# DRAFT

# Guidance for the Preparation of Toxicological Profiles

*Agency for Toxic Substances and Disease Registry*

*Department of Health and Human Services*

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# CONTENTS

# GENERAL

These guidelines assist authors and chemical managers with preparing toxicological profiles for the Agency for Toxic Substances and Disease Registry (ATSDR).

This section directs the use of font style, font size, headings, headers, footers, margins, page and paragraph spacing, table and figure properties, citations, and clear writing and plain language principles. The ATSDR chemical teams and contractors shall follow the National Center for Environmental Health (NCEH)-ATSDR Style Manual except when differing from this document, in which case the style as detailed in this document shall prevail. This document shall be provided to any contractor that writes for ATSDR and shall be reviewed by all ATSDR chemical managers and chemical teams.

## *Typeface and Point Size*

- Use 11-point Times New Roman for the text.

## *Headings*

- First level headings, must use all capital letters, be centered, and be **13-point bold Arial**
- Second level headings must use all capital letters, be left justified, and be **11-point bold Arial**
- Third level headings must use first-letter capitals, be left justified, and be **11-point bold Arial**
- Fourth level non-numbered template headings must use first-letter capitals, be left justified, and be **11-point bold Arial** followed by a period, two spaces, and then body text.
- Other subheadings must be use first-letter capitals, be left justified, and be ***11-point bold, italic Times New Roman*** font followed by a period, two spaces, and then body text.

## *Page Formatting*

- 1” margins all around
- Line spacing of 1.5
- Line numbering, restarting on each page, until final draft
- Boilerplate text is bold until final draft
- Widow/orphan control on
- Header from top and footer from bottom both set at 0.3”

The header font is in Arial 8-point, all caps. The header will contain the chemical name flush with the left margin and the page number flush with the right margin, followed by one blank line and then the number and name of the chapter, centered. There are two additional blank lines after the chapter title. Suppress the chapter title on the first page of the chapter.

The footer is in Arial 8-point font, all caps and is centered on the page. Pre- and post- public comment draft profiles use the following footer: ***DRAFT – DO NOT CITE OR QUOTE – [Month day, year]*** (define with the date code so that MS Word automatically populates with the date of the draft). Flush with the right margin will be the version number. Use the following footer for the final pre-public version: ***DRAFT FOR PUBLIC COMMENT***. The final post-public comment profile does not have a footer.

## *Editorial*

### Numbers

- Spell out numbers less than 10, except when they are used with a unit of measure or when they are in the same sentence with a number of 10 or more (e.g., one mouse was exposed to 1 mg/kg/day; 10 rats and 5 mice were exposed to 5 mg/kg/day).
- Use commas in numbers with four or more digits.
- When using scientific notation, do not use the "E" method (e.g., use $1x10^8$ instead of 1E8)
- Don't split numbers and units of measure across lines in the profile text or tables; use nonbreaking spaces or hyphens to keep from splitting.
- Don't repeat units of measure in ranges or series (e.g., 25, 50, or 100 ppm rather than 25 ppm, 50 ppm, or 100 ppm; 10 and 50% rather than 10% and 50%).

### Abbreviations, Acronyms, and Symbols

- When an abbreviation or acronym is used in a profile, the word or phrase should be spelled out the first time it used followed by the abbreviation or acronym in parenthesis. The acronym or abbreviation should be throughout rest of the text.
- Use full Latin name of species when first discussed in the profile (e.g., *Salmonella typhimurium*); and abbreviate thereafter (e.g., *S. typhimurium*).

- United States as a noun should always be spelled out; when used as an adjective, U.S. should be abbreviated.
- Most units of measure should be abbreviated (e.g., ppm, mg, µL, mmHg, nmol, °C, atm, etc.). However, it is preferable not to abbreviate common units of measure such as second, minute, hour, day, week, month, year, pound, ounce.
- In most cases, use symbols for alpha, beta, delta, gamma, and other Greek letters or mathematics terms. Note that either a lower-case x or the multiplication symbol can be used.
- The use of symbols for less than, greater than, less than or equal to, and greater than or equal to are preferred (e.g., 30 or more participants should be ≥30 participants).
- Do not use spaces around mathematical or other symbols (e.g., $p<0.01$; $n=9$; 10°C; $1x10^{-9}$).

## Hyphens, en dashes, and em dashes

- Use a hyphen between two words that work together to modify a noun (e.g., acute-duration exposure; dose-response relationship)
- It is preferred to not hyphenate words with prefixes (e.g., nonvolatile, nonresponsive, noncancer, multiphase, pretreatment, postexposure, undiluted); however, if the absence of a hyphen would create confusion regarding the meaning of the word, use the hyphen (e.g., un-ionized, not unionized).
- Use nonbreaking hyphens if necessary to keep hyphenated phrases from splitting across lines (e.g., 8-hour exposures; 1,2-dichloropropane).
- Use optional hyphens if necessary for long chemical names across lines (e.g., nitrosodiphenyl-amine).
- Use an en dash for ranges of numbers (e.g., 18–24 hours) in profile text and tables. Exceptions include when there is a “from/to” range (e.g., doses ranged from 12 to 20 mg/kg/day) or “between/and” range (e.g., between day 10 and 13).
- Use a regular hyphen in ranges of page numbers in the reference list.
- Use an em dash (with no spaces on either side to set off part of a sentence (e.g., This section is organized by route of exposure—inhalation, oral, and dermal).

