. Disposal of liquid wastes by injection underground—neither myth nor millennium. U.S. Geol. Surv. Circular 631, Reston, VA, 1969.

45. Warner, D. L., and Orcutt, D. H. Industrial wastewater injection wells in United States—status of use and regulation. In: Underground Waste Management and Artificial Recharge (J. Braunstein, Ed.), American Association Petroleum Geologists, US Geological Survey, International Association Hydrological Sciences, 2, 1973.

46. Grisak, G. E. Nitrates in shallow groundwater near Lethbridge. Tech. Rept. Environ. Protect. Serv., Edmonton, Canada, 1975.

47. Custer, G. S. Shallow Groundwater Salinization in Dry Land Farm Areas of Montana. Montana Universities Joint Water Resources Research Centre Rept. No. 79, Bozeman, MT, 1976.

48. Jackson, R. E., and Inch, K. J. Hydrogeochemical processes affecting the migration of radionuclides in a fluvial sand aquifer at the Chalk River Nuclear Laboratories. NHRI Paper No. 7 (Scientific Series No. 104). National Hydrological Research Institute Inland Waters Directorate, Ottawa, Canada, 1980.

49. Stumm, W., and Morgan, J. J. Aquatic Chemistry. John Wiley and Sons, New York, 1970.

50. Drever, J. I. The Geochemistry of Natural Waters. Prentice-Hall, Englewood Cliffs, NJ, 1982.

51. Romero, J. C. The movement of bacteria and viruses through porous media. Groundwater 8: 37-48 (1970).

52. Allen, M. J., and Geldreich, E. E. Bacteriological criteria for groundwater quality. Groundwater 13: 45-51 (1975).

53. Ogata, A. Theory of dispersion in a granular medium. U. S. Geol. Surv. Prof. Paper 411-1, Reston, VA, 1970.

54. Sudicky, E. A., Cherry, J. A., and Frind, E. O. Migration of contaminants in groundwater at a landfill: a case study, 4. A natural gradient dispersion test. J. Hydrol. 63: 81-108 (1983).

55. Lindorff, D. E. Groundwater pollution—a status report. Groundwater 17(1): 9-17 (1979).

56. Croll, B. T. The impact of organic pesticides and herbicides upon groundwater pollution. In: Groundwater Pollution in Europe (J. A. Cole, Ed.), Water Information Centre, Inc., Port Washington, NY, 1972, pp. 350-364.

57. Cherry, J. A. Groundwater occurrence and contamination in Canada. In: Canadian Aquatic Resources (M. Healey, Ed.), in press.

58. Davis, G. H., Lee, C. K., and Payne, B. R. Geohydrologic interpretations of a volcanic island from environmental isotopes. Water Resources Res. 6(1): 99-109 (1970).

59. Fried, J. J. Groundwater Pollution. American Elsevier Publications Co., New York, 1975.

60. Beak Consultants Limited. Groundwater Contamination in Canada: Selected Cases, Potential Sources and Protection Strategy. Final Report, DDS File No. 5255. KE-145-5-0138. Environment Canada, Hull, P.Q., 1986.

61. Cole, C. A., and Taylor, F. B. Possible acidification of some public groundwater supplies in Pennsylvania. Water Quality Bulletin, WHO Collaborating Centre on Surface and Groundwater Quality, Vol. 2, 1986, pp. 123-130.

62. Milde, G., Milde, K., Friesel, P., and Kiper, M. Basis and new development of the groundwater quality protection concepts in Central Europe. In: Proceedings of the International Conference on Groundwater and Man, Sydney, Australia, 1983, pp. 287-296.

63. Pupp, C. An Assessment of Groundwater Contamination in Canada, Part 1. Environment Interpretation Division, Environment Canada, 1985.

64. Pye, V. L., Patrick, R., and Quarles, J. Groundwater contamination in the United States. University of Pennsylvania Press, Philadelphia, PA, 1983.

65. Behnke, J. J., and Haskell, E. E. Groundwater nitrate distri-

bution beneath Fresno, California. J. Am. Water Works Assoc. 60(1): 477–480 (1968).

66. Okafor, N. Aquatic and Waste Microbiology. Fourth Dimension Publishers, Enugu, Nigeria, 1985.

67. Cullimore, D. R., and McCann, A. E. The identification, cultivation and control of iron bacteria in groundwater. In: Aquatic Microbiology (F. A. Skinner and J. M. Shewan, Eds.), Academic Press, London, 1977, pp. 219–261.

68. Hasselbarth, U., and Ludemann, D. Biological incrustation of wells due to mass development of iron and manganese bacteria. Water Treat. Exam. 21: 20–29 (1972).

69. Iwugo, K. O. Groundwater quality, treatment and pollution in Nigeria—the Lagos metropolis case study. In: Proceedings of Groundwater Resources Conference, Lagos, Nigeria, 1986, pp. 259–285.

70. Oyegoke, E. S. The hydrogeology of the Lagos metropolis. In: Proceedings of Groundwater Resources Conference, Lagos, Nigeria, 1986, pp. 69–80.

71. Lenvinson, A. A. Introduction of Exploration Geochemistry. Applied Publishing Limited, Calgary, Alberta, Canada, 1980, pp. 125–186.

72. Uma, K. O. Water Resources Potentials of Owerri and Its Environs. Unpublished M.Sc. Thesis, University of Nigeria, 1984.

73. Gupta, S. K., and Sharma, P., Eds. Current Trends in Arid Zone Hydrology. Today and Tomorrow's Printers and Publishers, New Delhi, India, 1978.

74. Heynen, J. D., and Trietsch, R. Appraisal of rural water supply projects in India. In: Proceedings of the 5th World Congress on Water Resources, Brussels, Belgium, 1985, pp. 567–576.

75. Pathak, B. D. Investigation of the hydrogeological problems in some mines in India. Mine Water 1: 181–189 (1985).

76. Nair, K. R., Manji, F., and Gitonga, J. N. The occurrence and distribution of fluoride in groundwaters of Kenya. In: Challenges of African Hydrology and Water Resources (D. E. Walling, S. S. D. Foster, and P. Wurzel, Eds.), IAHS Publ. No. 144, 1984, pp. 75–86.

77. Chilton, P. J., and Smith-Carington, A. K. Characteristics of the weathered basement aquifer in Malawi in relation to rural water supplies. In: Challenges in African Hydrology and Water Resources (D. E. Walling, S. S. D. Foster, and P. Wurzel, Eds.), IAHS Publ. No. 144, 1984, pp. 57–72.

78. Foster, S. S. D., Farr, J. F., and Lewis, W. J. A Detailed Evaluation of the Pollution Hazard to Village Water-Supply Boreholes

in Eastern Botswana. Geol. Surv. Rep. GS10/4. Report to Dept. of Water Affairs, Republic of Botswana, 1978.

79. DuPreez, J. W., and Barber, W. The Distribution and Chemical Quality of Groundwater in Northern Nigeria. Geol. Surv. Nigeria Bull. No. 36, Lagos, Nigeria, 1965.

80. Egboka, B. C. E., and Uma, K. O. Hydrogeochemistry, contaminant transport and tectonic effects at the Okposi-Uburu salt lake areas, Imo State, Nigeria. Hydrol. Sci. J. 36(2): 205–221 (1985).

81. Nwankwor, G. I., and Okpala, K. O. Assessment of the Pollution Risks in Parts of Otamiri and Aba River Waterworks, Imo State, Nigeria. Tech. Rep. Submitted to Imo State, Nigeria, Environmental Sanitation Authority, 1986.

82. Preul, H. C. Wastewater system alternatives for villages in lower Egypt. Proc. In: Proceedings of the 5th World Congress on Water Resources, Brussels, Belgium, 1985, pp. 357–366.

83. Al-Jabbari, M. H., and Al-Ansari, N. A. Dissolved load and ionic balance in the upper reaches of the Euphrates River. J. Water Resources (Iraq) 5(1): 98–116 (1986).

84. Salman, H. H., and Saadallah, A. S. Dust fallouts in central and southern parts of Iraq. J. Water Resources (Iraq) 5(1): 599–621 (1986).

85. Salman, A. M., Al-Ansari, N. A., and Al-Falluji, M. N. Bacterial density and related hydrological parameters of Tigris water within Baghdad. J. Water Resources (Iraq) 5(1): 622–640. (1986).

86. Brinkmann, W. L. F. Hydrogeochemistry of groundwater resources in central Amazonia, Brazil. In: Groundwater in Water Resources Planning. IAHS Publ. No. 142, 1983, pp. 67–85.

87. Ducloux, J. J. H. Groundwater exploration in the central zone of La Pampa province (Argentine). In: Groundwater in Water Resources Planning. IAHS Publ. No. 142, 1983, pp. 115–127.

88. Bender, H., Hobler, M., and Krampe, K. Groundwater resources evaluation and possibilities of their development in the area of the three towns (Khartoum Province, Sudan). In: Groundwater in Water Resources Planning. IAHS Publ. No. 142, 1983, pp. 55–66.

89. Walling, D. E., Foster, S. S. D., and Warzel, P., Eds. Challenges in African Hydrology and Water Resources. IAHS Publ. No. 144, 1984.

90. Deutsch, M. Natural controls involved in shallow aquifer contamination. Groundwater J. 3(3): 37–41 (1965).

91. Fernendez-Rubio, R. Mine Water, Vols. 1 and 2. Talleres Graficos ARTE, S.A. Maracena, Granada, 1985.

Articles
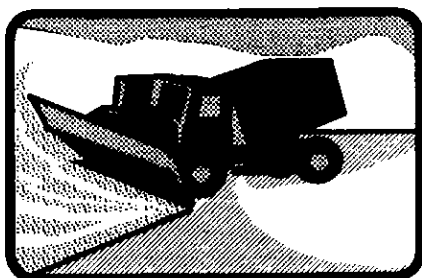
URBAN GEOLOGY 3. Groundwater Contamination Due To Road De-icing Chemicals — Salt Balance Implications

Ken W.F. Howard
Groundwater Research Group
University of Toronto
Scarborough Campus
Scarborough, Ontario MIC IA4

Janet Haynes
David L. Charlesworth and
Associates Inc.
Suite 110, 77 Mowatt Avenue
Toronto, Ontario M6K 3E3

ABSTRACT
Every year, roads and highways in Metropolitan Toronto receive more than 100,000 tonnes (t) of NaCl road de-icing chemicals. While much of this salt is flushed from the region every winter season by overland flow, a proportion will enter the sub-surface and eventually discharge to urban streams as baseflow. To determine annual retention rates of de-icing salts in an urban watershed, a chloride mass balance has been applied to the Highland Creek basin, a typical urban catchment in eastern Metropolitan Toronto. The catchment has an area of 104 km^2 and groundwater recharge is estimated to be 162 mm per year. Chloride input to the catchment was determined from municipal records. These show that the catchment receives approximately 10,000 t of chloride annually, predominantly in the form of NaCl de-icing chemicals which are applied to roads, highways and parking lots during the winter months. Chloride output was estimated from stream flow and electrical conductivity measurements recorded at 15-minute intervals over a two-year period.

The balance reveals that only 45% of the salt applied to the catchment is being removed annually and that the remainder is entering temporary storage in shallow sub-surface waters. If present rates of salt application are maintained, it is predicted that *average* steady-state chloride concentrations in ground waters discharging as springs in the basin will reach an unacceptable 426 ± 50 mg·L^{-1} possibly within a 20-year time frame. The value of 426 mg·L^{-1} represents a three-fold increase over present average baseflow concentrations, and is nearly twice the drinking water quality objective of 250 mg·L^{-1} maximum acceptable concentration.

INTRODUCTION
During the past 40 years, residents living in the snowbelt regions of Europe, Canada and the United States have come to expect bare-pavement driving conditions throughout the winter. As a result, millions of tonnes of de-icing agents, usually in the form of sodium chloride (NaCl), have been applied to urban roads and highways. The general assumptions have been that the majority of the applied salt is flushed from the basin every season by rain and snow-melt, and environmental impacts are minimal. There is increasing evidence, however, that a significant proportion of the salt may be retained in the basin,

entering the shallow sub-surface and migrating gradually to the watertable (*e.g.*, Diment *et al.*, 1973; Eisen and Anderson, 1980; Pilon and Howard, 1987). In such cases, serious degradation of groundwater quality could be anticipated (Howard and Beck, in press). The scenario shows an ominous resemblance to the situation in the United Kingdom and other European countries (Howard, 1985) where nitrate, innocently applied as fertilizer during post-war years, migrated through the unsaturated zone at a rate of ~1 m per year and eventually caused extensive contamination of major aquifers. In the case of NaCl de-icing chemicals, the most common concern is an increase in salinity to levels which would make the water unsuitable for human consumption and some industrial applications. There is also evidence, however, that sodium, the counter ion to chloride in most road de-icing salts, may have serious health implications. The ion has been strongly linked with the development of hypertension, a condition affecting perhaps 20% of the United States population (Moses, 1980; Craun, 1984; Tuthill and Calabrese, 1979). Elevated sodium intake has also been associated indirectly with hypernatraemia (World Health Organization, 1984). It is generally recommended that sodium concentrations in drinking water should not exceed 20 mg·L^{-1} for patients with hypertension or congestive heart failure.

Concern for contamination by deicing salts is particularly acute in the Toronto region, where >100,000 t of NaCl are applied to urban roads and highways every year. Within Metropolitan Toronto, major streams regularly contain several hundred mg·L^{-1} chloride; in winter months, values greater than 1000 mg·L^{-1} are common. Recent records suggest that values of this order have shown little change in recent years

and, while these levels are unacceptably high, there has been little stream quality evidence to indicate that the situation will become any worse.

It was Paine (1979) who first suggested that road de-icing chemicals may be accumulating in the sub-surface and could one day re-appear to cause serious damage to urban waterways. Paine performed a relatively coarse chloride mass balance on the Don River watershed (Fig. 1) and suggested that as little as 50% of the applied chloride was being removed from the basin annually and that the remainder was being stored in the shallow sub-surface. Follow-up studies by Pilon and Howard (1987), Eyles and Howard (1988), and Taylor et al. (1991) confirmed that concentrations of chloride as high as 14,000 mg·L⁻¹ were accumulating in shallow ground waters beneath Metropolitan Toronto, raising concern that the waters would ultimately enter local streams where serious and uncontrollable contamination would occur.

Recent and current studies at the University of Toronto have been concerned with the impact of urban development on groundwater quality and have focussed especially on the movement and behaviour of shallow ground waters containing elevated concentrations of chloride. An overall goal of the current work is to develop a series of numerical models that will permit the movement of de-icing salts in a catchment to be simulated and thereby allow the impact of alternative salting strategies to be evaluated. The success of these models depends, in turn, on an understanding of the nature of salt behaviour in a typical urban catchment, and, in particular, on an accurate knowledge of the rate at which salt is retained on an annual basis.

The retention rate of de-icing salts in a watershed is most reliably determined using a catchment mass balance approach. In this approach, salt input, represented by the mass of salt applied to the catchment during a specified time frame, is budgeted against salt output in the form of salt loads in the exiting stream. The net difference represents the mass of salt that is stored (retained) within the catchment.

In practice, the rates of salt retention will vary with time. At early times, the amount of salt entering the sub-surface will far exceed the amount leaving in baseflow. As time proceeds, salt con-
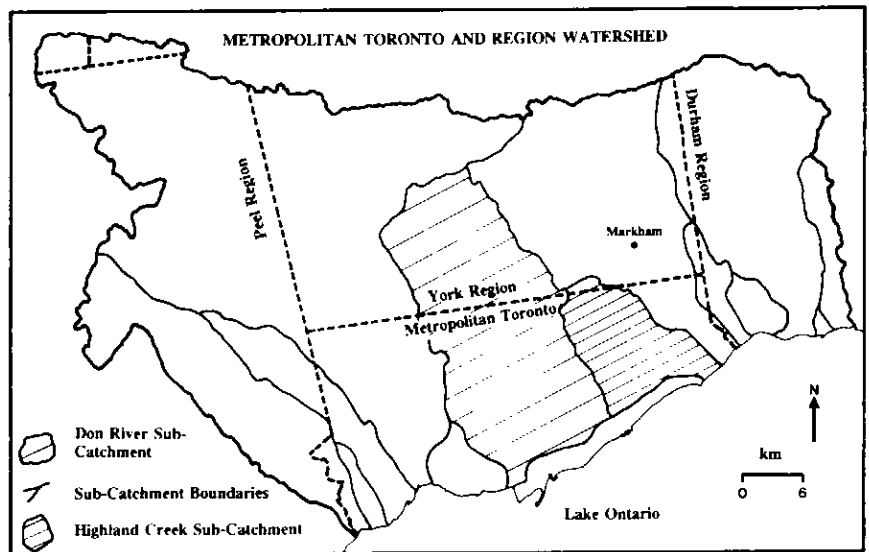
Figure 1 Fourteen sub-catchments of the Metropolitan Toronto and Region Watershed.
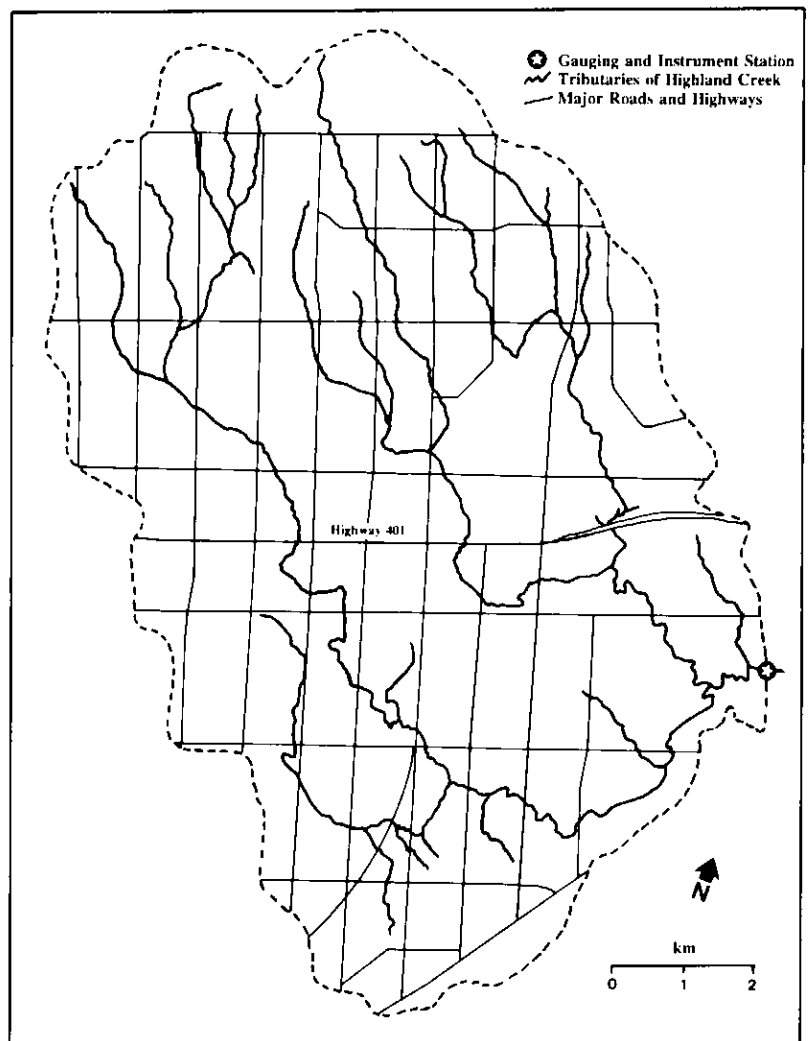
Figure 2 The Highland Creek basin showing distribution of major salted roads and highways. The basin is highly urbanized; minor salted roads (not shown) comprise 75% of the total length of salted roads and highways in the basin.

centrations in baseflow will increase and, on an annual basis, losses will start to approach the amounts entering the sub-surface. Steady state will be reached when the net rate of salt retention equals zero and the inflow of salt matches outflow. While a simple salt balance for a specific drainage system will allow the rate of salt retention to be determined for any one time period, corrections for baseflow contributions to the drainage system must be made if the annual rate of contribution of salt to the sub-surface is to be estimated.

The accuracy of the balance is determined by the quality, frequency and time frame of the data used. Previous studies, conducted in differing types of catchment, have produced figures for the amount of salt (chloride) retained annually, ranging from 19% to 65% (Wulkowicz and Saleem, 1974; Scott, 1980; Paine, 1979). Diment *et al.*, (1973) showed that the amount retained can fluctuate each year, finding that unusually high summer rainfall in the second year of a two-year study resulted in much higher (20%) salt removal than seen in the first year. A criticism of much of the earlier work, however, is that the balances were conducted over a relatively short time (often one year or less) and/or used daily stream flow data. The latter is of particular concern in urban catchments, where stream flow rates and chemical concentrations can vary by three orders of magnitude within a

matter of hours. In the present study, conducted on Highland Creek from March 1989 to April 1991, this shortcoming has been surmounted by installation of a data logger to collect the necessary data at 15-minute intervals.

STUDY AREA

The chloride mass balance study was performed on Highland Creek basin, one of 14 major sub-catchments in the Metropolitan Toronto and Region watershed (Fig. 1). Annual precipitation in this catchment is approximately 850 mm and Thornthwaite evapotranspiration is estimated to be 600 mm. The catchment has a total area of 104 km^2 and an Environment Canada gauging station is located 5 km upstream of its discharge into Lake Ontario. A review of storm sewer drainage areas and topographic data suggests that the catchment area upstream of the weir has an area of approximately 82 km^2. This area was used for the mass balance calculations.

The basin is almost entirely urbanized (Fig. 2), with recreational open space along the main Creek valley and some remaining undeveloped land in the extreme northeast. The surface sediments are predominantly silty sand till (Karrow, 1967), but recent flood plain deposits occur extensively along the valley floor. The most important aquifers include the Scarborough Sands and the Lake Iroquois terrace deposits (Ontario Ministry of Natural Resources, 1980).

Locally these are separated by the Sunnybrook diamict. Total aquifer thickness is estimated to be 30 m and the water table depth varies up to 20 m. Groundwater flow velocities are thought to be in the range of 10-100 m per year.

The basin is crossed by Highway 401 (12 lanes wide), and by a grid of two- and four-lane arterial roads about 1.5-2 km apart. These arteries and numerous secondary roads are regularly salted throughout the winter season by five agencies: the Ontario Ministry of Transportation (MTO), Metropolitan Toronto, City of Scarborough, Town of Markham, and the Regional Municipality of York. The basin receives ~17,000 t of NaCl road de-icing chemicals each year. This represents approximately 200 g of NaCl for every square metre of the catchment.

Approximately two years of average daily stream flow data for Highland Creek are shown in Figure 3. Baseflow contributions vary between 0.3 $m^3 \cdot s^{-1}$ and 1.2 $m^3 \cdot s^{-1}$, peaking during the spring, and average 0.42 $m^3 \cdot s^{-1}$ over the year. This represents an average annual recharge to the 82 km^2 catchment of 162 mm. If all of the applied salt were to enter the sub-surface *via* recharge, average steady-state sodium and chloride concentrations in ground water would approach 500 $mg \cdot L^{-1}$ and 800 $mg \cdot L^{-1}$, respectively.

INSTRUMENTATION

Because no reasonably priced, reliable and rugged chloride sensor was available, electrical conductivity, which is directly related to chloride concentration, was selected as a surrogate for monitoring in the field. Work began in July of 1988 when a YSI conductivity cell was installed at the Environment Canada weir to take advantage of the flow data which were already being collected at 15-minute intervals. Within days, the meter was damaged by a nearby lightning strike and the probe was buried in the stream bed. A second attempt to obtain data was made in March 1989, when an IC Controls temperature-compensated conductivity probe, model CC01, was installed near mid-stream at the weir. This probe is designed specifically for industrial applications, with both the sensor and sensor electronics sealed in PVC. The probe was connected by cable to a conductivity meter which was housed in the Environment Canada monitoring station, and pro-
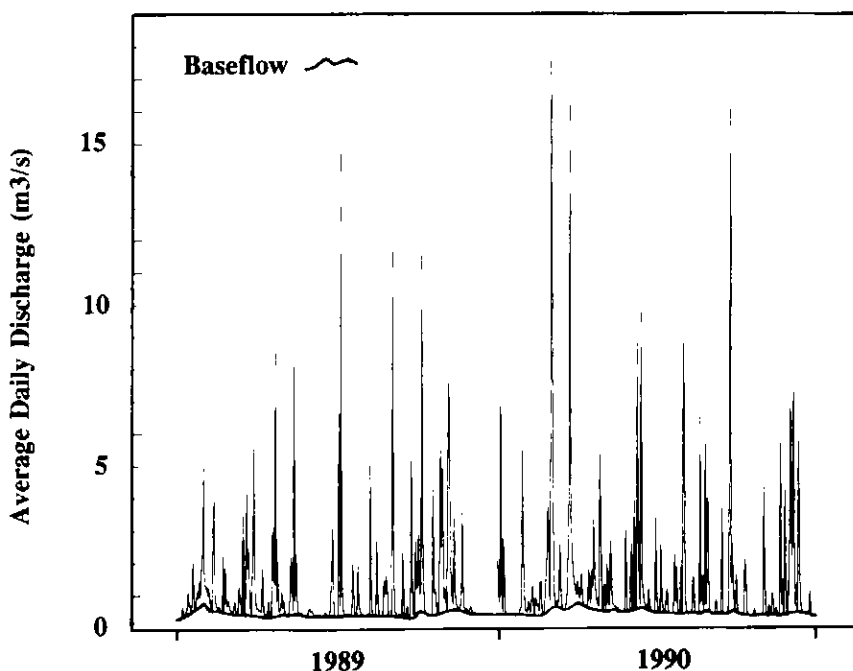

Figure 3   *Highland Creek stream hydrograph for the period 1 January 1989 to 30 November 1990.*

vided a continuous output of electrical voltage as a measure of the conductivity. This output was recorded every 15 minutes by a Lakewood LE7110 data logger, which is capable of storing up to six months of data. When possible, data were retrieved from the data logger monthly using a laptop computer.

The probe sensors were cleaned every 3-4 weeks with a very fine abrasive. Algal growth that accumulates during the summer on the protective mesh around the probe tip was removed manually and appears to have had no effect on the probe function.

Calibration

The probe was initially calibrated and tested for drift in the laboratory using NaCl solutions of known conductivity. After installation in the creek, however, a consistent difference was observed between conductivity values calculated from the *in situ* conductivity readings and values measured from stream samples. This discrepancy is largely the result of monitoring in a flowing stream *versus* calibrating in static solutions. A small correction factor is now applied to the field measurements.

To permit conversion of measured electrical conductivity values to chloride concentration, samples of water were collected from the creek on a regular basis and analyzed for both parameters As shown in Figure 4, the data correlate well. The best line fit is represented by a second order quadratic of the form:

Cl concentration (mg·L $^{-1}$) =
$0.0000025 * E_c^2 + 0.31 * E_c - 96$ (1)
where E_c is the electrical conductivity in µS/cm at 25°C.

CHLORIDE BALANCE CALCULATION

The chloride balance study was carried out from December 1988 to April 1991, inclusive, and included three winter seasons' salting. It was performed by estimating the total chloride input to the catchment over a pre-determined period and subtracting the amount of this chloride leaving the watershed during the same period by overland flow. The difference represents the mass of chloride retained, at least temporarily, in surface waters, soils and subsurface waters. In the study, the amount of chloride leaving the basin through stream sediment load was assumed to be negligible. Corrections were made, however, to account for the mass of chloride leaving the basin as baseflow, since very little of this chloride would have originated from the previous salting season.

Chloride Input Data

Road salt is the major source of chloride entering the basin. It is applied as pure
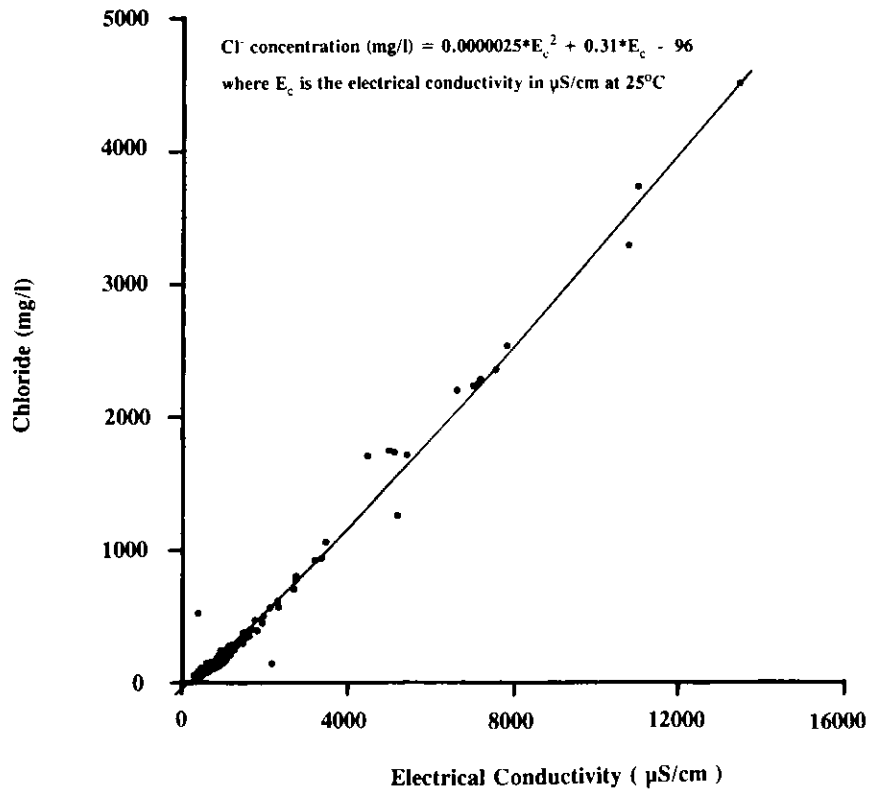
Figure 4 *Relationship between chloride and electrical conductivity used for site calibration.*

Table 1	Annual rates of NaCl application to the Highland Creek catchment.				
	SODIUM CHLORIDE				CHLORIDE
Salting Season	Applied to Roads/ Highways (t)	Applied to Parking Lots (t)	Domestic Use (t)	Basin Total (t)	Basin Total (t)
1988-89	15,031	2104	150	17,285	10,486
1989-90	16,095	2253	161	18,510	11,228
1990-91	13,149	1841	131	15,122	9,173
Annual Average	14,758	2066	147	16,972	10,295

salt (NaCl) or as a salt/sand mixture. Daily salt application was determined from the yard records of the five agencies applying salt. Where salt/sanding routes straddled the catchment boundary, the total salt applied was apportioned to the catchments on the assumption that rates of application remained consistent along the routes. Total salt application in the study area

during the winters of 1988-89, 1989-90 and 1990-91 is shown in Table 1. The annual distribution of chloride application from 1 November 1989 to 30 April 1991 is shown in Figure 5. It is estimated that these figures are accurate to ±5%.

Also included in Table 1 are estimates of other sources of chloride input. These include the amount of salt applied to parking lots, for which it was assumed

that rates of application for shopping centre lots were the same as those for major roads, and for other lots, one-half this rate (Scott, 1980). Salt applied to parking lots represented about 14% of the total salt applied. Application by private home-owners was estimated by multiplying the approximate number of single-family residences by an average annual rate of 4 kg/household. This represented <1% of the total salt application.

Other sources of chloride are relatively insignificant. Chloride concentration in precipitation around Metropolitan Toronto during 1986 averaged 0.2 mg·L^{-1} (Ontario Ministry of the Environment, 1988), which converts to ~0.1% of the total chloride input. A calcium chloride de-icing additive is used occasionally in the area, but contributes <0.1% of the total chloride input. There are no known sanitary-storm sewer interconnections in the basin (Scarborough Works; pers. comm.). Depending on their chemical formulation, fertilizers may contain a significant amount of chloride; however, this potential contribution is not thought to be significant and has not been quantified in this study.
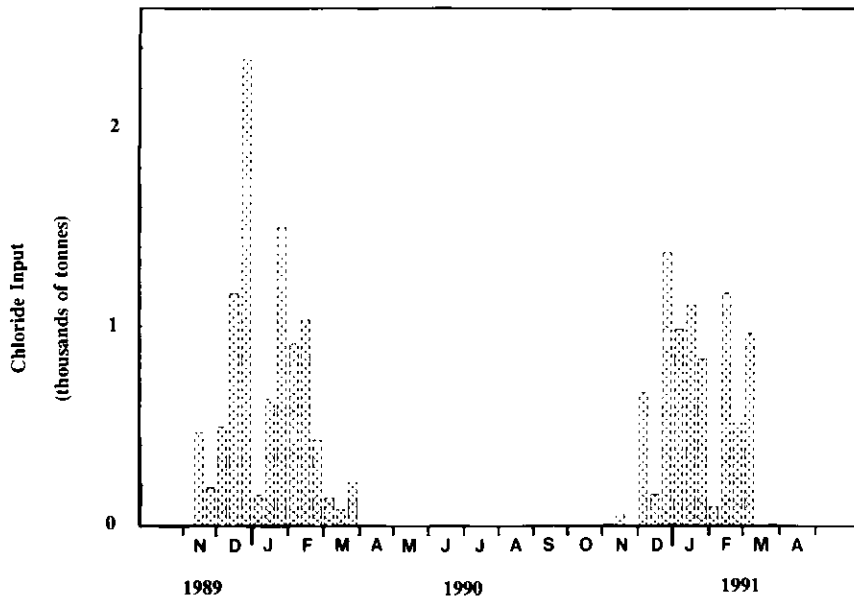
Figure 5   Temporal distribution of chloride input for the period 1 November 1989 to 30 April 1991. Data are plotted for periods ending on the tenth, twentieth and last day of each month.

Chloride Output Data

Chloride discharge or "loading" in a stream can be calculated by:

$$CI = Cm * Q \tag{2}$$

where Cm is mass concentration and Q is stream discharge. This calculation was performed for 15-minute intervals using a Lotus 123 spreadsheet. Totals were output three times per month, at the end of the tenth, twentieth and last day of each month. Estimates for the period from 11 March 1989 to 30 April 1991 are shown on Table 2; data for the period from 1 November 1989 to 30 April 1991 are plotted in Figure 6. Some values in Table 2 are marked with an asterisk to indicate that some data were lost during the time period: data extrapolation was required in those cases.