## Miscellaneous

- Use a comma in series of three or more (e.g., rats, mice, and guinea pigs; 1, 3, or 10 ppm)

- Use brackets within parentheses, not parentheses within brackets.
- Italicize technical phrases in a foreign language, such as *in vivo, in vitro, in utero, ad libitum, in situ.*
- Italicize Latin names of species (e.g., *Escherichia coli*).
- Do not italicize et al., e.g., i.e., etc.
- Capitalize Section, Chapter, Table, and Figure.
- Use mg/kg/day rather than mg/kg-day.
- Avoid using "man" for "human(s)."
- Avoid "human volunteer" (omit the word "human"); animals cannot volunteer.
- It is repetitive to use “oral gavage;” omit the word “oral” and just use “gavage.”

### *Table Naming and Formatting*

Table names must have short titles that do not end with periods. A subtitle may have a longer explanation of the contents.

The following table name is appropriate.

**Table 5-X. NHANES Statistics for Mono-2-Ethylhexyl Phthalate (MEHP) Concentrations in the U.S. Population**

However, the following table name is too long and may not be the primary title.

~~**Table 5-X. Geometric Mean and Selected Percentiles of MEHP Urine Concentrations (in µg/L) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2012.**~~

The table title must be 12-point bold Arial font, within the 1st row of the table, and styled to show up in the list of tables. Table titles have first letter capitalized and centered, and there is a hard return after the title. The title row has light blue shading (Red 222, Green 234, Blue 246). The header row has light gray shading (Red 242, Green 242, Blue 242) and text that is first letter capitalized and left justified in 11-point Arial font with light gray shading (Red 242, Green 242, Blue 242). Both title and header rows are defined so that they repeat over multiple pages. Use a darker gray shade (Red 217, Green 217, Blue 217), if necessary, for rows that divide sections (e.g., acute, intermediate, chronic). The font for data entries in the table shall be Arial 10-point. There should be no blank cells so as not to confuse the reader; use

consistent language to denote no data, no effect, not detected, etc. Tables are center justified and 6.5 inches for portrait and 9 inches for landscape. The table text is single-spaced and all cell margins are set at 0.02 inches. There are horizontal lines separating data rows in tables; there is no horizontal line above the table title row or below the footer row. No vertical lines are used in tables. If applicable, footnotes shall be lettered (not numbered) and be defined in the last row of the table in 9-point Arial font. There is a blank line followed by the footnotes, followed by another blank line and then definitions of acronyms (in alphabetical order, hard spaces around the equal signs so they don't split across lines), followed by another blank line and then the source (e.g., Source: TRI16 2017). Tables should stand alone; therefore, all acronyms used in a table should be defined, regardless of whether they were previously defined in the text.

### *Figure Naming and Formatting*

Figure names must have short titles. A subtitle may have a longer explanation of the contents. Figure titles are 12-point Arial font, centered, first letter capitalized, and styled to show up in the list of figures. If possible, the font size for figures should be a minimum of 10-point.

With the need to address people with disabilities (508 compliance of the Rehabilitation Act), colors distinguishable when printed in black-and-white are necessary in all figures. Use patterns to distinguish different datasets when necessary.

All tables and figures, as demonstrated in the Exhibits, must be included in a toxicological profile unless: (1) data do not exist (e.g., there are no physiologically based pharmacokinetic [PBPK] or pharmacodynamic [PD] models) or (2) there is agreement between the lead chemical manager and author for its removal. Upon deciding to remove a table, the lead chemical manager will be required to discuss this decision with his/her supervisor and their branch chief. The final decision on exclusion of any table/figure is with their branch chief. Including additional tables/figures, will be at the discretion of the author and chemical manager. An example table that you may want to include could be one that provides details on the distribution of the chemical of interest within the body based on single and multiple administrations and doses.

### *Citations and References*

Citations are in the body of the text and direct the reader to references. Use the author-date format for in text citations. Use "and" for two authors. When there are three or more authors list the primary author's

last name with et al. and the year. Citations shall be in parentheses unless referring to the study as a proper noun. Below are examples.

***One Author (Including Institutional Authors):***

> Using PBPK modeling, NTP (2006) analyzed the relative contribution …[…]…and colon in rats following oral exposure to bromodichloromethane.

***Two Authors:***

> Three pathways have been identified for the metabolism of bromo-dichloromethane: (1) cytochrome P450 oxidation to phosgene (Allis and Zhao 2002);…[…]

***Three or More Authors:***

> In C57BL/6 mice, inhalation exposure to ≥30 ppm for 1 week resulted in decreases in body weight gain (Torti et al. 2001).

Citations of references that have supplementary materials shall include both references. For instance, if there is a Jones (2017a) study and a Jones (2017b) that is the supplemental material, the author must cite both (e.g., Jones 2017a, 2017b). Order string reference citations alphabetically and separate each reference by semi-colons (e.g., Abby et al. 2001; Green 2012a, 2012b; White et al. 2000).

For more information regarding references, see Chapter 8.