Also shown in Table 2 are estimates of the mass of chloride entering the stream as a result of baseflow. These were determined by multiplying the average baseflow for the time interval of interest by the estimated chloride concentration. Normally, this would be the chloride concentration in the stsream at a time, usually following several days of dry weather, when stream flows are maintained entirely by baseflow. Most of

Figure 6   Temporal distribution of chloride output in Highland Creek for the period 1 November 1989 to 30 April 1991. Data are plotted for periods ending on the tenth, twentieth and last day of each month.

6

the chloride contained in the baseflow is unlikely to have originated during the previous winter's salting, and instead represents chloride that has accumulated in the basin over its history of salting. As such, the baseflow contributions must be subtracted from the total chloride load to calculate the amount of chloride retained in the basin as a result of the previous winter's salting. A summary of the results is shown in Table 3.

RESULTS AND CONCLUDING DISCUSSION

Urbanized catchments of the Metropolitan Toronto and Region watershed have, for several decades, received more than one hundred thousand tonnes of de-icing salts annually. For much of this time, it has been assumed that most of this salt is flushed from the catchments each season by overland flow and that sub-surface impacts are minimal. However, recent evidence of elevated chloride in ground water beneath the catchments suggests that a significant proportion of the applied salt may be retained in the basin each season and may, therefore, be responsible for the observed degradation in groundwater quality.

The salt balance performed on the Highland Creek basin of Metropolitan Toronto has generated a large volume of

Table 2		Chloride mass balance components for the period 1 December 1988 to 31 October 1991.								
Month	Period	Input (t)	Baseflow load (t)	Output[1] (t)	Input (t)	Baseflow load (t)	Output[1] (t)	Input (t)	Baseflow load (t)	Output[1] (t)
		1988								
Dec.	1-10	228 62	–	–						
	11-20	1802 65	–	–						
	21-31	973.62	–	–						
		1989				**1990**			**1991**	
Jan.	1-10	1761 81	–	–	173.83	47 25	96.54*	1136 75	94 05	319 84
	11-20	526 17	–	–	728.48	47.25	1359 52*	1276 50	94 50	684.27
	21-31	412 93	–	–	1725.53	47 25	381 74*	962 56	90.00	117 50
Feb.	1-10	1031 09	–	–	1052.91	47 25	291 94*	111 95	81 00	147 93
	11-20	239 59	–	–	1189 35	76.32	51 33	1347 16	43 20	429.98
	21-28	1453 80	–	–	494.25	75 60	558 13	597 11	61 20	166 43
March	1-10	735 89	–	–	160 74	87.08	123 51	111 99	81 90	103.40*
	11-20	1024 05	112 50	810 70	95 32	135 00	49.88	0 67	70 20	37 09*
	21-31	164 70	121 50	588 53	251 77	44.55	34 93	9 00	79 56	134 65*
Apr.	1-10	130 88	108.00	112 21	0 00	40.50	24 70	0 00	40 50	140.76*
	11-20	0 00	99 00	109 54	0.00	26 64	28.93	0 00	108.00	51 73
	21-30	0 00	45.00	29 60	0 00	54.00	62 38	0 00	112 50	36 11
May	1-10	0 00	61 20	235.56	0 00	51.75	319 30	–	–	–
	11-20	0 00	76 50	201.86	0.00	56 25	427 58	–	–	–
	21-31	0 00	45 90	187 65	0 00	63.00	289.23	–	–	–
June	1-10	0 00	45.00	104 50*	0 00	79.20	56 15	–	–	–
	11-20	0 00	36 00	92 94*	0.00	75 26	23.45	–	–	–
	21-30	0 00	34 20	47 23	0.00	64.80	110 19	–	–	–
July	1-10	0 00	45 36	49.14	0.00	69.84	14.00	–	–	–
	11-20	0 00	48 60	18 70	0.00	73 80	36 68	–	–	–
	21-31	0 00	54 00	9 17*	0.00	55.44	38 98	–	–	–
Aug.	1-10	0 00	63.00	353 27*	0.00	79.70	22 13	–	–	–
	11-20	0.00	54.72	174.85	0.00	85.32	55 15	–	–	–
	21-31	0 00	51 98	87 81	0 00	72.00	11 96	–	–	–
Sept.	1-10	0 00	51.84	60.61	0 00	38 93	9 07	–	–	–
	11-20	0.0	70.20	14.62	0.00	32.63	27 76	–	–	–
	21-30	0 00	36 99	63.30	0.00	35.10	11 56	–	–	–
Oct.	1-10	0.00	47 25	66.64	0 00	31 86	3 87	–	–	–
	11-20	0 00	27 00	2 01	0 00	60 75	30 18	–	–	–
	21-31	0 00	16.88	1 14	0.00	64 08	5.49	–	–	–
Nov.	1-10	0 00	86.76	67 49	10.89	43.88	12.98			
	11-20	534 28	67 50	138.83*	70 76	88 20	50 47			
	21-30	217 00	67.50	182.03*	0.00	44 10	3 51*			
Dec.	1-10	571 04	54 00	52.50	773 43	70.56	34.43*			
	11-20	1340.71	60.75	23.50	181.72	61.74	22 42*			
	21-31	2,693.18	70 20	14 60*	1582 66	52.92	67 85*			

Notes: 1 Output has been corrected for baseflow load.
– Not determined.
* Some data infilling required.

Table 3	Chloride balance summary for the period 1 November 1988 to 31 October 1991.					
	Salting Season	Total Input (t)	Total Output (t)	Baseflow Load (t)	Corrected Output[1] (t)	Salt Output (as % of salt applied during salt season)	Total %
1988-89	Winter (1 Nov.–30 April)	10,486	2137[2]	486[2]	1651[2]	>15[2]	>34
	Summer (1 May–31 Oct.)	NIL	2889	867	2022	19	
1989-90	Winter (1 Nov.–30 April)	11,228	4562	1135	3427	31	45
	Summer (1 May–31 Oct.)	NIL	2699	1089	1609	14	
1990-91	Winter (1 Nov.–30 April)	9173	3651	1318	2333	26	>26
	Summer (1 May–31 Oct.)	NIL	–	–	–	–	

Notes   [1] Output has been corrected for baseflow load.
[2] Data only available for March and April.
– Not determined.

reliable data. Most of the data gaps are due to the failure of flow logging instruments; these gaps were usually filled by correlation with data from neighbouring catchments. In total, a full chloride balance was completed for 26 months, extending over three salting seasons.

The results (Table 3) are consistent over the period of study. Each year, during the winter period (1 November to 30 April), approximately 10,000 t of chloride was applied to the Highland Creek catchment. Only 45% of this was removed by surface run-off before the following winter, when a new salting season began. Most of the chloride removed was flushed from the catchment during the winter in which it was applied. In the 1989-90 salting season, for example, 3427 t of chloride left the catchment by overland flow before the end of April, representing 31% of the total chloride applied. A further 1609 t (or 14% of the total) were removed by summer rain between 1 April and 30 October. Data are incomplete for the salting seasons 1988-89 and 1990-91, but the available results are comparable to those for 1989-90. For example, in 1990-91, 26% of the total chloride applied left the basin during the winter months; in 1988-89, 19% of the total chloride applied was removed during the summer.

If only 45% of the salt applied to the catchment is being removed annually, then the remainder is being stored, pre-sumably in underlying ground waters. The rate of accumulation will depend on the rate of groundwater movement in the basin. While the total mass of chloride entering the sub-surface is greater than the mass of chloride leaving as baseflow to the stream, chloride will accumulate in the ground water, and groundwater chloride concentrations will increase. For the period of study, total input of chloride far exceeds output (including baseflow load) and significant accumulation occurs. Eventually, chloride in the ground water will reach a level at which annual baseflow losses will match the amount of chloride entering the sub-surface. At this stage, steady-state will be reached and no further deterioration of groundwater or stream water quality will occur. Numerical modelling will allow the rates of change of ground water and streamwater quality to be predicted with more certainty. Assuming an annual recharge of 162 mm, an aquifer thickness of 30 m, a specific yield of 20%, and groundwater flow velocities of 100 m per year, deployment of analytical transient solutions for the transport of contaminants from continuous line sources (Domenico and Schwartz, 1990) indicates that steady state concentrations could be achieved within 60 years of initial salt application, or approximately 20 years from the present.

When steady state is reached, inflow and outflow of chloride will be in balance and the mass of chloride stored within the basin will remain unchanged. The stored chloride may not be evenly distributed within the sub-surface and water quality stratification will occur depending on the local groundwater flow regime. If conditions in the catchment remain the same and present rates of salt application are maintained, however, a simple division of the total chloride entering the sub-surface by the annual recharge reveals that average chloride concentrations in ground waters discharging as baseflow in the basin will reach 426 ± 50 mg•L^{-1}. This value represents a three-fold increase over present average baseflow concentrations, and is nearly twice the drinking water quality objective of 250 mg•L^{-1} maximum acceptable concentration.

ACKNOWLEDGEMENTS

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REFERENCES

Craun, G.F., 1984, Health aspects of groundwater pollution, *in* Bitton, G. and Gerba, C.P., eds., Groundwater Pollution Microbiology: John Wiley, New York, p. 135-179.

Diment, W.H., Bubeck, R.C. and Deck, B.L., 1973, Some Effects of De-icing Salts on Irondequoit Bay and its Drainage Basin: Highway Research Record 425, p. 23-25.

Domenico, P.A. and Schwartz, F.W., 1990, Physical and Chemical Hydrogeology: John Wiley and Sons, New York, 824 p.

Eisen, C. and Anderson, M.P., 1980, The effects of urbanization on groundwater quality, Milwaukee, Wisconsin, U.S.A., *in* Jackson, R.E., ed., Aquifer Contamination and Protection: UNESCO Press, Paris, SRH no. 30, p. 378-390.

Eyles, N. and Howard, K.W.F., 1988, A hydrochemical study of urban landslides caused by heavy rain: Scarborough Bluffs, Ontario, Canada: Canadian Geotechnical Journal, v. 25, p. 455-466.

Howard, K.W.F., 1985, An approach to recognizing low level contamination of groundwaters in shallow sedimentary aquifers: Water Pollution Research Journal of Canada, v. 20, p. 1-11.

Howard, K.W.F. and Beck, P., in press, Hydrogeochemical implications of groundwater contamination by road de-icing chemicals: Journal of Contaminant Hydrology, in press.

Karrow, P.F., 1967, Pleistocene Geology of the Scarborough Area: Ontario Department of Mines, Geological Report 46, 107 p.

Moses, C., 1980, Sodium in Medicine and Health, A Monograph: Reese Press Inc., Baltimore, 122 p.

Ontario Ministry of the Environment, 1988, Acidic precipitation in Ontario study, cumulative precipitation chemistry listings 1986: Ministry of the Environment, Report ARB-034-88.

Ontario Ministry of Natural Resources, 1980, Quaternary Geology of Toronto and Surrounding Area: Preliminary Map P. 2204.

Paine, R.L., 1979, Chlorides in the Don River Watershed Resulting from Road De-Icing Salt: University of Toronto, Institute for Environmental Studies, Snow and Ice Control Working Group, Working Paper SIC-3, 23 p.

Pilon, P.E. and Howard, K.W.F., 1987, Contamination of Subsurface Waters by Road De-icing Salts: Water Pollution Research Journal of Canada, v. 22, p. 157-171.

Scott, W.S., 1980, Road Salt Movement into Two Toronto Streams: ASCE Journal of Environmental Engineering, v. 106, p. 547-560.

Taylor, L., Howard, K.W.F. and Chambers, L., 1991, The use of spring-dwelling ostracodes as bio-monitors for inorganic contamination of groundwaters along an urban-rural transect in southern Ontario: Proceedings of the Toronto '91 GAC-MAC Annual Conference, Toronto, May 1991.

Tuthill, R.W. and Calabrese, E.J., 1979, Elevated sodium levels in the public drinking water as a contributor to elevated blood pressure levels in the community: Arch. Environmental Health, v. 34, p. 197-203.

World Health Organization, 1984, Guidelines for Drinking Water Quality, Vol. 1. Recommendations: World Health Organization, Geneva, 130 p.

Wulkowicz, G.M. and Saleem, Z.A., 1974, Chloride Balance of an Urban Basin in the Chicago Area: Water Resources Research, v. 10, p. 974-982.

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Water Science School

Aquifers and Groundwater

A huge amount of water exists in the ground below your feet, and people all over the world make great use of it. But it is only found in usable quantities in certain places underground — aquifers. Read on to understand the concepts of aquifers and how water exists in the ground.

• **Water Science School HOME** • **Groundwater topics** •

Groundwater and aquifers

I hope you appreciate my spending an hour in the blazing sun to dig this hole at the beach. It is a great way to illustrate the concept of how, below a certain depth, the ground, if it is permeable enough to hold water, is saturated with water. The upper surface of this zone of saturation is called the water table. The saturated zone beneath the water table is called an aquifer, and aquifers are huge storehouses of water. What you are looking at in this picture is a "well" that exposes the water table, with an aquifer beneath it. Of course, I am cheating here, as at the beach, the level of the water table is always at the same level as the ocean, which is

A hole dug at the beach is a great way to illustrate the concept of how, below a certain depth, the ground, if it is permeable enough to hold water, is saturated with water. The upper surface of this zone of saturation is called the water table. (Credit: Howard Perlman, USGS)

Status - Completed

Contacts

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Water

just below the surface of
the beach.

Groundwater is one of our most valuable resources—even though you probably
never see it or even realize it is there. As you may have read, most of the void
spaces in the rocks below the water table are filled with water. These rocks have
different porosity and permeability characteristics, which means that water does not
move around the same way in all rocks below ground.

When a water-bearing rock readily transmits water to wells and springs, it is called
an aquifer. **Wells** can be drilled into the aquifers and water can be pumped out.
Precipitation eventually adds water (recharge) into the porous rock of the aquifer.
The rate of recharge is not the same for all aquifers, though, and that must be
considered when pumping water from a well. Pumping too much water too fast
draws down the water in the aquifer and eventually causes a well to yield less and
less water and even run dry. In fact, pumping your well too much can even cause
your neighbor's well to run dry if you both are pumping from the same aquifer.

Visualizing groundwater

In the diagram below, you can see how the ground below the water table (the blue
area) is saturated with water. The "unsaturated zone" above the water table (the
gray area) still contains water (after all, plants' roots live in this area), but it is not
totally saturated with water. You can see this in the two drawings at the bottom of the
diagram, which show a close-up of how water is stored in between underground rock
particles.
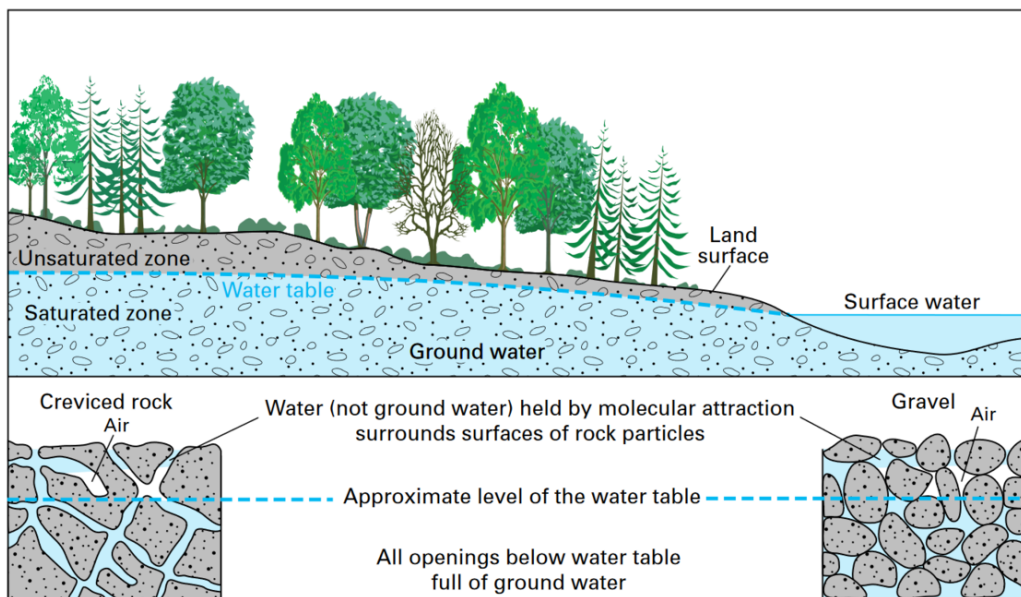
How ground water occurs in rocks.

How Groundwater Occurs

Credit:USGS, Public domain

Sometimes the porous rock layers become tilted in the earth. There might be a
confining layer of less porous rock both above and below the porous layer. This is an

example of a confined aquifer. In this case, the rocks surrounding the aquifer confines the pressure in the porous rock and its water. If a well is drilled into this "pressurized" aquifer, the internal pressure might (depending on the ability of the rock to transport water) be enough to push the water up the well and up to the surface without the aid of a pump,

Learn the basics about water using our Water Primers!

- A Primer on Water (PDF)
- A Primer on Ground Water (PDF)
- Ground water and the Rural Homeowner

sometimes completely out of the well. This type of well is called artesian. The pressure of water from an **artesian well** can be quite dramatic.

A relationship does not necessarily exist between the water-bearing capacity of rocks and the depth at which they are found. A very dense granite that will yield little or no water to a well may be exposed at the land surface. Conversely, a porous sandstone may lie hundreds or thousands of feet below the land surface and may yield hundreds of gallons per minute of water. Rocks that yield freshwater have been found at depths of more than 6,000 feet, and salty water has come from oil wells at depths of more than 30,000 feet. On the average, however, the porosity and permeability of rocks decrease as their depth below land surface increases; the pores and cracks in rocks at great depths are closed or greatly reduced in size because of the weight of overlying rocks.

Aquifers and wells

The illustration shows an artesian well and a flowing artesian well, which are drilled into a confined aquifer, and a water table well, which is drilled into an unconfined aquifer. Also shown are the Piezometric surface in the confined aquifer and the impermeable, confining layer between the confined and unconfined aquifer. (Credit: Environment and Climate Change Canada)

Pumping can affect the level of the water table

Groundwater occurs in the saturated soil and rock below the water table. If the aquifer is shallow enough and permeable enough to allow water to move through it at a rapid-enough rate, then people can drill wells into it and withdraw water. The level of the water table can naturally change over time due to changes in weather cycles and precipitation patterns, streamflow and geologic changes, and even human-induced changes, such as the increase in impervious surfaces on the landscape.

The pumping of wells can have a great deal of **influence on water levels below ground**, especially in the vicinity of the well, as this diagram shows. If water is withdrawn from the ground at a faster rate that it is replenished, either by infiltration from the surface or from streams, then the water table can become lower, resulting in a "cone of depression" around the well. Depending on geologic and hydrologic conditions of the aquifer, the impact on the level of the water table can be short-lived or last for decades, and it can fall a small amount or many hundreds of feet. Excessive pumping can lower the water table so much that the wells no longer supply water—they can "go dry."

Water movement in aquifers

Water movement in aquifers is highly dependent of the permeability of the aquifer material. Permeable material contains interconnected cracks or spaces that are both numerous enough and large enough to allow water to move freely. In some permeable materials groundwater may move several meters in a day; in other places, it moves only a few centimeters in a century. Groundwater moves very slowly

Schematic showing a cone of depression around the well, usually the result of overpumping. (Credit: Tara Gross, USGS)

through relatively impermeable materials such as clay and shale. (Source: Environment Canada)

After entering an aquifer, water moves slowly toward lower lying places and eventually is discharged from the aquifer from springs, seeps into streams, or is withdrawn from the ground by wells. Groundwater in aquifers between layers of

poorly permeable rock, such as clay or shale, may be confined under pressure. If such a confined aquifer is tapped by a well, water will rise above the top of the aquifer and may even flow from the well onto the land surface. Water confined in this way is said to be under artesian pressure, and the aquifer is called an **artesian aquifer**.

Visualizing artesian pressure

Here's a little experiment to show you how artesian pressure works. Fill a plastic sandwich baggie with water, put a straw in through the opening, tape the opening around the straw closed, point the straw upward *(but **don't** point the straw towards your teacher or parents!)* and then squeeze the baggie. Artesian water is pushed out through the straw.
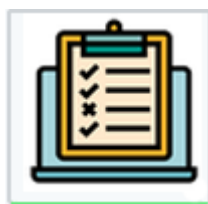
Do you think you know about groundwater?
Take our **Groundwater true/false quiz**, part of our **Activity Center**.

Quiz icon made by mynamepong from www.flaticon.com

Below are other science topics related to aquifers and groundwater.

Date published: OCTOBER 9, 2018
Status: Completed

Groundwater Information by Topic

Groundwater is one of our most valuable resources—even though you probably never see it or even realize it is there. There is water somewhere beneath your feet no matter where on Earth you live. Groundwater starts as precipitation, just as surface water does, and once water penetrates the ground, it continues moving, sometimes quickly and sometimes very slowly. Eventually groundwater emerges...

Contacts: Ask USGS

Date published: NOVEMBER 8, 2018
Status: Completed

Groundwater True/False Quiz

How much do you know about the water below your feet? Take our Groundwater True/False Quiz and find out.

Contacts: Ask USGS
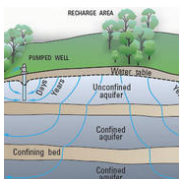
Date published: JUNE 18, 2018
Status: Completed

Groundwater Storage and the Water Cycle

The ground stores huge amounts of water and it exists to some degree no matter where on Earth you are. Lucky for people, in many places the water exists in quantities and at depths that wells can be drilled into the water-bearing aquifers and withdrawn to server the many needs people have.

Note: This section of the Water Science School discusses the Earth's "natural" water cycle...

Contacts: Ask USGS

Date published: JUNE 11, 2018
Status: Completed

How Do Hydrologists Locate Groundwater?

As a non-existent proverb states: "*Humans don't live by surface water alone.*" For thousands of years, people have also relied on groundwater to serve their every need. Groundwater is invaluable for many uses, from irrigation to drinking-water supply. But, you can't see groundwater, so how do water scientists know where it is in order to be able to drill wells and pump it out for use...

Contacts: Ask USGS
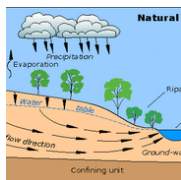
Date published: JUNE 11, 2018
Status: Completed

Groundwater Flows Underground

Millions of cubic miles of water exists in the ground. You can't see it, but not only is it there, it is always moving around -- mostly downward, but also horizontally. Moving groundwater helps keep rivers full of water and allows for people to draw out water via wells. Moving groundwater is an important part of the water cycle.

Contacts: Ask USGS

Date published: JUNE 6, 2018
Status: Completed

Groundwater Wells

Wells are extremely important to all societies. In many places wells provide a reliable and ample supply of water for home uses, irrigation, and industries. Where surface water is scarce, such as in deserts, people couldn't survive and thrive without groundwater, and people use wells to get at underground water.

Contacts: Ask USGS
Attribution: Water Resources
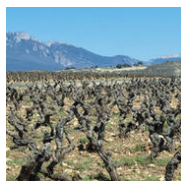
Date published: JUNE 6, 2018
Status: Completed

Groundwater Decline and Depletion

Groundwater is a valuable resource both in the United States and throughout the world. Groundwater depletion, a term often defined as long-term water-level declines caused by sustained groundwater pumping, is a key issue associated with groundwater use. Many areas of the United States are experiencing groundwater depletion.

Contacts: Ask USGS
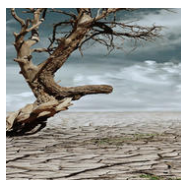
Date published: JUNE 6, 2018
Status: Completed

Drought and Groundwater Levels

Groundwater decline is a real and serious problem in many places of the Nation and the world. When rainfall is less than normal for several weeks, months, or years, the flow of streams and rivers declines, water levels in lakes and reservoirs fall, and the depth to water in wells increases.

Contacts: Ask USGS
Attribution: Water Resources

Below are publications associated with aquifers and groundwater. In addition to those below, Water sources: groundwater by Environment and Climate Change Canada may be of interest.
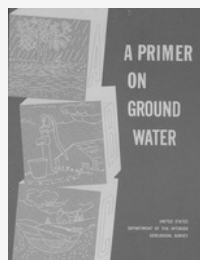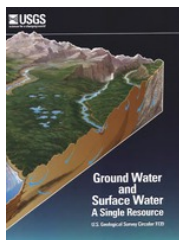
Year Published: 1963

A primer on ground water

Most of us don't have to look for water. We grew up either in big cities where there was a public water supply, or in small towns or on farms where the water came from wells. But there are some people to whom finding a new supply of water is vitally important.

Baldwin, Helene L.; McGuinness, C.L.
Attribution: United States of America
View Citation ⌄

Year Published: 1998

Ground water and surface water: A single resource

The importance of considering ground water and surface water as a single resource has become increasingly evident. Issues related to water supply, water quality, and degradation of aquatic environments are reported on frequently. The interaction of ground water and surface water has been shown to be a significant concern in many of these issues....

Winter, Thomas C.; Harvey, Judson W.; Franke, O. Lehn; Alley, William M.
Attribution: Water, Dakota Water Science Center, Water Resources, Environmental Health, Toxic Substances Hydrology Program, United States of America
View Citation ⌄
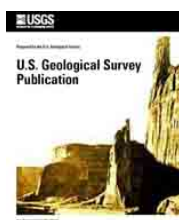
Year Published: 1994

Ground water and the rural homeowner

As the salesmen sang in the musical The Music Man, "You gotta know the territory." This saying is also true when planning to buy or build a house. Learn as much as possible about the land, the water supply, and the septic system of the house before buying or building. Do not just look at the construction aspects or the beauty of the home and...

Waller, Roger M.
Attribution: Water, Water Resources, United States of America, Water Science School
View Citation ⌄
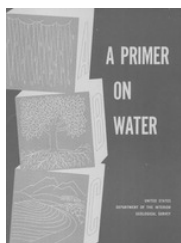
Year Published: 1960

A primer on water

When you open the faucet you expect water to flow. And you expect it to flow night or day, summer or winter, whether you want to fill a glass or water the lawn. It should be clean and pure, without any odor.You have seen or read about places where the water doesn't have these qualities. You may have lived in a city where you were allowed to water...

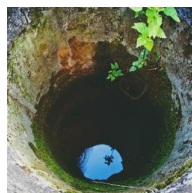Leopold, Luna Bergere; Langbein, Walter Basil

View Citation ⌄

Below are multimedia items associated with aquifers and groundwater.

APRIL 7, 2016

Artesian Water and Artesian Water

Artesian well used for irrigation, Rapid City, South Dakota (January 1956).

AUGUST 21, 2015

Groundwater is accessible through a "well"

Below a certain depth, the ground, if it is permeable enough to hold water, is saturated with water. The upper surface of this zone of saturation is called the water table. The saturated zone beneath the water table is called an aquifer, and aquifers are huge storehouses of water. What you are looking at in this photo is a "well" that exposes the water table, with an

...
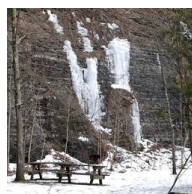
FEBRUARY 16, 2013

Water seeping between rock layers

The ground beneath our feet is not just rock, or at least, not just one kind of rock. Many different types of rock exist, and they have very different properties. Often, different types of rocks exist in horizontal layers beneath the land surface. Some layers are more porous than others, and at a certain depth below ground the pores and fractures in these rocks can be

...
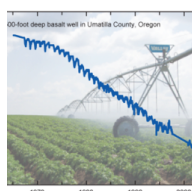
Long term pumping can lower the water level of aquifers.

Pumping has removed water from storage in basalt aquifers and caused declines in many areas of the Columbia Plateau.

Northport Middle School Closes: Benzene Found in Septic Systems/ Relocation Info. Updated

Environment & Nature   Times of Huntington   Times of Huntington-Northport   Town of Huntington

by Donna Deedy - January 18, 2020

Northport Middle School closed after contamination concerns. File photo

Northport-East Northport Union Free School District Superintendent Rob Banzer has decided, effective immediately, to close Northport Middle School for the remainder of the 2019-20 school year after P.W. Grosser Consulting, the environmental firm who has been testing soil around the school property, found on Saturday elevated levels of benzene in two separate septic systems on site.

Classes for the Northport Middle School students were cancelled for Tuesday, Jan. 21 and Wed. Jan. 22, and will resume on Jan. 23 in new locations.

"It is important to note that preliminary air testing indicated no observable detection of volatile organic compounds or VOCs, which includes benzene, inside the building, or from soil samples, as well as at the source of the septic tanks," Banzer said in an email notice to parents sent at 4:00 p.m. Saturday afternoon. "However, in the best interest of students and staff and in consideration of ongoing testing and remediation, the building will be closed for the balance of the school year."

During an unscheduled workshop with board members Wednesday, Jan. 15, Banzer presented and reviewed a decisive contingency relocation plan for Northport Middle School students that ultimately became necessary to implement just days later.

The plan, developed with goals identified by all stakeholders, maintains the school's curriculum, allows for spring sports, and enables students to access science labs. It was considered the best, least disruptive option.

As discussed during the workshop, transportation is feasible, but may require that some students change buses at the William J. Brosnan School building on Laurel Avenue. Additional drivers and buses might alleviate the need for transferring, Banzer said, but could be tough to secure.

"Although a great deal of the plan is already in place, we will need Tuesday and Wednesday to refine the logistics for staff and students, including scheduling, transportation and food service," Banzer stated in his note to parents.

As explained to parents and reviewed in the Jan. 15 workshop:

- Northport Middle School 8th graders will relocate to a special wing of the high school.
- Northport Middle School 7th graders will relocate to East Northport Middle School.

Originally, Northport Middle School 6th graders were expected to be relocated to either Norwood Avenue or Bellerose Elementary schools. But, as explained in a letter sent to parents Jan. 20, the district opted to keep all of the 6th graders together at Norwood Avenue school. The gifted and talented program will instead be relocated to Bellerose

Suffolk County Department of Health Services requires that the site be remediated to remove the benzene. The health department also requires remediation for high levels of mercury and silver found in the leaching pools outside of the schools G-wing. Remediation plans are still under development.

Many parents have been conflicted about sending their children to the school. Students and staff have complained about unidentified foul odors that regularly surface inside the building. Some parents, retired teachers and community members blame chemicals previously identified on school grounds as a potential cause for their illnesses. As the environmental investigation continues, some parents are breathing a sign of relief.

"We are happy to know that the testing can be completed with the children and staff relocated to safe locations," said Bethany Watts.

Donna Deedy

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EXHIBIT LLLL

Effects of Highway-Related Pollutant on the Groundwater Quality of Turfy Swamps in the Changbai Mountain Area

Hong Wang, Lei Nie, Yan Xu,[*] Chao Du, Tao Zhang, and Yuzheng Wang

Construction Engineering College, Jilin University, Xi Min Zhu Street, Changchun 130026, Jilin, China; wanghong15@mails.jlu.edu.cn (H.W.); nielei@jlu.edu.cn (L.N.); duchao16@mails.jlu.edu.cn (C.D.); zhangtao16@mails.jlu.edu.cn (T.Z.); wangyz16@mails.jlu.edu.cn (Y.W.)

[*]Correspondence: xuyan8102@jlu.edu.cn

Abstract

Transportation activities such as fuel consumption, vehicle wear and road deicing can detrimentally affect the groundwater quality of fragile roadside wetland environments including. Nineteen parameters (Cu, Pb, Zn, Cd, Cr, Ni, Hg, As, pH, TDS, Ca^{2+}, Mg^{2+}, Na^+, K^+, SO_4^{2-}, Cl^-, HCO_3^-, NO_3^- and F^-) were determined in groundwater samples from turfy swamps impacted by highway traffic from Jiangyuan (JY), Longquan (LQ), and Huangsongdian (HSD). Our results indicate that the metals Cu, Pb, Zn, Cr, Cd, the ions Na^+, K^+ and Cl^- in groundwater were negatively affected by highway transportation, and the maximum affected distance of these pollutants varied from 15 to 100 m. The content of most of these pollutants in roadside groundwater decreased exponentially with the distance from the highway, as did the heavy metal pollution index HPI and C_d. The values of HPI and C_d in these three sites ranged from 46.8 to 78.4 and −4.9 to −2.9, respectively. The low pollution levels of heavy metals are related to the strong adsorption capacity of turfy soil towards metals. In any case, road transport activities increased the Cu, Pb, Zn, Cr, Cd, Na^+, K^+ and Cl^- content in roadside groundwater in turfy swamp. With the increase of highway operation time, it will inevitably have a great influence on the groundwater quality of these wetlands. Therefore, the long-term monitoring is necessary to protect the sustainable development of turfy swamp.

Keywords: turfy swamp, heavy metals, groundwater quality, highway transportation

1. Introduction

Environmental pollutants from traffic infrastructure are an important subject in ecological and environmental sciences, due to their toxic effects on the biosphere [1,2,3]. It is generally accepted that highway transportation activities such as the combustion of liquid fuels, vehicular component abrasion and the weathering of pavement materials can release fine particles containing Zn, Pb, Cd, Cu and Cr [4,5,6,7]. These particles containing heavy metals are deposited into soil, water and plant near the

highway through dry and wet deposition [8]. In addition, some studies have reported that road-salting activities can increase the contents of Na^+, K^+, Cl^- and NO_3^- in roadside groundwater and even cause severe Cl^- contamination [9,10,11,12]. These highway-related pollutants are persistent and non-degradable, and therefore can remain in the roadside environment for long periods of time. When their accumulations exceed a certain level, they will pose a threat to the microbiota, flora and fauna in turfy swamps [13].

Since the 1970s, concern about highway-related pollutants has spread beyond academia [14,15,16]. Studies of highway-related pollutants have focused on the identification of type of pollutants and their distribution influenced by distance from the roadway [17,18,19]. Zhang et al. [8] discovered that soil concentrations of Zn, Cd, Cu, As and Pb were the most affected by highway transport in the Tibetan plateau. Earon et al. [12] demonstrated that the metals Fe, Al, Zn, Mn, Pb, Ni, Cr, Cd and the ions Ca^{2+}, SO_4^{2-}, K^+, Na^+, Mg^{2+}, NO_3^-, and Cl^- in groundwater are significantly related to road transportation, decreasing exponentially as the distance from the roadside increases. In addition, many monitoring studies have demonstrated Pb, Cu, Cr, Cd and Zn pollution are severe in diverse roadside environments, including Beijing [4], Hong Kong [20], Mexico [21], and Yorkshire, England [22]. However, all these studies were mainly concentrated on roadside soil, with relatively little attention being paid to the effects of traffic pollution on groundwater. As far as we know, environmental pollutant levels in turfy swamp groundwater coming from highways have not been reported.