## *Keep Your Writing Uncluttered and Clearly Organized*

Structure and organization play key roles in reading comprehension.

- Make each sentence about one thing or serve one purpose.
- Each paragraph should also be about only one thing. Don't overcrowd paragraphs with multiple topics.

- Where possible, use a topic sentence at the beginning of the paragraph (i.e., a sentence that summarizes or expresses the main idea of the paragraph).
- Focus on a limited number of key points such as threshold for risk, health effects, health tests, and environmental concentrations.
- Eliminate or reduce unnecessary words and jargon as can be reviewed on page 50 of the NCEH/ATSDR Style Guide.

## Recommended Clear Writing Principles

### *Good Writing Practices*

- Use bulleted or numbered lists and meaningful headings to chunk information into organized sections.
- Try to minimize use of abbreviations and acronyms. For example, if you use something only twice, maybe you don't need to use the acronym; just spell it out both times.
- Consider using tables to make complex material easier to understand.
- Use transitions to get from one paragraph to the next.

### *The "Do Not" List*

Don't use the term "respectively" for sentences with more than 3 groups. It puts too much burden on the reader; it's a comprehension speed bump.

> They reported that for an infant cardiopulmonary bypass, pediatric hemodialysis, exchange transfusion, and extracorporeal membrane oxygenation, serum DEHP concentrations ranges were 1.1–5.1, 0.4–4.2, 5.4–21.5, and 18–98 µg/mL, respectively.

Instead, place each value with its unit measure next to each object, like seen below.

> They reported that serum DEHP concentration ranges for an infant were: cardiopulmonary bypass 1.1–5.1 µg/mL, pediatric hemodialysis 0.4–4.2 µg/mL, exchange transfusion 5.4–21.5 µg/mL, and extracorporeal membrane oxygenation 18–98 µg/mL.

- Don't cluster a bunch of nouns together. This practice often impedes reading comprehension; e.g., Formaldehyde chronic inhalation guidelines. Better – Formaldehyde, chronic-inhalation guidelines. Or try, chronic-inhalation guidelines for formaldehyde.

- Don’t use slashes ( / ). Slashes sometimes cause reader confusion or are unnecessary.
- Don’t add in extra, unnecessary words, descriptions, or jargon.

## Attachment/Exhibit Considerations

Attachments and exhibits in this document are supplemental materials that further refine or display the toxicological profile contents. Attachments often explain scientific considerations, whereas exhibits display the formatting and content of figures and tables.

# PURPOSE AND SCOPE OF ATSDR TOXICOLOGICAL PROFILES

The toxicological profiles are summaries of ATSDR's evaluations concerning whether, and at what levels of exposure, adverse health effects occur and levels at which no adverse effects occur. The profiles include information about exposure and environmental fate that may help readers determine the significance of levels found in the environment. The primary users of these documents are health assessors who need succinct interpretations of toxicological data for such purposes as responding to telephone inquiries from the public and assessing a specific problem at a Superfund site, among others. Other health professionals/clinicians may also be users of this document.

Toxicological profiles provide syntheses and interpretations of data, which distinguishes them from ordinary reviews. Interpretations are useful for those health professionals who may not have the resources to gather and consider all the toxicological data themselves.

Interpreting data often requires judgment and implicit assumptions that are more a matter of policy than objective science. Specifically, the profiles incorporate ATSDR's evaluations of the validity of particular studies and the inferences that are made from them. To this end, the profiles do not provide all of the information necessary to support these evaluations; presenting data in sufficient detail to allow users to weigh all of the evidence themselves is incompatible with the concept of a "profile." Nor do the profiles present details on selected studies (except as noted in this document), because the absence of a discussion of other studies would not allow users to form independent judgments of the meaning of the data.

However, in order to increase the transparency of this process, ATSDR has begun incorporating systematic review methodology into profile development. In some cases, only a limited systematic review may be feasible or necessary. For example, a full systematic review may be needed only for the health endpoint associated with a Minimal Risk Level (MRL), while other endpoints could include only a limited systematic review that does not include all of the systematic review elements. Furthermore, because of profile complexity (e.g., a chemical with multiple congeners), not all profiles are amenable to systematic review. These profiles will rely on the weight-of-evidence method mentioned above.

## Innovations

Risk assessment is constantly undergoing improvement in techniques and methodology that is consistent with the biological and physical fields that it encompasses. Recent innovations to the field include the use

of germ free animals, epigenetics, paradigm shifts in epidemiology, and use of transcriptomics data. Each of these innovations helps to target potentially deleterious chemicals and their most probable effect endpoints. In so doing, human risk assessment is better refined and efforts are expended on meaningful investigation. With this updated guidance, some of these innovations will be discussed with new tables and text suggested for the toxicological profiles written after this publication.

# QUALITY CRITERIA FOR ANIMAL AND HUMAN STUDIES

ATSDR has adopted the National Research Council's (NRC's) "Guidelines for Assessing the Quality of Individual Studies," which appear in Toxicity Testing: Strategies to Determine Needs and Priorities, published by NRC in 1984. ATSDR agrees with the NRC that judging the quality of past and future studies solely by today's standards is inappropriate. The NRC considers a report of scientific findings adequate for use in health hazard assessment if the report meets the following basic criteria (refer to Attachment A):

- A clear description of all elements of exposure is provided.
- Results in test subjects are predictive of human response, and test subjects are sensitive to the effects of the substance.
- Controls are comparable with test subjects in all respects except the treatment variable.
- Endpoints answer the specific questions addressed in the study, and observed effects are sufficient in number or degree to establish a dose-response relationship useful in estimating the hazard to the target species.
- Both the design and the interpretation of the study allow for appropriate statistical analysis of the data.