Wetland ecosystems have strong hydrological dependence, with the healthy water quality and hydrochemical characteristics being a determining factor in their sustainable development [23]. Turfy swamp is a unique natural ecosystem formed by interaction between water and land system [24]. Therefore, groundwater is extremely essential for survival of all living organisms in turfy swamps, which is sensitive to outside influences. Even small-scale human activities can result in significant environmental changes [25,26,27]. In the Changbai Mountain area, most of the turfy swamps are well preserved. However, increasing highway construction activities have caused many environmental problems in turfy swamps in recent decades. For example, Wang et al. [28] reported that metals Zn, Cr, Cu and Cd have reached moderate pollution in roadside soil in turfy swamp areas. However, the effect of highway on the heavy metal and hydrochemical characteristic of groundwater in turfy swamp is not clear. To formulate effective measures for turfy swamp protection, investigating the source and pollution states of highway-related pollutants in groundwater of turfy swamps is crucial.

This paper quantifies the impact of highway transport on groundwater in turfy swamps in the Changbai Mountain area from the two aspects of heavy metal and hydrochemical characteristics. Based on findings from a large-scale field investigation, we identify the types of highway-related pollutants in turfy swamp groundwater, and explore the relationships between highway-related pollutants concentrations and roadside distance, and lastly assess the heavy metal contamination levels of turfy swamp groundwater.

2. Materials and Methods

2.1. Site Description

This study was performed in the Changbai Mountain area, where a large variety of turfy swamps can be found. This region is the main headstream of the Mudan River and an important water conservation area in Jilin Province. Three sampling sites without any other confounding anthropogenic pollution sources were selected for collection of groundwater samples: Jiangyuan (JY, N43°7′, E128°1′), Longquan (LQ, N42°25′, E126°36′), and Huangsongdian (HSD, N43°39′, E127°39′) (Figure 1). At the JY site, the climate is cool and humid, with moderate illumination and abundant rainfall; the average annual temperature and precipitation are 2.6 °C and 632 mm. G201 highway with daily traffic volume greater than 2000 invades the edge of the turfy swamps. The climate characteristics at the LQ site are

similar to JY site, with average annual temperature 3.7 °C and precipitation 947 mm; the S302 highway with daily traffic volume greater than 1500 invades the middle of the turfy swamp. For HSD, the average annual temperature and precipitation are 3.4 °C and 709 mm; G12 highway passes through the edge of the turfy swamp, with daily traffic volume greater than 1100. Regarding specific information about these three sites, readers may refer to Wang et al. [28].

Figure 1

Study area and sampling sites in the Changbai Mountain area, Jilin Province.
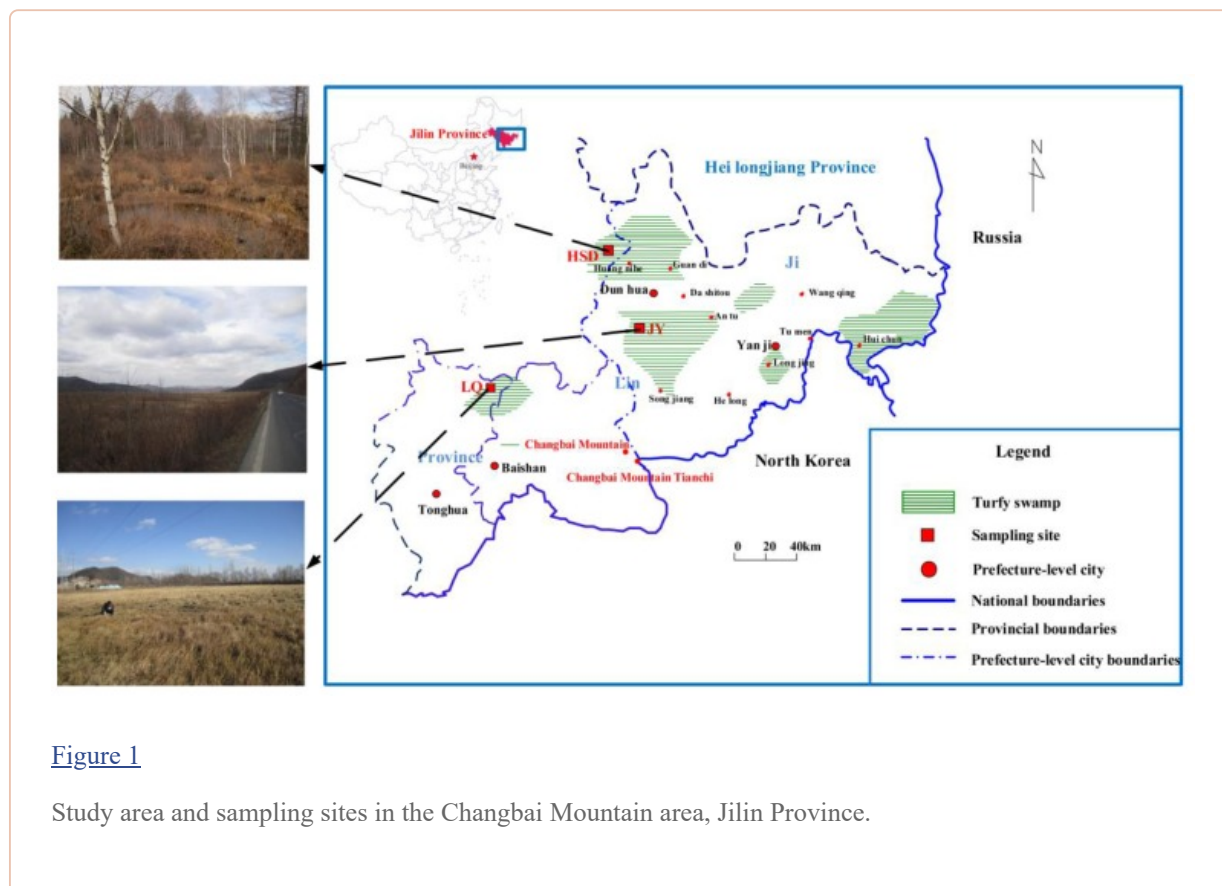
2.2. Sampling and Experimental Methods

Groundwater samples were collected in July–August 2017 at distances of 5, 15, 50, and 200 m, from the highway, respectively at the three sites. Before sampling, three PVC tubes with a diameter of 160 mm were embedded in the soil at each distance, separated by 100 m, exposing 50 cm above ground and embedding 60 cm underground. Many holes with a diameter of 4 mm were drilled in the PVC tube wall to allow the groundwater to naturally infiltrate into the tube. In order to prevent sediment, branches and rainwater from entering the tubes PVC, the PVC outer wall was covered with gauze, and the tube end was covered with a plastic cloth fixed with a nylon rope. After groundwater samples were collected using the erect water collectors, the samples were filtered using a 0.45 μm filter membrane, and then transported to the laboratory at 2–5 °C in a cooler. Nineteen physical and chemical parameters, including Cu, Pb, Zn, Cd, Cr, Ni, Hg, As, pH, TDS, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, SO_4^{2-}, Cl^{-}, HCO_3^{-}, NO_3^{-} and F^{-}, were analyzed. The pH and total dissolved solids (TDS) were determined in the field using a portable measuring instrument (HI98195, HANNA, Padua, Italy). All samples were collected and stored according to the standard for detection of groundwater quality (DZ/T 0064.2-1993); the specific preservation and test methods are shown in Table 1. All samples were tested within 15 days after sampling at the Jilin University Testing Center.

Table 1

Storage requirements and test methods for groundwater samples.

Parameter	Sampling Volume	Container Material	Preservation Method	Test Method
Cu, Pb, Zn, Cd, Ni	500 mL	P	Add concentrated HNO_3 and adjust pH to 1–2	ICP-MS
Cr	100 mL	P	Add NaOH and adjust pH to 8–9	ICP-MS
Hg	250 mL	B	Add concentrated HNO_3 and adjust pH to 1–2	Atomic florescence spectrum
As	250 mL	B	Add concentrated H_2SO_4 and adjust pH to 1–2	ICP-MS
$Ca^{2+}, Mg^{2+}, Na^+, K^+, SO_4^{2-}, Cl^-, HCO_3^-, F^-$	500 mL	P	Original preservation	$Ca^{2+}, Mg^{2+}, Na^+, K^+$: ICP-AES Cl^-, F^-, SO_4^{2-}: Ion chromatography method HCO_3^-: Acid-base titration method
NO_3^-	100 mL	P	Original preservation	Ion chromatography method

P. Polypropylene bottles; B. Borosilicate bottle; Original preservation: After sampling, no chemical reagents are added.

2.3. Data Analysis Methods

A regression model was used to describe the relationship between the content of highway-related pollutants and the distance from the roadside using Origin 9.0 software (Origin Lab, Northampton, MA, USA). The high R^2 values indicate that this model can well quantify the distribution characteristics of highway-related pollutants contents in groundwater.

To evaluate the pollution level of groundwater in turfy swamp, the heavy metal pollution index HPI and C_d were calculated by the following formulas [29]:

$$HPI = \frac{\sum_{i=1}^{n} W_i Q_i}{\sum_{i=1}^{n} W_i} \qquad (1)$$

$$(2)$$

$$Q_i = \sum_{i=1}^{n} \frac{(M_i(-)I_i)}{(S_i - I_i)} \times 100$$

where Q_i and W_i are the subindex and unit weight of the i-th element. n is the number of elements considered. M_i is the measured value of the ith metal, I_i and S_i are ideal and standard values of the ith parameter, respectively. The sign $(-)$ indicates the numerical difference of the two values, ignoring the algebraic sign. To calculate W_i the groundwater class III standards (GB/T 14848-2017) were used for Cu, Pb, Zn, Cd, Cr, Ni, Hg and As.

The C_d index indicates the relative contamination of different metals separately and manifests the combined effects of all metals, is calculated as follows:

$$C_d = \sum_{i=1}^{n} C_{fi} \tag{3}$$

$$C_{fi} = \frac{C_{Ai}}{C_{Ni}} - 1 \tag{4}$$

where C_{Ai} and C_{Ni} are the measured value and upper allowable value of ith metal (N refers to the normative value). C_{Ni} is considered as the standard permissible value (S_i) formerly introduced in HPI calculation.

The experimental data were subjected to statistical analysis using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA), including the description analysis, K-S test, ANOVA, cluster analysis, factor analysis and Pearson correlation analysis. The ANOVA and LSD test were used to identify the significant differences of the measured parameters among different sites. The correlation analysis was used to explore the relationship between the measured parameters and distance from highway. The cluster analysis and factor analysis was performed to identify the interrelationship among these parameters in groundwater of turfy swamp. Euclidean distance and Ward's method were used in cluster analysis, and the primary data were standardized using Z score.

3. Results and Discussion

3.1. Concentration of Metals and Hydrochemical Parameters in Groundwater

The basic statistical descriptions of heavy metals contents for different turfy swamp sites in roadside groundwater are summarized in Table 2. The maximum concentrations of all heavy metals in this area are lower than the drinking water quality of China (GB/T 14848-2017). The low contents of metals may be related to turfy soil with high organic matter content. The concentrations of Cu, Pb, Zn, Cr, Cd and Hg (58.3 μg L^{-1}, 26.3 μg L^{-1}, 114.2 μg L^{-1}, 1.8 μg L^{-1}, 5.4 μg L^{-1}) at LQ turfy swamp are the highest among the three sites, while the Ni and As contents (8.9 μg L^{-1}, 8.7 μg L^{-1}) in JY are the highest. The ANOVA results indicated that the contents of most metals at the JY and LQ sites were significantly higher than those at the HSD one ($p < 0.05$). This finding is in accordance with the traffic volume in these three sites. The daily traffic volume at HSD is lower than that at JY and LQ site as previously reported by Wang et al. [28].

Table 2

Descriptive statistics of heavy metal concentrations ($\mu g\ L^{-1}$) in groundwater of turfy swamps.

	Cu	Pb	Zn	Cd	Cr	Hg	Ni	As
JY site								
Mean	48.5a	23.2ab	100.0ab	1.57a	5.0a	0.7b	8.9a	8.7a
SD	7.4	5.0	10.6	0.18	1.4	0.1	1.0	0.8
Min	35.2	15.4	80.3	1.26	3.4	0.5	7.7	7.2
Max	61.5	30.5	118.3	1.91	7.3	0.8	10.8	9.9
CV (%)	15.2	21.5	10.6	11.5	26.8	6.5	8.5	8.6
LQ site								
Mean	58.3a	26.3a	114.2a	1.75a	5.4a	0.08b	6.9b	3.1b
SD	10.5	5.1	8.0	0.22	0.7	0.001	0.8	0.3
Min	43.3	17.6	97.3	1.41	4.23	0.07	5.8	2.4
Max	78.1	34.1	126.8	2.33	6.81	0.08	8.3	3.6
CV (%)	18.1	19.2	17.0	13.1	13.0	3.5	10.8	10.2
HSD site								
Mean	20.6b	16.5b	91.0b	1.27b	6.6a	3.0a	6.9b	8.0a
SD	1.9	0.8	8.1	0.12	1.4	0.1	0.4	0.6
Min	17.3	15.3	79.0	1.14	4.1	2.4	6.3	7.3
Max	23.6	18.1	106.2	1.51	9.2	3.6	7.9	9.7
CV (%)	9.3	5.1	8.9	9.93	21.2	8.3	6.5	7.6
ACV (%)	14.2	15.3	12.2	11.50	20.3	6.1	8.6	8.8
Class III	1000	50	1000	5	50	1	20	10

The data with different superscript lower case letters have significant differences ($p < 0.05$) in the columns; JY. Jingyu site; LQ. Longquan site; HSD. Huangsongdian site; CV. Coefficient of variation; ACV. Average CV; Class III. The water used for the second level reserve of groundwater source for drinking in China.

As shown in [Table 3](#), the hydrochemical analysis results indicated that the mean concentrations of TDS (218.9 mg L^{-1}), Ca^{2+} (29.3 mg L^{-1}), Mg^{2+}(5.8 mg L^{-1}), Na^{+} (2.9 mg L^{-1}), K^{+} (1.2 mg L^{-1}), SO$_4$$^{2-}$ (11.6 mg L^{-1}), Cl^{-} (5.5 mg L^{-1}), HCO$_3$$^{-}$ (179.8 mg L^{-1}), NO$_3$$^{-}$ (0.44 mg L^{-1}), F^{-} (0.16 mg L^{-1}) of the entire region were lower than the drinking water quality standards. The ACV% of these measured parameters followed the sequence K^{+} > Cr > Na^{+} > Cl^{-} > Pb > Cu > Zn > Cd > Mg^{2+} > pH > As > Ni > NO$_3$$^{-}$ > F^{-} > Hg > SO$_4$$^{2-}$ > Ca^{2+} > TDS > HCO$_3$$^{-}$ and the CV% of Cu, Pb, Zn, Cr, Cd, Na^{+}, K^{+}, Cl^{-} are significantly higher than those of the remaining parameters in the three sites. This result indicated that the concentrations of Cu, Pb, Zn, Cr, Cd, Na^{+}, K^{+} and Cl^{-} varied greatly in different distances

from the highway in turfy swamps, which leads us to speculate that concentrations of Cu, Pb, Zn, Cr, Cd, Na^+, K^+ and Cl^- in groundwater were most likely related to human activities along the highway in the turfy swamp areas.