Apply these criteria where appropriate to judgments on the quality of data from epidemiological investigations and other scientific studies of relevance to ATSDR's toxicological profiles.

The reliability of epidemiological data in hazard identification is increased when results are obtained from studies that have the following characteristics (refer to Attachment B):

- Derived from well-designed and well-executed case-control or cohort studies that are free from bias.
- Display a strong association unlikely to be due to chance variation.
- Follow a logical, temporal sequence of exposure-response.
- Replication occurred in a variety of settings.
- Exhibit a dose-response relationship, using valid estimates of exposure and dose.
- Are toxicologically plausible.
- Where possible, include an examination of causality.

In addition, ATSDR recognizes the following desirable factors of studies or reports of scientific findings as set forth in the NRC guidelines:

- Minimalization of subjective elements.
- Peer review of scientific papers and of reports is desirable. *Note: The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates the peer review of toxicological testing results that ATSDR uses.*
- Results reported have increased credibility if they are supported by findings from other investigations.
- Similarity of results to those of tests conducted on structurally related compounds increases scientific confidence.
- Evidence of adherence to good laboratory practices improves confidence in results.

# GENERAL GUIDANCE FOR PREPARING A TOXICOLOGICAL PROFILE

When preparing a toxicological profile, avoid describing studies. Rather present data as a cohesive summary of the results, including a discussion of refuting studies. If a conclusion is uncertain or controversial, a brief description of the studies that are the basis for the uncertainty may be included. The description should be limited to those factors that are necessary to summarize the issue; do not include all details of the study. Avoid long string references; use a maximum of three to five citations per sentence or refer the reader to relevant tables or other sections of the profile (e.g., see Table 2-1 for additional citations) when more than five citations

Consider all data when making conclusions. ***Support all conclusions with references to original literature, not reviews. Refer to an abstract only if the original paper is not obtainable. Discuss current abstracts in the "Ongoing Studies" sections of Chapter 6. Disregard older abstracts if not followed up in the literature.***

An individual designated by the contractor works with the designated ATSDR representative to obtain epidemiological studies, health surveys, and current chemical regulations and guidelines.

Exhibit 1 provides a general outline for the toxicological profiles. Note that the outline presented in Exhibit 1 lists the topics that should be included in every toxicological profile. It follows the organization of the table of contents for toxicological profiles but does not match the table of contents exactly. Specifically, the outline contains some subheadings that do not appear in the table of contents.

- The phrase "ATSDR boilerplate" denotes a heading or text that must be included in every toxicological profile. All required material is provided in this guidance document and the attached exhibits. Boilerplate material is presented in a bold type to distinguish it from guidance information. It is in bold in drafts of profiles, but not in bold for clearance or final documents.

As noted above, this guidance document contains both guidance information and boilerplate material. Although some section titles in this document match the boilerplate titles used in toxicological profiles, others do not either because they address more than one profile subsection or because they represent more general guidance.

This guidance document uses the term "lesion(s)." We define lesion to be inclusive of pathologic changes to tissues including but not limited to atrophy, hyper-trophy/-plasia, patches of multifocal disease, and wounds/injuries. Do not include cancer as a lesion as toxicological profiles have a separate section for cancer and the main objective of the health effects section is to provide information on non-cancerous health effects.

## Targeted Profiles

ATSDR has developed a product called "targeted profiles." These profiles are quick updates to an already existing profile. Literature searches for Health Effects (Chapter 2) and Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions (Chapter 3) are completed from 2 years prior to the last profiles' publication to the present. Additionally, an update is completed for the Regulations and Guidelines chapter (i.e., Chapter 7).

We recommend that contractors begin by taking the old profile and associated Addendum document, if available, and pasting content into the appropriate places of the toxicological profile outline. It is important to do this so that old data that are still relevant are not lost in this process.

ATSDR has updated toxicological profiles to include more figures. These new figures require correlation between chapters. In review of the profile, ATSDR personnel will be checking that all figures, tables, and text (especially in Chapters 1, 2, 5, and 6 correlate with each other. ATSDR personnel already review literature in the profile and check for consistency with profile content. We ask ourselves questions like, are we consistently relaying messages (text and figures) throughout the profile?

## Appendix Considerations

ATSDR is producing some profiles that include a systematic review of the health effects data. Appendix C presents the systematic review framework and is only included in the profile if a systematic review was conducted. Contractors must be aware of which appendices to include in a particular toxicological profile and letter the appendices accordingly. In cases where there is no systematic review, the appendices are Appendix A–Appendix F. When a systematic review is included, the appendices are Appendix A–Appendix G. See below for a more explanatory table.

## Table of Appendices

| Appendix | Appendix Name for Literature Review Profile | Appendix Name for Literature and Systematic Review Profile |
|---|---|---|
| A | ATSDR Minimal Risk Level Worksheets | ATSDR Minimal Risk Level Worksheets |
| B | Literature Search Framework for [Substance x] | Literature Search Framework for [Substance x] |
| C | User's Guide | Framework for ATSDR's Systematic Review of Health Effects Data for [Substance x] |
| D | Quick Reference for Health Care Providers | User's Guide |
| E | Glossary | Quick Reference for Health Care Providers |
| F | Acronyms, Abbreviations, and Symbols | Glossary |
| G | Not applicable | Acronyms, Abbreviations, and Symbols |

The development of the toxicological profile begins with the front matter as indicated below. The writing and editing process for the toxicological profiles includes a minimum of three drafts before "camera-ready" status. At such time, the author shall use Attachment C to ensure a complete and accurate product before delivery to ATSDR.

# FRONT MATTER

The front matter of the profile contains, in order, the following:

- Title Page (Exhibit 2)
- Foreword (Exhibits 3A and 3B)
- Version History (Exhibits 4A and 4B)
- Contributors & Reviewers (Exhibits 5A and 5B)
- Contents (Exhibit 6)
- List of Figures (Exhibit 7)
- List of Tables (Exhibit 8)

Please refer to the exhibits, as hyperlinked, to view guidance for these sections.

# CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

The purpose of Chapter 1—Relevance to Public Health—is to provide to the reader an executive summary-type overview of the nature, manufacture, uses, general population exposures, and health effects of the substance under review. When confronted with making a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health, a public health professional shall be able to obtain from this chapter sufficient information about the profiled substance. The chapter assists in determining whether it is necessary to further evaluate the exposure scenario and detail the biologically significant health effects.

This chapter shall be limited to information presented in the other chapters of the toxicological profile. The presentation in this chapter shall be sufficient to provide a public health official with information that would be germane to making an initial assessment of a particular environmental scenario, but should not contain a level of detail that goes beyond this purpose. For a more detailed discussion, the reader can refer to the other chapters of the profile.

Chapter 1 shall be concise (10–15 pages depending on the substance being profiled), yet informative. This chapter shall have three sections:

1.1 Overview and U.S. Exposures
1.2 Summary of Health Effects
1.3 Minimal Risk Levels (MRLs)

This chapter may have four figures and one table. Exhibit 9, Chapter 1 Figures and Tables demonstrates these visuals.

## Targeted Profile Considerations

The chapter will begin with the boilerplate (below) in Section 1.1, **if and only if**, it is a targeted toxicological profile.

**ATSDR's *Toxicological Profile for [Substance X]* was released in [YEAR]. In order to update the literature in this profile, ATSDR conducted a literature search focused on health effects information as described in Appendix B. Chapters 2, 3, and 7 were revised to reflect the most**

**current health effects and regulations/guidelines data. In some cases, other sections of the profile were updated as needed or for consistency with the updated health effects data. However, the focus of the update to this profile is on health effects information.**

## 1.1 OVERVIEW AND U.S. EXPOSURES

Background information, chemical general statement, chemical uses, potential exposure pathways, and biomarkers of exposure.

Chapter 1 begins with a brief identification of the substance. For substances that are essential elements or nutrients, information to this effect should be included up front in the overview. Provide a brief discussion of the basis for essentiality, including the recommended dietary allowance and normal reference laboratory values.

- Chemical identification and uses? If banned, or no longer produced, say so and give some specifics dates.

  *"DDT is an organochlorine insecticide that has found a broad range of agricultural and nonagricultural applications in the United States and worldwide beginning in 1939. In 1972, DDT use was banned in the United States and in many parts of the world, except for use in controlling emergency public health problems. DDT is still used in certain parts of the world to control vector-borne diseases, such as malaria."*

- How is the general public likely to be exposed? What are the likely sources of exposure and important pathways for humans? Include ambient air and drinking water levels. If available, provide dietary intakes. Utilize data from large-scale studies or surveys (e.g., U.S. Environmental Protection Agency [EPA] Office of Drinking Water, Food and Drug Administration [FDA] market basket studies, etc.). Avoid data from individual studies; however, if this is the only information that is available, it is appropriate to include a range of values.

  *"The estimated dietary intake of PCBs for an average adult was about 0.03 µg/kg/day in 1979, but this had declined to <0.001 µg/kg/day by 1991."*

- What common lab tests (e.g., blood, urine) are available to determine whether exposure occurred? (i.e., biomarkers of exposure). Include normal baseline values, if available.

  *"The mean total mercury levels in whole blood and urine of the general population are approximately 1–8 and 4–5 μg/L, respectively. Recently, the International Commission on Occupational Health (ICOH) and the International Union of Pure and Applied Chemistry (IUPAC) Commission on Toxicology determined that a mean value of 2 μg/L was the background blood level of mercury in persons who do not eat fish. These blood and urine levels are "background" in the sense that they represent the average levels in blood in the general population and are not associated with a particular source for mercury. However, the intra- and inter-individual differences in these biomarkers are substantial, possibly due to dental amalgams (urine) and ingestion of contaminated fish (blood). Long-term consumption of fish is the source of nearly all of the methylmercury measured in the general population, and individuals in communities with high fish consumption rates have been shown to have blood levels of 200 μg/L, with daily intake of 200 μg mercury."*

If available, provide specific insights about the substance. For example, Chapter 1 for a substance such as lead should include its use as a gasoline additive and resulting dispersal throughout the environment; its use in solder and impact to canned foods and water pipes; folk remedies; lead-based paint, etc. Such a historical perspective would be informative to the reader but also indicate any present-day impacts (e.g., although many of these uses are banned, leaded paint is still prevalent in older housing and of special concern for young children who exhibit hand to mouth behavior. As lead-based paint deteriorates, it contributes to indoor lead dust that accumulates floors where children crawl; lead solder may still be of concern in older housing as well).