Table 3

Descriptive statistics of hydrochemical parameters concentrations (mg L^{-1}) in groundwater of turfy swamps.
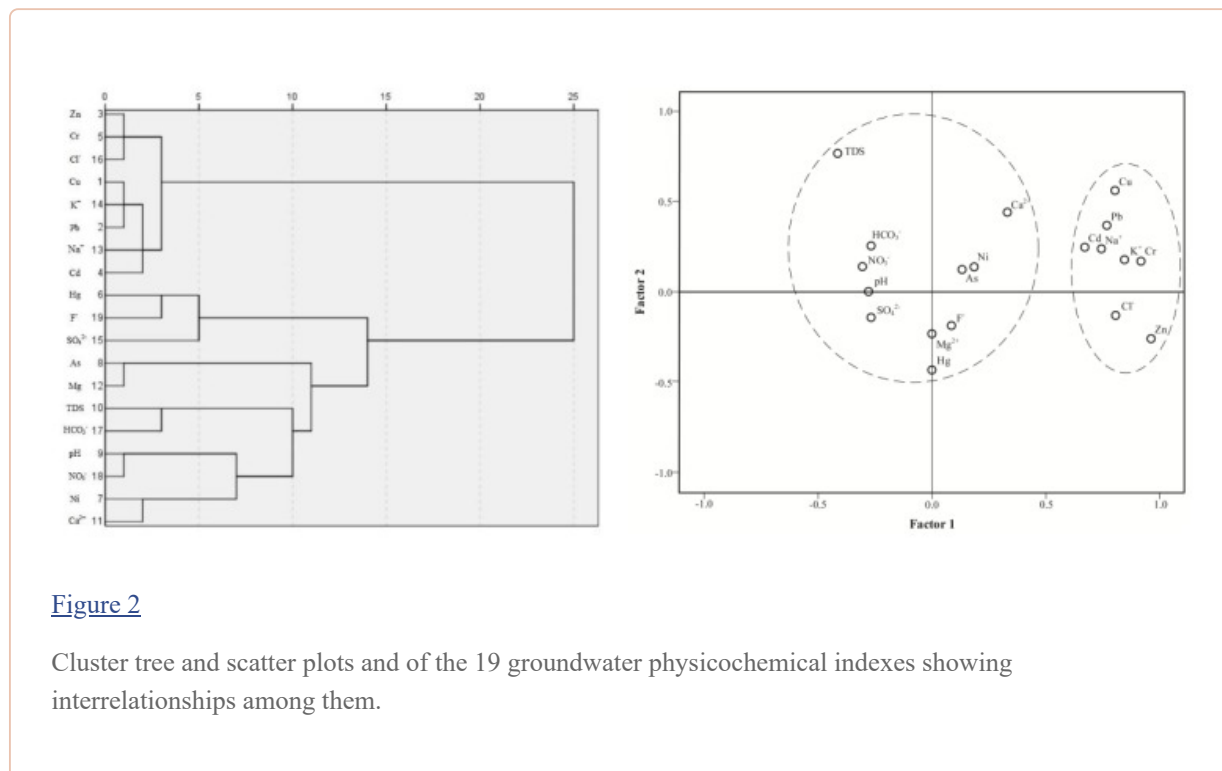
	pH	TDS	Ca^{2+}	Mg^{2+}	Na^+	K^+	SO_4^{2-}	Cl^-	HCO_3^-	NO_3^-	F^-
JY site											
Mean	5.6	186.8	15.6	7.2	3.1	1.4	12.4	4.4	202.1	0.43	0.11
SD	0.5	3.1	0.9	0.7	0.7	0.5	0.5	0.6	3.1	0.05	0.02
Min	4.4	180.0	14.3	6.5	2.3	0.9	11.9	3.4	195.2	0.37	0.09
Max	6.3	191.0	17.3	8.9	4.6	2.6	13.3	5.6	207.5	0.52	0.15
CV (%)	9.0	1.6	5.9	9.3	21.7	36.2	4.0	13.2	1.6	12.83	9.14
LQ site											
Mean	5.9	271.6	35.7	5.0	2.8	1.2	11.3	8.1	204.8	0.56	0.06
SD	0.7	11.5	2.2	0.6	0.5	0.4	0.7	1.6	4.6	0.03	0.001
Min	4.4	255.1	31.9	4.2	1.8	0.7	10.4	5.4	196.3	0.44	0.04
Max	6.9	296.3	38.3	6.5	3.4	1.9	12.3	10.4	210.6	0.74	0.07
CV (%)	12.5	4.2	6.1	11.9	18.2	30.5	6.2	19.6	2.3	5.01	7.63
HSD site											
Mean	6.2	198.4	36.6	5.2	2.8	1.1	11.1	3.9	132.5	0.33	0.31
SD	0.4	4.2	1.5	0.7	0.5	0.2	0.7	1.0	3.9	0.02	0.01
Min	5.6	189.3	34.1	3.6	1.9	0.9	9.4	3.0	126.5	0.29	0.29
Max	6.7	204.0	38.5	6.0	3.7	1.5	12.3	5.7	138.3	0.37	0.33
CV (%)	6.8	2.1	4.1	9.4	19.5	18.7	6.6	25.1	2.9	7.47	4.35
ACV (%)	9.4	2.7	5.4	10.2	19.8	28.4	5.6	19.3	2.2	8.44	7.04
Total	5.9	218.9	29.3	5.8	2.9	1.2	11.6	5.5	179.8	0.44	0.16
Class III	6.5–8.5	1000	150		200		250	250		20	1.0

[Open in a separate window](#)

JY. Jingyu site; LQ. Longquan site; HSD. Huangsongdian site; CV. Coefficient of variation; ACV. Average CV; Class III. The water used for the second level reserve of groundwater source for drinking in China.

3.2. Interrelationships among Measured Parameters in Groundwater in Turfy Swamp

Cluster analysis and factor analysis were performed on the 19 parameters determined in roadside groundwater to identify a shared source of these parameters in turfy swamps (Figure 2). As previously reported by Lee et al. [30] and Khan et al. [31], elements in the same group are expected to be from a common anthropogenic or natural source and a lower cluster distance indicates a more significant association. Two different cluster groups were presented at the three sites, Cu, Pb, Zn, Cr, Cd, Na$^+$, K$^+$ and Cl$^-$ with a distance between 5 and 10 indicated that these parameters are from the same source. The remaining parameters are clustered at a high distance criterion between 10 and 15, possibly due to environmental heterogeneity of turfy swamp.

Figure 2

Cluster tree and scatter plots and of the 19 groundwater physicochemical indexes showing interrelationships among them.

The results of factor analysis indicated that Cu, Pb, Zn, Cr, Cd, Na$^+$, K$^+$ and Cl$^-$ in groundwater are significantly related to each other. A total of two factors with an eigenvalue >1 were extracted, accounting for 89.00% of the total variance (Figure 2). Factor 1, accounting for 72.91% of the variance, positively related to Cu, Pb, Zn, Cr, Cd, Na$^+$, K$^+$ and Cl$^-$. The remaining parameters (pH, TDS, Ni, Hg, As, Mg^{2+}, Ca^{2+}, SO$_4^{2-}$, HCO$_3^-$, NO$_3^-$ and F$^-$) were positively related to factor 2 (16.08%). As a whole, the result of classification based on cluster analysis is consistent with the results from factor analysis.

Many studies have demonstrated that Cu, Pb, Zn, Cr, Cd, Na$^+$, K$^+$ and Cl$^-$ are indicators of roadside contaminated environment [32,33,34,35]. Although the cluster analysis and factor analysis were inadequate to conclude whether the pollutants originated from highway transport activities, the Cu, Pb, Zn, Cr, Cd, Na$^+$, K$^+$ and Cl$^-$ appear to have derived from a similar source, which may be related to traffic. Some studies have shown that highway-related pollutants derived from traffic had a significant negative correlation with distance to the highway [5,6,7], As shown in Table 4, there were significant negative correlations between Cu, Pb, Zn, Cr, Cd, Na$^+$, K$^+$, Cl$^-$ and the distance to the highway through the whole analysis. However, individual indicators did not show significant negative correlations in the different research sites, possibly partly due to the limited number of sampling points. Collectively, the results suggest that Cu, Pb, Zn, Cr, Cd, Na$^+$, K$^+$, Cl$^-$ in the groundwater were related to traffic activities.

Table 4

Pearson correlation coefficients between the affected parameters and distances in groundwater.

Site	Cu	Pb	Zn	Cd	Cr	Na^+	K^+	Cl^-
JY	−0.698 *	−0.666 *	−0.397 *	−0.822 *	−0.671 *	−0.438	−0.528 *	−0.407 *
LQ	−0.797 **	−0.871 **	−0.385	−0.349 *	−0.554	−0.541	−0.606 *	−0.386
HSD	0.208	−0.160	−0.411 *	−0.427	−0.306	−0.488 *	−0.476	−0.450 *
Whole	−0.340 *	−0.440 **	−0.264 *	−0.341 *	−0.416 *	−0.456 **	−0.484 **	−0.288 **

* Correlation is significant at the 0.05 level (two-tailed); ** Correlation is significant at the 0.01 level (two-tailed).

3.3. The Nonlinear Regression Model of Highway-Related Pollutants

The nonlinear regression model ($y = a_0 + a_1 \times a_2^x$) was proposed by Wang et al. [36] to describe the distribution characteristics of traffic-related metals contents in turfy swamp soil and plant. As shown in Figure 3, there were two different distribution patterns of the highway-related pollutants in groundwater. The contents of most parameters showed an exponential decline with increasing distance from the road edge in these three sites, whereas the contents of Cu and Pb in HSD site fluctuated with increasing distance from the highway edge. The high R square values showed that this model was also suitable for characterizing the relationship between highway-related pollutants and roadside distance in groundwater.
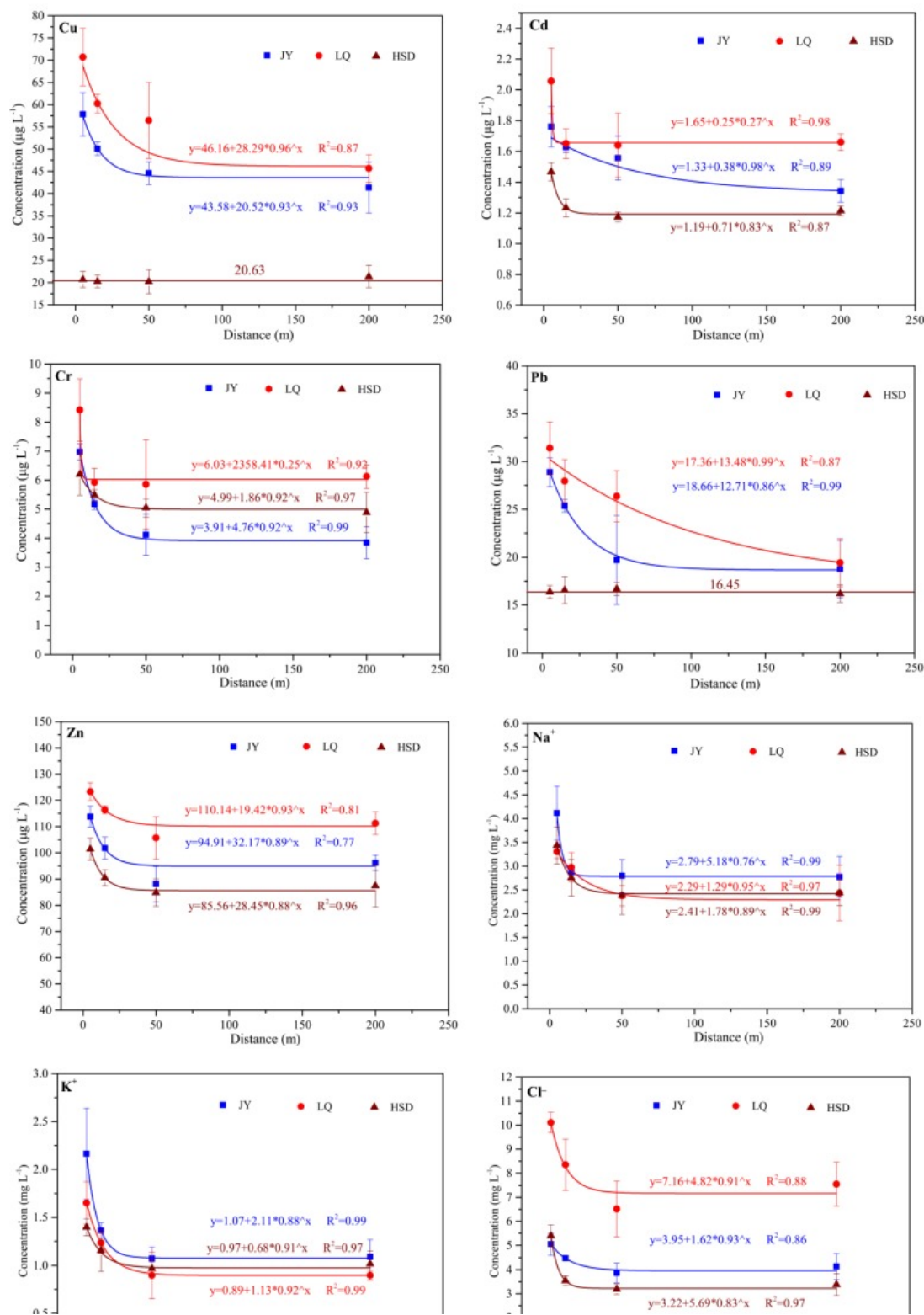
Figure 3

Regression curves of highway-related pollutants concentrations in groundwater in turfy swamp.

The maximum affected distance of highway-related pollutants diffusion is an important parameter for formulating corresponding wetland protection measures. As shown in Figure 3, the contents of Cu, Zn, Cr become the background content at 15 to 50 m distance from the road edge in these three sites. However, the range of maximum affected distance for Pb and Cd varied from 15 to 100 m.

The affected distances of Cu, Zn, Cr, Pb and Cd in groundwater are consistent with those in turfy soil as previously reported by Wang et al. [36]. For Na^+, K^+, and Cl^-, no other study has reported their contents showed an exponential decrease with increasing distance, and reached background contents at 15 to 50 m distance from road edge. Only Earon et al. [12] reported that Na^+, K^+ and Cl^- concentrations gradually decreased in groundwater with increasing distance from the road edge, but they did not give a specific distribution function.

As shown in Table 5, the background contents of Cu, Pb, Zn, Cr, Cd, Na^+, K^+, Cl^- at different turfy swamp sites varied, which may be related to the environmental heterogeneity of turfy swamps. In this study, the concentrations of pollutants at all test points were lower than the class III of China Environmental Guidelines, but the concentration of these pollutants in roadside groundwater are obviously higher than the background concentration, which means that the maximum sorption capacity of pollutants is reached and the some pollutants are precipitated from the turfy soil [37,38]. However, this proposal should be interpreted with caution and will require verification during future follow-up studies.

Table 5

The background contents of Cu, Pb, Zn, Cr, Cd, Na^+, K^+, Cl^- in groundwater in different turfy swamp sites.

Site	Cu ($\mu g\ L^{-1}$)	Pb ($\mu g\ L^{-1}$)	Zn ($\mu g\ L^{-1}$)	Cd ($\mu g\ L^{-1}$)	Cr ($\mu g\ L^{-1}$)	Na^+ ($mg\ L^{-1}$)	K^+ ($mg\ L^{-1}$)	Cl^- ($mg\ L^{-1}$)
JY	43.6	18.7	94.9	1.3	3.9	2.8	1.1	4.0
LQ	46.2	17.4	110.1	1.7	6.0	2.3	0.9	7.2
HSD	20.6	16.5	85.6	1.2	5.0	2.4	1.0	3.2

3.4. Identification of Metals Pollution Degree in Groundwater

HPI and C_d are effective tools to evaluate the groundwater pollution because they combines several parameters to obtain a particular value which can be compared with the critical value. In this study, the HPI was calculated in different distances from highway using the class III of the China Environmental Guidelines (GB/T 14848-2017), based on Cu, Pb, Zn, Cd, Cr, Ni, Hg and As. The HPI results are shown in Figure 4. The range and mean values of HPI for the groundwater samples in the three sites were 54.1–78.3 and 66.4 (JY), 46.8–72.9 and 60.4 (LQ), and 57.2–66.2 and 62.4 (HSD), respectively. The results showed that the HPIs for all the samples were below the critical limit of 100 proposed for drinking water by Prasad and Bose [39].
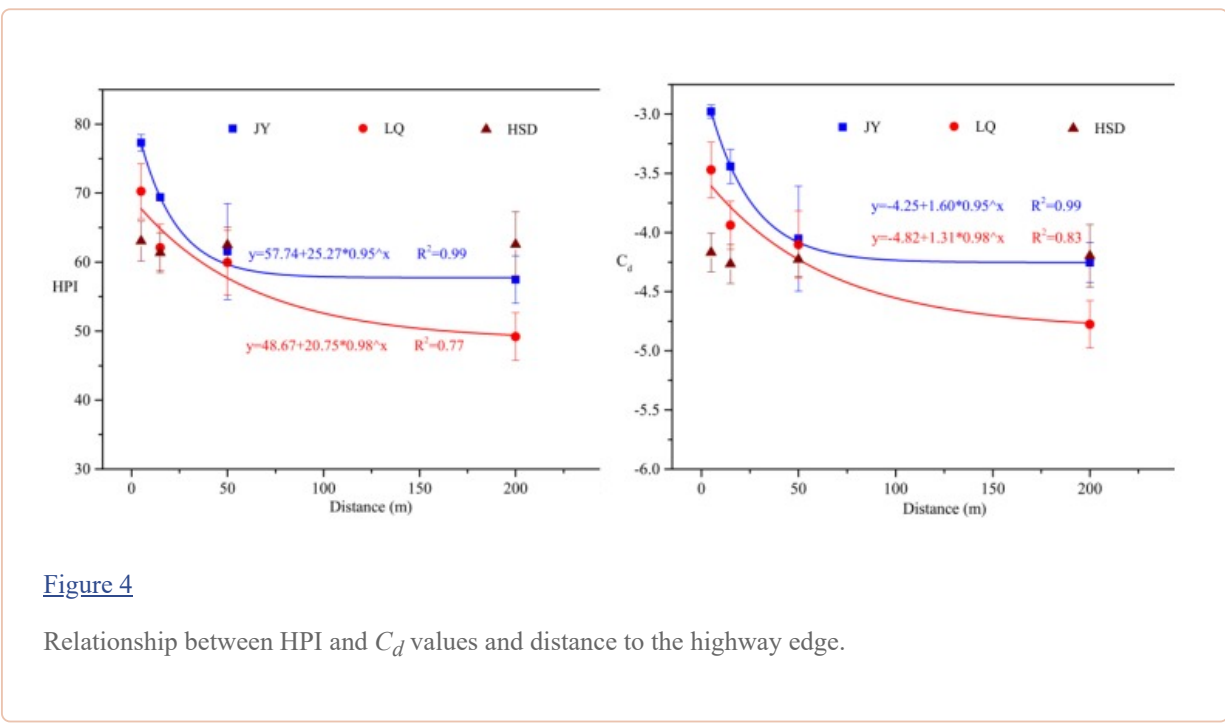
Figure 4

Relationship between HPI and C_d values and distance to the highway edge.