## 1.2 SUMMARY OF HEALTH EFFECTS

Begin this section with an introductory paragraph on the exposure type that provides the ***most notable*** health effects information. Are there epidemiological studies? What endpoints have been evaluated? Generalize the number of experimental studies identified. What type of exposure had the majority of studies and are there data for all routes of exposure (inhalation, oral, and dermal)? At the discretion of the chemical manager, differences in the health effects observed between pathways may be included in this section.

Develop and insert thermometer figure(s) of health effects (Exhibit 9, Figure 1-1) and discuss this figure by identifying the most sensitive effects. ***When a systematic review is completed***, indicate that a systematic review of these endpoints resulted in the following hazard identification conclusions (see Appendix C for additional information).

- X effects are [presumed, suspected, known] health effect for humans
- Y effects are [presumed, suspected, known] health effect for humans
- The data are inadequate to conclude whether Y effects will occur in humans

For the bullets, list as many endpoints as necessary. In the same order as the bullets, continue with non-numbered subsections of **ONLY** the **MAJOR** system/target organs and provide a more detailed summary. The following paragraph demonstrates a major effect discussion.

***Hepatic Effects.*** Results from numerous inhalation and oral animal studies support the identification of the liver as a presumed target in humans. Oral studies in rats and mice have found marked increases in serum enzymes (e.g., alanine aminotransferase [ALT], aspartate aminotransferase [AST], and sorbitol dehydrogenase) and centrilobular hepatocellular vacuolar degeneration in rats following acute exposure (Condie et al. 1983; Keegan et al. 1998; Lilly et al. 1994, 1996; Thornton-Manning et al. 1994).

Format this section in the following manner:

- The name of the health effect shall be in ***bold font and italicized***. Follow it with a period and two spaces.
- The discussion begins on the same line.

The discussion should be a general discussion of scientific findings based on the weight of evidence of studies.

## 1.3 MINIMAL RISK LEVELS (MRLS)

Begin this section with a brief paragraph that discusses whether MRLs were derived for each duration/exposure route and calls out the MRL summary table and refer the reader to Appendix A for greater detail, for example:

*Due to absence of inhalation studies, data were not available for deriving inhalation MRLs. The oral database was considered adequate for derivation of an intermediate-duration MRL for 1,2-diphenyhydrazine, but not for acute- or chronic-duration oral exposure. The MRL value is summarized in Table 1-1 and discussed in greater detail in Appendix A.*

MRLs shall only be part of the Relevance to Public Health Chapter and Appendix A, which contains the MRL worksheets. The second chapter on health effects will refer the reader to Appendix A for more information on the MRLs.

In this MRL section, describe the exposure route databases as either "limited" or "adequate." Develop, insert, and refer to the Summary of Sensitive Targets Figure(s) (see Exhibit 9, Figures 1-2 and 1-3) for each exposure route that has data. ***The data in these figures goes beyond the endpoints considered for MRLs. It shall include the lowest sensitive endpoints, even if these are cancer or death***. The data presented will be a judgement call based on which endpoints are most sensitive and reliable. A review of references may reveal the endpoints. Do not place this/these figure(s) in the appendix or in a worksheet. However, the chemical manager, in coordination with the contractor, has discretion on whether to include the figure(s) and how many figures to include. In instances when there are not enough data, the chemical manager may opt to not have these figures or limit them.

Create an MRL table (see Exhibit 9, Table 1-1) that includes the exposure duration, MRL, critical effect, point of departure, uncertainty factor, references, and units for each exposure type. Do not use bullets for the MRL(s).

# CHAPTER 2. HEALTH EFFECTS

## OVERVIEW

The sections within this chapter are:

2.1 Introduction
2.2 Death
2.3 Body Weight
2.4 Respiratory
2.5 Cardiovascular
2.6 Gastrointestinal
2.7 Hematological
2.8 Musculoskeletal
2.9 Hepatic
2.10 Renal
2.11 Dermal
2.12 Ocular
2.13 Endocrine
2.14 Immunological
2.15 Neurological
2.16 Reproductive
2.17 Developmental
2.18 Other Noncancer
2.19 Cancer
2.20 Genotoxicity
2.21 Mechanisms of Action *(needed only when there is/are similar mechanism(s) of action that occur in multiple health effects)*

Chapter 2 summarizes information regarding the health effects of the profiled substance. The intent is to provide information useful to community-level public health officials, toxicologists, and concerned citizens.