The contamination index (C_d) was used as a reference of estimating the degree of metal pollution [40]. The range and mean values of C_d of the groundwater samples were respectively −4.5–−2.9 and −3.7 (JY), −4.9–−3.3 and −4.1 (LQ), and −4.8–−4.4 and −4.7 (HSD). C_d may be grouped into three classes [41] as follows: high ($C_d > 3$), medium ($C_d = 1$–3) and low ($C_d < 1$). In this study, all analyzed samples do not exceed 0, suggesting that they are below the water quality standards, which is consistent with the results of HPI analysis. As reported previously by Wang et al. [28], the degree of metals in turfy soil reached heavily polluted level. In this study, highway-related metals in groundwater are almost no pollution, which may be explained by the strong sorption capacity of turf soil to heavy metals. Many studies have shown that turfy soil has strong potential for heavy metal remediation, and it can effectively reduce heavy metal content in contaminated soil [42,43,44].

In addition, it is worth noting that the values of HPI and C_d decline exponentially with the increase of distance from the highway at these three sites (Figure 4). There are significant negative correlations between the pollution indexes (HPI and C_d) and distance to highway in the JY and LQ site (Table 6). This result can be explained by the lower traffic volume of HSD compared to JY and LQ. In order to investigate the key metals contributing to the computed indices, correlation analysis was performed between the indices (HPI and C_d) and heavy metal concentrations. In JY and LQ, Cu, Pb Zn, Cd and Cr show significant correlations with all the indices, suggesting that these metals are the major contributory parameters (Table 6). For the HSD site, Zn, Cd and Cr are the main contributors to HPI and C_d. Collectively, these results are consistent with the distribution pattern, suggesting that the pollution level is related to traffic activities.

Table 6

Correlation coefficients for the distance and metal concentrations with indices values.

	JY		LQ		HSD	
	HPI	C_d	**HPI**	C_d	**HPI**	C_d
Distance	−0.750 *	−0.739 *	−0.854 **	−0.868 **	0.038	0.047
Cu	0.778 **	0.798 **	0.912 **	0.910 **	0.258	0.221
Pb	0.976 **	0.976 **	0.980 **	0.983 **	0.599 *	0.646 *
Zn	0.660 *	0.666 *	0.621 *	0.620 *	0.213	0.338
Cd	0.718 **	0.694 *	0.642 *	0.623 *	0.739 **	0.608 *
Cr	0.821 **	0.812**	0.621*	0.622 *	0.612 *	0.715 **
Hg	0.203	0.183	0.289	0.282	0.291	0.333
Ni	0.179	0.256	−0.456	−0.460	0.307	0.352
As	0.049	0.082	−0.128	−0.140	0.513	0.562

* Correlation is significant at the 0.05 level (two-tailed); ** Correlation is significant at the 0.01 level (two-tailed).

Although the pollution degree of heavy metals in groundwater of turfy swamp is very low, the concentrations of Cu, Pb, Zn, Cr, Cd, Na^+, K^+ and Cl^- in groundwater near the highway edge are obviously higher than the background concentration values. The correlation analysis between the two pollution index (HPI and C_d) and metals also showed that Cu, Pb, Zn, Cr and Cd are the main contributors to the heavy metal pollution in the groundwater, so it cannot be ignored that operation of highways proximal to turfy swamps may produce heavy metal accumulation in groundwater.

4. Conclusions

Nineteen physical and chemical parameters (Cu, Pb, Zn, Cd, Cr, Ni, Hg, As, pH, TDS, Ca^{2+}, Mg^{2+}, Na^+, K^+, SO_4^{2-}, Cl^-, HCO_3^-, NO_3^- and F^-) were tested in groundwater to evaluate the impact of highway transportation on groundwater environments of proximal turfy swamps. Cu, Pb, Zn, Cr, Cd, Na^+, K^+ and Cl^- were identified as highway-related pollutants. However, neither the concentrations of pollutants, nor the HPI and C_d indexes, exceeded the water quality standard. The concentration of pollutants were greatest at the edge of the highway followed by an exponential decay away from the highway with the affected distances between 15 to 100 m dependent on location and pollutant. Given the sensitivity and vulnerability of turfy swamps, long-term monitoring of pollution in roadside groundwater is important to establish recommendations for minimal distances of roadways from wetlands.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Duong T.T.T., Lee B.K. Determining contamination level of heavy metals in road dust from busy traffic areas with different characteristics. J. Environ. Manag. 2011;92:554–562. doi: 10.1016/j.jenvman.2010.09.010. [PubMed] [CrossRef] [Google Scholar]

2. Sezgin N., Ozcan H.K., Demir G., Nemlioglu S., Bayat C. Determination of heavy metal concentrations in street dusts in Istanbul E-5 highway. Environ. Int. 2004;29:979–985. doi: 10.1016/S0160-4120(03)00075-8. [PubMed] [CrossRef] [Google Scholar]

3. Zhang H., Zhang Y., Wang Z., Ding M., Jiang Y., Xie Z. Traffic-related metal(loid) status and uptake by dominant plants growing naturally in roadside soils in the Tibetan plateau, China. Sci. Total Environ. 2016;573:915–923. doi: 10.1016/j.scitotenv.2016.08.128. [PubMed] [CrossRef] [Google Scholar]

4. Chen X., Xia X., Zhao Y., Zhang P. Heavy metal concentrations in roadside soils and correlation with urban traffic in Beijing, China. J. Hazard. Mater. 2010;181:640–646. doi: 10.1016/j.jhazmat.2010.05.060. [PubMed] [CrossRef] [Google Scholar]

5. Adachi K., Tainosho Y. Characterization of heavy metal particles embedded in tire dust. Environ. Int. 2004;30:1009–1017. doi: 10.1016/j.envint.2004.04.004. [PubMed] [CrossRef] [Google Scholar]

6. Hjortenkrans D., Bergbäck B.G., Häggerud A. Metal emissions from brake linings and tires: Case studies of Stockholm, Sweden 1995/1998 and 2005. Environ. Sci. Technol. 2007;41:5224–5230. doi: 10.1021/es070198o. [PubMed] [CrossRef] [Google Scholar]

7. Zaharescu D.G., Hooda P.S., Soler A.P., Fernandez J., Burghelea C.I. Trace metals and their source in the catchment of the high altitude Lake Respomuso, Central Pyrenees. Sci. Total Environ. 2009;407:3546–3553. doi: 10.1016/j.scitotenv.2009.02.026. [PubMed] [CrossRef] [Google Scholar]

8. Zhang H., Wang Z., Zhang Y., Ding M., Li L. Identification of traffic-related metals and the effects of different environments on their enrichment in roadside soils along the Qinghai-Tibet highway. Sci. Total Environ. 2015;521–522:160–172. doi: 10.1016/j.scitotenv.2015.03.054. [PubMed] [CrossRef] [Google Scholar]

9. Howard K., Haynes J. Groundwater contamination due to road de-icing chemicals—Salt balance implications. Geosci. Can. 1993;20:1–8. [Google Scholar]

10. Cunningham M.A., Snyder E., Yonkin D., Ross M., Elsen T. Accumulation of deicing salts in soils in an urban environment. Urban Ecosyst. 2008;11:17–31. doi: 10.1007/s11252-007-0031-x. [CrossRef] [Google Scholar]

11. Blomqvist G. Ph.D. Thesis. Royal Institute of Technology; Stockholm, Sweden: 2001. De-Icing Salt and the Roadside Environment: Air-Borne Exposure, Damage to Norway Spruce and System Monitoring. [Google Scholar]

12. Earon R., Olofsson B., Renman G. Initial effects of a new highway section on soil and groundwater. Water Air Soil Pollut. 2012;223:5413–5432. doi: 10.1007/s11270-012-1290-6. [CrossRef] [Google Scholar]

13. Wang G., Yan X., Zhang F., Zeng C., Gao D. Traffic-related trace element accumulation in roadside soils and wild grasses in the Qinghai-Tibet Plateau, China. Int. J. Environ. Res. Public Health. 2013;11:456–472. doi: 10.3390/ijerph110100456. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

14. Motto H.L., Daines R.H., Chilko D.M., Motto C.K. Lead in soils and plants: Its relationship to traffic volume and proximity to highways: discussions follow. Environ. Sci. Technol. 1970;4:231–237. doi: 10.1021/es60038a009. [CrossRef] [Google Scholar]

15. Koeleman M., Vd Laak W.J., Ietswaart H. Dispersion of PAH and heavy metals along motorways in the Netherlands—An overview. Sci. Total Environ. 1999;235:347–349. doi: 10.1016/S0048-9697(99)00253-3. [PubMed] [CrossRef] [Google Scholar]

16. Blomqvist G., Johansson E.L. Airborne spreading and deposition of de-icing salt—A case study. Sci. Total Environ. 1999;235:161–168. doi: 10.1016/S0048-9697(99)00209-0. [CrossRef] [Google Scholar]

17. Zhang H., Wang Z., Zhang Y., Hu Z. The effects of the Qinghai-Tibet railway on heavy metals enrichment in soils. Sci. Total Environ. 2012;439:240–248. doi: 10.1016/j.scitotenv.2012.09.027. [PubMed] [CrossRef] [Google Scholar]

18. Yan X., Gao D., Zhang F., Zeng C., Xiang W., Zhang M. Relationships between heavy metal concentrations in roadside topsoil and distance to road edge based on field observations in the Qinghai-Tibet Plateau, China. Int. J. Environ. Res. Public Health. 2013;10:762–775. doi: 10.3390/ijerph10030762. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

19. Fakayode S., Olu-Owolabi B. Heavy metal contamination of roadside topsoil in Osogbo, Nigeria: Its relationship to traffic density and proximity to highways. Environ. Geol. 2003;44:150–157. doi: 10.1007/s00254-002-0739-0. [CrossRef] [Google Scholar]

20. Li X., Poon C., Liu P.S. Heavy metal concentration of urban soils and street dusts in Hong Kong. Appl. Geochem. 2001;16:1361–1368. doi: 10.1016/S0883-2927(01)00045-2. [CrossRef] [Google Scholar]

21. Morton-Bermea O., Hernández Álvarez E., Gaso I., Segovia N. Heavy metal concentrations in surface soils from Mexico City. Bull. Environ. Contam. Toxicol. 2002;68:383–388. doi: 10.1007/s001280265. [PubMed] [CrossRef] [Google Scholar]

22. Akbar K.F., Hale W.H.G., Headley A.D., Athar M. Heavy metal contamination of roadside soils of Northern England. Soil Water Res. 2006;1:158–163. [Google Scholar]

23. Carol E.S., Kruse E.E. Hydrochemical characterization of the water resources in the coastal environments of the outer Río de la Plata estuary, Argentina. J. S. Am. Earth Sci. 2012;37:113–121. doi: 10.1016/j.jsames.2012.02.009. [CrossRef] [Google Scholar]

24. Nie L., Su Z.D., Xu L.N., Yang X.R. Formation environment and distribution characteristics of main swamp turfy soil in China. J. Jilin Univ. (Earth Sci. Ed.) 2012;42:1477–1484. doi: 10.13278/j.cnki.jjuese.2012.05.029. In Chinese. [CrossRef] [Google Scholar]

25. Cobbett C.S. Phytochelatins and their roles in heavy metal detoxification. Plant Physiol. 2000;123:825–832. doi: 10.1104/pp.123.3.825. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

26. Schützendübel A., Polle A. Plant responses to abiotic stresses: Heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot. 2002;53:1351–1365. doi: 10.1093/jexbot/53.372.1351. [PubMed] [CrossRef] [Google Scholar]

27. Kalay M., Ay Ö., Canli M. Heavy metal concentrations in fish tissues from the Northeast Mediterranean Sea. Bull. Environ. Contam. Toxicol. 1999;63:673–681. doi: 10.1007/s001289901033. [PubMed] [CrossRef] [Google Scholar]

28. Wang H., Nie L., Xu Y., Lv Y. The effect of highway on heavy metal accumulation in soil in turfy swamps, Northeastern China. Water Air Soil Pollut. 2017;228:292. doi: 10.1007/s11270-017-3486-2. [CrossRef] [Google Scholar]

29. Mohan S.V., Nithila P., Reddy S.J. Estimation of heavy metals in drinking water and development of heavy metal pollution index. J. Environ. Sci. Health Part A. 1996;31:283–289. doi: 10.1080/10934529609376357. [CrossRef] [Google Scholar]

30. Lee C.S.L., Li X., Shi W., Cheung S.C.N., Thornton I. Metal contamination in urban, suburban, and country park soils of Hong Kong: A study based on GIS and multivariate statistics. Sci. Total Environ. 2006;356:45–61. doi: 10.1016/j.scitotenv.2005.03.024. [PubMed] [CrossRef] [Google Scholar]

31. Khan M.N., Wasim A.A., Sarwar A., Rasheed M.F. Assessment of heavy metal toxicants in the roadside soil along the N-5, National Highway, Pakistan. Environ. Monit. Assess. 2011;182:587–595. doi: 10.1007/s10661-011-1899-8. [PubMed] [CrossRef] [Google Scholar]

32. Ma J.H., Chu C.J., Li J., Song B. Heavy metal pollution in soils on railroad side of Zhengzhou-Putian section of Longxi-Haizhou railroad. Pedosphere. 2009;19:121–128. doi: 10.1016/S1002-0160(08)60091-0. [CrossRef] [Google Scholar]

33. Eimers M.C., Croucher K.N., Raney S.M., Morris M.L. Sodium accumulation in calcareous roadside soils. Urban Ecosyst. 2015;18:1213–1225. doi: 10.1007/s11252-015-0454-8. [CrossRef] [Google Scholar]

34. Bukowiecki N., Gehrig R., Hill M., Lienemann P., Zwicky C.N., Buchmann B., Weingartner E., Baltensperger U. Iron, manganese and copper emitted by cargo and passenger trains in Zürich (Switzerland): Size-segregated mass concentrations in ambient air. Atmos. Environ. 2007;41:878–889. doi: 10.1016/j.atmosenv.2006.07.045. [CrossRef] [Google Scholar]

35. Davila A., Rey D., Mohamed K., Rubio B., Guerra A. Mapping the sources of urban dust in a coastal environment by measuring magnetic parameters of Platanus hispanica leaves. Environ. Sci. Technol. 2006;40:3922. doi: 10.1021/es0525049. [PubMed] [CrossRef] [Google Scholar]

36. Wang H., Nie L., Xu Y., Li M., Lv Y., Wang H. Traffic-emitted metal status and uptake by Carex meyeriana Kunth and Thelypteris palustris var. pubescens Fernald growing in roadside turfy swamp in the Changbai Mountain area, China. Environ. Sci. Pollut. Res. 2018;19:18498–18509. doi: 10.1007/s11356-018-1990-6. [PubMed] [CrossRef] [Google Scholar]

37. Mishra S.R., Chandra R., Kaila A.J., Darshi B.S. Kinetics and isotherm studies for the adsorption of metal ions onto two soil types. Environ. Technol. Innov. 2017;7:87–101. doi: 10.1016/j.eti.2016.12.006. [CrossRef] [Google Scholar]

38. Kumpiene J., Lagerkvist A., Maurice C. Stabilization of Pb- and Cu-contaminated soil using coal fly ash and peat. Environ. Pollut. 2007;145:365–373. doi: 10.1016/j.envpol.2006.01.037. [PubMed] [CrossRef] [Google Scholar]

39. Prasad B., Bose J.M. Evaluation of the heavy metal pollution index for surface and spring water near a limestone mining area of the lower himalayas. Environ. Geol. 2001;41:183–188. doi: 10.1007/s002540100380. [CrossRef] [Google Scholar]

40. Al-Ani M.Y., Al-Nakib S.M., Ritha N.M., Nouri A.M., Assima A. Water quality index applied to the classification and zoning of Al-jaysh canal, Baghdad—Iraq. J. Environ. Sci. Health Part A Environ. Sci. Eng. 1987;22:305–319. doi: 10.1080/10934528709375351. [CrossRef] [Google Scholar]

41. Edet A.E., Offiong O.E. Evaluation of water quality pollution indices for heavy metal contamination monitoring: A study case from Akpabuyo-Odukpani area, Lower Cross River Basin (southeastern Nigeria) GeoJouranl. 2003;57:295–304. doi: 10.1023/B:GEJO.0000007250.92458.de. [CrossRef] [Google Scholar]

42. Ruiying D., Yanhong W., Mingdeng T., Mengjun L., Shaoying A. Remediation effects and evaluation of peat amendment on Pb and Cd contaminated in vegetable soil. Ecol. Environ. Sci. 2015;24:1893–1897. doi: 10.16258/j.cnki.1674-5906.2015.11.021. [CrossRef] [Google Scholar]

43. Carolin C.F., Kumar P.S., Saravanan A., Joshiba G.J., Naushad M. Efficient techniques for the removal of toxic heavy metals from aquatic environment: A review. J. Environ. Chem. Eng. 2017;5:2782–2799. doi: 10.1016/j.jece.2017.05.029. [CrossRef] [Google Scholar]

44. Dousova B., Buzek F., Rothwell J., Krejcova S., Lhotka M. Adsorption behavior of arsenic relating to different natural solids: Soils, stream sediments and peats. Sci. Total Environ. 2012;433:456–461. doi: 10.1016/j.scitotenv.2012.06.063. [PubMed] [CrossRef] [Google Scholar]

INLAND WETLAND AND WATERCOURSE REVIEW AREAS

James G. MacBroom, P.E.[1]
November, 2002

INTRODUCTION

Inland Wetland Agencies (IWA) have traditionally regulated activities within mapped inland wetlands that are defined by soils, vegetation, or presence of water bodies. The 1995 revisions to the Inland Wetland and Watercourse Act specifically enabled the IWA to also regulate upland activities that would likely impact wetlands or watercourses. The recent Connecticut Supreme Court decision in the case of Queach Corporation vs. Branford Inland Wetlands Commission reaffirmed this authority. This clarification in the authority to regulate upland areas generates interest in how to evaluate the functions and values of upland areas with respect to the wetland or watercourse and how to assess the impact of proposed activities.