Authors of Chapter 2 should be familiar with both (1) the main literature search strategy and any supplemental search strategies used and (2) whether these searches are likely to have missed any relevant

resources. *Authors of Chapter 2 are responsible for instigating supplemental literature searches as necessary.* The need for a supplemental literature search may become obvious at any time during toxicological profile development.

The chapter consists of endpoint-specific discussions of available epidemiological and toxicological health effects data involving inhalation, oral, or dermal exposure to the profiled substance; data for other routes of exposure may also be included to address data gaps. The text is support by multiple figures and tables; see Exhibit 10, Chapter 2 Figures and Tables for examples of typical tables and figures. Number the tables sequentially from the beginning of the chapter.

Chapter 2 has three main parts: introduction (Section 2.1), discussion of health effects (Sections 2.2–2.20), and mechanism of action (either as Section 2.21 or along with the health effects in Sections 2.2–2.20). Below is information that is common to Sections 2.2–2.19 and the mechanisms of action discussions. In the numbered sections of this chapter are detailed descriptions of the appropriate content for each Chapter 2 section.

The purpose of Sections 2.2 through 2.19 is to discuss the health effects associated with the substance and the degree of certainty attached to that association. *Place emphasis on providing a synthesis and evaluation of the weight of evidence available on each topic, rather than a detailed description of the studies.* Scientifically prudent judgments, interpretations, and reasoned speculations are both appropriate and desirable.

Evaluate all epidemiological and animal studies for quality before including in the toxicological profile. Attachment A contains criteria for evaluating the quality of human studies and Attachment B contains criteria for evaluating the quality of animal studies. Report the quality studies in either human observation data table(s) or the Levels of Significant Exposure (LSE) tables and figures. Please see the example LSE tables and figures in Exhibit 10, Chapter 2 Figures and Tables (Tables 2-1, 2-2, and 2-3 and Figures 2-3 and 2-4).

- Include information describing reliable studies in the tables. Reserve text for conclusions, discussions, and explanations.
- Only provide information on health effects that increases the understanding regarding pathogenesis and mechanisms of toxicity.

- State when there is ambiguous data.

The text should consist of conclusions and supporting data to determine whether the effect occurs or not, and whether the data are reliable. Focus should be first on conclusions about occurrence in humans followed by results observed in other species. Compare and contrast effects observed in humans with those observed in laboratory animals (e.g., "These effects are consistent with the observed renal toxicity of [Substance x] in humans"). If data permit, discuss the differences in exposure levels that produced effects in humans and animals (i.e., whether the levels of effect are similar or whether there are large differences in susceptibility to adverse effects). Do not discuss extrapolation of animal studies to humans in this section; the appropriate place is Chapter 1 (Relevance to Public Health). Compare health effects and effect levels for different species and strains, if relevant. This, along with the toxicokinetic data in Section 3.1, should provide the basis for discussion of relevant species in Chapter 2 and data needs in Chapter 6 (Adequacy of the Database).

The text should not simply repeat information in LSE tables. Rather, the text should focus on a weight-of-evidence evaluation of whether the effect does or does not occur in humans or animals. *All studies in LSE tables and figures should be discussed (or at least referred to, if the substance is data-rich) in the text.* Discussion should include any reliable studies that provide qualitative data but were not included in the LSE table due to lack of quantitative data. The identified limitations should be included in the text and the supplemental document. Epidemiological studies and case reports often fall into this category. For data-poor substances, include a discussion of the strengths and weaknesses of the available data on a study-by-study basis. For data-rich substances, provide a more general discussion of the strengths and limitations of the available data, presenting an overall sense of the weight of evidence. Discuss whether different data sets are quantitatively similar, or whether no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) estimates vary widely between studies or species. If estimates vary widely, discuss likely or possible reasons.

When information suggests that an effect occurs but the dose-response relationship is unclear, discuss this issue in the text. For example, if there are two well-conducted studies, one that reports renal tubular necrosis at 25 and 250 ppm chronic exposure, and another that reports no effect at 50 and 100 ppm but records renal necrosis at 500 ppm chronic exposure, present that information in the text. Repeating every NOAEL for sections in which there are many data is unnecessary. Provide the lowest levels of effect within a duration and endpoint. Emphasize differences in effects (type and level of occurrence).

If there is relevant variation, discuss differences between species, strains, and sex in the response to the substance. Consider physiological differences for humans, animals, and strains when assessing effects. This is especially important in discussing strains used for cancer studies. Do not give the exposure regimen (e.g., hour/day or days/week) for every effect in the LSE tables and figures. Use terms such as "intermittent" and "continuous" for inhalation when describing the type of exposure. For oral studies, discuss differences in effects observed that might or can be attributed to the method of administration (food, capsule, water, gavage, bolus versus continuous) and vehicle used. The discussion of studies and levels in which effects occurred should flow smoothly (i.e., information should be used in context to support the conclusion). Feasible or well-accepted explanations of, or reasons for, differences in toxicity that are provided by the authors of the studies should be presented and the appropriate references cited. Use introductory statements to provide a summary of the information under each heading or a summary paragraph to provide conclusions on human and animal data (see the Benzene Toxicological Profile as an example).

Discuss reliable studies showing no effect or negative data for a specific end-point in which positive data exist. Where the database is limited, present studies that are not in the tables and figures and address the limitations. In cases where limitations exist, but the studies are still considered reliable enough to include in the tables and figures, provide a brief explanation of the limitations.