Evaluation of upland areas should include: hydrologic functions including protecting stream banks from erosion, providing flood water conveyance, providing groundwater recharge and storage; water quality functions including providing shade to moderate water temperature, trapping sediment, renovating surface water runoff and isolating pollution sources; ecological functions including providing sources of woody detritus for streams, terrestrial habitat, wildlife corridors, nesting sites, and protection of rare or endangered species; and cultural values including aesthetics, recreation and educational opportunities. Evaluating the scientific functions and values of wetlands and their adjacent upland areas often requires review of the watersheds natural resources and technical assistance.

The assessment and regulation of upland areas beyond the boundaries of wetlands and waterbodies is not a new role for IWA. Many IWAs have had regulated upland areas, popularly known as buffers, adjacent to wetlands for many years, often specifying a fixed width regulated area parallel to wetland boundaries. The designation and use of upland review areas has been suggested to IWAs in the Connecticut Department of Environmental Protection "Guidelines For Upland Review Area Regulations" recommends use of a 100-foot wide review area. The preparation of a town wide wetland inventory of watershed plan would help identify areas of special interest or concern and inform all parties where and under what circumstances upland review areas are applied.

BUFFER ZONE HIERARCHY

It is not uncommon for the riparian areas to be thought of as having two or more sub-areas based upon their primary function. The first 25± feet of upland adjacent to a wetland or watercourse are usually the most important. This inner portion of the zone includes stream banks that may be subject to periodic inundation and may convey and or store floodwaters. Bank vegetation provides root mass that stabilizes banks and the

canopy reduces rainfall energy. It is the interface between aquatic and terrestrial habitat and its vegetation that provides shade to moderate water temperature fluctuations.

Vegetative zones up to 50± feet wide are important as a source of coarse woody debris and particulate material that serves as a source of organic energy for the base of the food chain. The first 50 feet adjacent to a wetland is also important for the treatment of surface water runoff which moves as sheet flow through vegetated areas that filter, absorb, infiltrate and attenuate of non-point source pollutants.

The use of increasingly wide buffer zones has diminishing benefits to wetlands and watercourses. Zones in excess of 100 feet have been reported in the literature primarily for protection of wetland dependent mobil wildlife rather than for direct water resources protection. This raises the logical issue of to what extent should an IWA regulate a non-wetland habitat for species such as amphibians that use a combination of aquatic and upland terrestrial habitats.

The Metropolitan Washington Council of Governments has published a three-part procedure for estimating buffer widths. This model for water quality (sediment) protection considers the slope of the land, vegetation density, adjacent land uses and sediment type with resulting buffer widths ranging from 50 feet for low gradient sandy soils to 200 feet for steep silty soils. It is noted that vegetative buffers are not effective in trapping clay sediment particles, which can travel hundreds of feet (MWCOG, 1995).

Buffer zones in urban areas are primarily for the protection of stream banks, renovation of runoff, providing shade and woody detritus, and aesthetics. The literature suggests that these functions are often accomplished in relatively narrow zones of 25 to 75 feet in width. In suburban areas, dominant land uses are often single-family residential lots with on-site sewage disposal systems and water supply wells. The Connecticut Public Health Code requires sewage disposal systems to be 50 feet from an "open watercourse," which could include wetlands with exposed surface water, and to be 100 feet from water supply reservoirs. The US Environmental Protection Agency recommends a 50-100 foot separation distance between sewage disposal systems and surface water.

Evaluation of Upland Areas

The author recommends a five-step process to help guide the review, regulation, and management of the upland areas in a structured manner. The five steps are to evaluate existing natural resources associated with the wetland and/or watercourse; evaluate upland site conditions such as soils, slope and vegetation; set clearly defined conservation goals and objectives consistent with the Inland Wetland and Watercourse Act; assess the scope of the proposed activities and their potential impacts; and evaluate potential mitigation measures that avoid, minimize or compensate for potential adverse impacts.

The first step in establishing an equitable regulated area is to inventory and assess the wetland and watercourse resources, including their local and watershed wide values. Typical metrics include type of wetland (marsh, swamp, bog, open water, etc.), water quality classification, water supply usage, fauna and flora, presence of rare or endangered species, floodwater storage or conveyances, recreational use, etc. There are numerous models that can be used to organize the data. The author recommends that communities with upland review areas develop guidelines for how to identify areas or activities of special concern. There are numerous wetland evaluation models available to help inventory and assess wetlands functions and values including the CTDEP Bulletin #9, the US Army Corps of Engineers Descriptive Approach and the HGM methodology. However, there are few established methods for evaluation of adjacent upland areas. Resource evaluations are most valuable when comparative data is available for other local wetlands/watercourses, allowing one to compare wetland values to reference sites. The above task should be performed in coordination with, the staff of those towns that seek to regulate broad areas. Ideally, watershed management plans should be prepared at the inter-municipal level to coordinate basin activities that affect wetlands, flooding, water supply, waste disposal, open space, greenways etc. Individual applicants for activities in upland areas may not even own or abut the down gradient wetlands and often lack permission to enter and inspect private property or reference sites.

The second step is to assess the upland site of the proposed activity and the area leading to wetlands or watercourses. Specific geophysical issues that affect the performance of upland areas include soil types, soil erodibility, slopes, vegetation, depth to groundwater, watershed area, runoff rates and drainage patterns. For example, steep slopes and low permeability soils influence soil erosion and sediment transport, while dense natural vegetation and irregular micro-topography help to reduce sediment travel distances. Similarly, highly pervious soils minimize natural surface runoff and erosion, but result in a large increase in runoff if they are paved over.

The performance of upland areas for water quality protection varies with site conditions. Upland areas with steep slopes (over ten percent) have rapid flow velocities that tend to channelize overland flow, reducing opportunities for water infiltration, nutrient uptake or absorption of pollutants. Wider areas or less intense land uses are recommended for highly erodible soils with a high silt or clay content, or where there is thin vegetation.

At the conclusion of Steps 1 and 2, one can assess whether the adjacent upland review area contributes to the wetland or watercourse functions, leading to setting goals and objectives for balancing land use and resource conservation. Logical questions include whether the wetland has high value functions, is it rare, is it part of a continuous corridor, does it have true riparian characteristics or is it a perched groundwater site on a hillside? Does the upland review area support or supplement the wetland or watercourse? Is the wetland or watercourse dependent upon the adjacent upland area and to what extent? These questions can be difficult to address and incorporate into the application process unless one has a basic understanding of the overall watershed.

Low impact activities within the upland area would include selective vegetation removal, passive recreation, water supply wells, narrow crossings such as roads, utilities, agriculture, pathways and water dependent activities. Activities with potentially large impacts include clear cutting vegetation, extensive earthwork, buildings, hazardous materials, excessive use of lawn products, parking lots and wastewater disposal systems. Some potential impacts can be limited by sensitive site design and erosion controls.

Temporal impact factors include the duration of the activity and the season in which it occurs. Short duration activities with temporary impacts may be more tolerable than long-term activities of a lower intensity. Similarly, in-water activities during the spawning, breeding, or migratory periods may be of greater significance than the same activities during the off-season.

Mitigation efforts begin with good site design to avoid unnecessary negative impacts. A simple example is to cross wetlands or watercourses at their lowest value area, often at their narrowest point. There is a need to emphasize low impact design to reduce the dependency on buffer zones. Low impact techniques include minimizing impervious cover, building vertically with a smaller footprint, use of narrower roads, avoiding non-functional curbs, use of grass swales instead of enclosed pipes, and use of storm water infiltration systems. It is desirable to avoid direct discharges of stormwater runoff from impervious areas into watercourses. Pollution prevention, through the use of substitute materials, safe storage and proper disposal, is an important measure to reduce pollutants. Phased construction to minimize the disturbed area and rapid soil stabilization are important, plus best management measures for soil erosion prevention, sediment control, and runoff treatment.

There is extensive literature on the performance of buffer zones in relation to specific functions. However, much of the data is limited to regional geographic areas or vague, poorly defined land uses. As a result, summaries of the literature tend to be generalized and provide a wide range for buffer widths. It is apparent that published widths and performance vary depend on their intended function and site conditions. A recent publication by the U.S. Army Corps of Engineers for example, recommends 5 to 30 meters for water quality protection, 10 to 20 meters for stream bank stabilization, 3 to 10 meters for input of woody detritus, 20 to 150 meters for flood attenuation, and 30 to 500 meters for habitat (Fischer, 2000). The non-profit Center for Watershed Protection summary of buffer widths in 36 communities and found a median width of 100 feet.

As noted by the US Army Corps of Engineers, there is insufficient information in the literature to rigorously relate buffer widths to upland land use and riparian functions. The process thus requires professional judgment.

EMERGING ISSUES

The use of effective mitigation measures is an important factor to consider in reviewing potential project impacts. For instance, research on water quality and sediment impacts generally neglect the use of best management practices which could include erosion silt fence, sediment basins, hydro seed, grit chambers, and others. Best management practices for storm water runoff are being emphasized by the new NPDES Phase II regulations and by the DEP Office of Long Island Sound.

The 1995 revisions to the General Statutes included vernal pools as a regulated area and allow IWA to review their impact areas. Vernal pools are a seasonal landscape feature whose unique properties, fauna and flora are most visible during a short period in the spring. Consequently, there are seasonal limitations that may impede comprehensive site assessments, IWA staff inspections and the public review process. There has been some discussion, but no resolution, concerning mandatory timing of site assessments.

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References

Ammam, Alan, Editor. "Method for the Evaluation of Inland Wetlands in Connecticut," DEP Bulletin No. 9, Hartford, Connecticut. August, 1990.

Connecticut Department of Public Health. "Connecticut Public Health Code, Regulations and Technical Standards for Subsurface Sewage Disposal Systems," Hartford, Connecticut. January, 2000.

Fischer, Richard, and Fischenich, Craig. "Design Recommendations for Riparian Corridors and Vegetated Buffer Strips," US Army Engineer Research and Development Center, Vicksburg, MS. April, 2000.

Herson-Jones, Lorraine, et.al. "Riparian Buffer Strategies for Urban Watersheds," Metropolitan Washington Council of Governments, prepared for USEPA, Washington, DC. December 1995.

U.S. Army Corps of Engineers, New England Division. "Buffer Strips for Riparian Zone Management," prepared for State of Vermont. Waltham, Massachusetts. January, 1971.

U.S. Army Corps of Engineers. "The Highway Methodology Workbook, Wetland Functions and Values, A Descriptive Approach". November, 1995.

[1] The author is a Water Resources Engineer and Vice President of Milone & MacBroom, Inc., and author of: "The River Book," published by CTDEP.

POSITION STATEMENT

A Statement on Upland Review Areas for Connecticut Municipal Inland Wetlands and Watercourse Commissions

BACKGROUND

Under the Inland Wetlands and Watercourses Act, Connecticut's municipalities regulate proposed development activities in or affecting wetlands and watercourses. CACIWC has received numerous inquiries from wetland commissioners and their staff for guidance in establishing upland review area provisions in their municipal wetland regulations.

The term Upland Review Area was developed and put forth by Connecticut DEP in a 1997 guidance document (*Guidelines - Upland Review Area Regulations – Connecticut's Inland Wetlands & Watercourses Act*) which encourages inland wetland agencies to review activities proposed in upland areas surrounding wetlands and watercourses wherever such activity is likely to impact or affect wetlands and watercourses. Activities that can adversely affect adjacent wetlands and watercourses include, but are not limited to the following: land clearing, soil compaction, excavation, fill, changes in run-off volume and pollutant discharges.

It is important to note that the extension of the upland review area through text amendments to the municipal regulations does not prohibit construction and development within these areas and, therefore, is **not** an unconstitutional "taking" of property. The expanded area simply enables the IWWA to review and evaluate potential impacts of development proposals within this area.

POSITION

CACIWC supports inclusion of a 100 foot Upland Review Area in the municipal inland wetlands regulations as a <u>minimum area</u> of review to properly assess the potential impacts of a proposed activity on inland wetlands and watercourses. Further, CACIWC supports the existing regulations that authorize wetland agencies to regulate activities in other upland areas that are "likely to impact or affect a wetland or watercourse."

Establishing an Upland Review Area in the municipal regulations in which the agency regularly evaluates proposed activities and their likely impact on adjacent wetlands and watercourses provides a consistent framework for regulating and permitting activities. Inclusion of an Upland Review Area in the municipal regulations also serves to notify the public and potential applicant as to what activities adjacent to inland wetlands and watercourses require an application for permit

RATIONALE

Enabling Legislation
The preamble to the Inland Wetlands and Watercourses Act (Chapter 440, Sec 22a-36 to 22a-45) states the rationale and authority for wetlands and watercourses protection in Connecticut.

> **"The wetlands and watercourses are …. essential to an adequate supply of surface and underground water; to hydrological stability and control of flooding and erosion; to the recharging and purification of groundwater; and to the existence of many forms of animal, aquatic and plant life."**

The preamble also enables local inland wetlands and watercourses commissions to "...protect the citizens of the state by making provisions for the protection, preservation, maintenance and use of the inland wetlands and watercourses by

- minimizing their disturbance and pollution;
- maintaining and improving water quality…;
- preventing damage from erosion, turbidity or siltation;
- preventing loss of fish and other beneficial aquatic organisms, wildlife and vegetation and the destruction of the natural habitats thereof;
- deterring and inhibiting the danger of flood and pollution;
- protecting the quality of wetlands and watercourses for their conservation, economic, aesthetic, recreational and other public and private uses and values; and
- protecting the state's potable fresh water supplies from the dangers of drought, overdraft, pollution, misuse and mismanagement…"

Connecticut Department of Environmental Protection
The 1997 guidance document published by the Connecticut Department of Environmental Protection *Guidelines Upland Review Area Regulations - Connecticut's Inland Wetlands & Watercourses Act* provides the rationale for a 100-ft. upland review area. It states that "the DEP believes that a 100 foot-wide upland review area is sufficient for reviewing construction activities in areas surrounding wetlands or watercourses because most of the activities which are likely to impact or affect these resources will be located in that area."

Court Decisions
In *Queach Corporation v. Town of Branford Inland Wetlands Commission* (2001) the Connecticut Supreme Court upheld the 100 foot upland review area with this language in the decision: "Thus, we conclude that the 100 foot upland review area imposed by the regulation is a valid administrative devise reasonably designed to enable the commission to protect and preserve the wetlands located within [Branford], in fulfillment of its duty under the [act]." And "We note that the department Guidelines for Upland Review Area

Regulations under Connecticut's Inland Wetland and Watercourses Act, the testimony before the commission, and the broad purpose of the act, provided ample evidence for the commission to approve the 100 foot setback."

Scientific Support

Over the last 20 years, a large body of scientific evidence has determined that protection of the riparian area adjacent to rivers and streams is critical to controlling flooding, erosion, excess sedimentation, and maintaining the hydrologic balance of those rivers and streams. Based upon these studies, the CT DEP Inland Fisheries Division published a position statement "Utilization of 100 Foot Buffer Zones to Protect Riparian Areas in Connecticut" by Brian D. Murphy that sets policy for the Division that a 100 foot protective buffer is a minimum setback along perennial streams. Similar scientific evidence supports the establishment and maintenance of a minimum 100 foot vegetated buffer to protect inland wetlands from non point source pollution impacts. Please visit our website (www.caciwc.org) for links to additional documents detailing the importance and function of riparian areas.

Municipal Decisions

Many communities in Connecticut have extended the upland review area to 100 feet, Middletown, Bristol, Cromwell, Middlefield, Glastonbury and Rocky Hill. Other municipalities have adopted larger regulatory areas around specific water bodies. New Milford and Sherman established a 200 foot regulatory area around Candlewood Lake. Killingworth developed a 500 foot regulatory review area around vernal pools, and Vernon set a 200 ft upland review area along two rivers, the Tankerhoosen and the Hockanum, and five stream tributaries. Also of note is the Town of Burlington that established an upland review area of greater than 600 feet due to the preponderance of steep slopes and the significant sources of groundwater within the community. The quantity and quality of local water resources vary from town to town. Local communities need to evaluate the wetlands and watercourse resources within their own communities, and based upon that evaluation, establish appropriate distances for review of potential regulated activities.

CACIWC's mission is **"To promote the statutory responsibilities of Connecticut Conservation Commissions and Inland Wetlands Commissions and to foster environmental quality through education and through the conservation and protection of wetlands and other natural resources."**

Approved by the Board of Directors July 2006.