Provide in text any definition of concepts that are more difficult, medical terms, or medical conditions. In general, ATSDR prefers to avoid the use of nonstandard acronyms (that are not in Appendix G). In cases where the text would flow better, substitute certain terms that occur routinely in Chapter 2 with scientifically acceptable acronyms. When writing, define an acronym the first time it is used.

Do not discuss MRLs in Chapter 2, though you may refer the reader to Appendix A for more information.

Based on the available data, organize the health effects data into one of the following styles. One way to write this section is to organize toxicity information according to exposure route and/or exposure duration. If using this style, the order of the exposure routes are inhalation, oral, dermal, and other routes of exposure (if applicable). Alternatively, discuss the health effect data across routes or durations. This second style may be especially useful for chemical profiles that have the same health effects even though the route of exposure is different. This style is also useful for epidemiological studies that provide little information to indicate a specific route or level of exposure. Discuss all proposed changes in format or

anticipated problems with the categories with the chemical manager. In all cases, provide as much information as possible on effects in humans before discussing effects in animals.

Mention the incidence of effect and/or severity in the text for animal and human studies wherever confusion could arise regarding the interpretation of concentration or dose responses (e.g., if a concentration or dose response is in the text but not in the table, provide the evidence).

For profiles discussing more than one substance, the principal author must discuss the organization of text, tables, and figures with the chemical manager prior to completion of the first draft and the consistency review. The organizational scheme should allow for a clear and concise presentation of data. Consider the degree of information available for each form or compound, the categories in which the compounds are grouped (e.g., inorganic versus organic, cis versus trans, trivalent versus hexavalent), differences in toxicity, mode of action, and the relationship of the substances discussed (e.g., metabolites) when organizing and presenting material. Organize carefully to present the least confusion to the reader. Express doses in terms of the element and include the compound when reporting effect levels in the text, tables, and figures. For example, text should read, "Rats received 3.18 mg silver/kg/day as silver nitrate" rather than "Rats received 5 mg/kg/day of silver nitrate."

Place each study in one or several of the subsections listed in the Chapter 2 outline. Use the "Other Noncancer" heading to address effects on other tissues or organ systems. Where effects fall under more than one category, choose the most appropriate category in which to discuss the effects and cross-reference the discussion in the other categories. For example, dermal sensitization creates immunological and dermal effects. Address dermal sensitization as a dermal effect and cross-referenced to the immunological effects. Further, discuss sensitization reactions and the mechanism of the allergic response. If the chemical manager and principal author feel that doing so provides a better picture of the toxic potential of the profiled substance, the same effect can appear under more than one system category in the LSE tables and figures. However, developmental studies where exposures occurred under prenatal (only) or prenatal and postnatal conditions should include effects to the developing organism or offspring under "developmental" in the table and not under specific effect categories, such as neurological.

All numbered headings must appear, in order, even if there are no data. Use the following boilerplate when no data are located for major headings under health effects (e.g., death, hepatic, immunological effects).

**No studies were located regarding [health effect] in [humans and/or animals] after exposure to [Substance x].**

When no human or animal data are located for three or more major (numbered) headings in a row, collapse them in order.

**No studies were located regarding the following health effects in human or animals after exposure to [Substance x]:**

2.16 Reproductive
2.17 Developmental
2.18 Other Noncancer

### Epidemiological Studies

Summarize epidemiological studies that do not contain external exposure estimates in table(s). If it is possible to sort by health effect then, at the discretion of the chemical manager, split the human health effects table into smaller tables within each health effects section. At a minimum, the Health Effects in Humans tables will detail the reference, study population, exposure, and results. Include epidemiological studies that have reliable external exposure estimates in the LSE tables. In consultation with the chemical manager, use forest plots (Exhibit 10, Figure 2-2) to present risk ratios.

### Levels of Significant Exposure (LSE) Tables and Figures

LSE tables and figures summarize health effects data by exposure route, duration of exposure, and endpoint. The figures graphically illustrate levels of exposure associated with those effects. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs for less serious and serious health effects, or cancer effect levels (CELs). When data are available, create LSE tables for inhalation, oral, and dermal exposure route data. Create LSE figures for inhalation and oral exposure route data, only. The LSE tables and figures are computer-generated based on the supplemental document. See Attachment D for guidance on the supplemental document and Attachment E for guidance on the LSE tables and figures.

Exhibit 10, Chapter 2 Figures and Tables (Tables 2-1, 2-2, and 2-3 and Figures 2-3 and 2-4) show examples of LSE tables and figures. Include a User's Guide for these tables and figures (see Appendix D) in the profile.

### Classification of Endpoints as NOAELs, Less Serious LOAELs, or Serious LOAELs

The judgment of whether an endpoint is a NOAEL or a LOAEL depends in part upon the toxicity that occurs at other doses in the study or in other studies, and in part upon knowledge regarding the mechanism of toxicity of the substance. ATSDR defines the term "adverse health effect" in its Biennial Report, Volume II, to the Assistant Secretary for Health, U.S. Public Health Service. The quote from it is as follows: "a harmful or potentially harmful change in the physiologic function, physiologic state, or organ structure that may result in an observed deleterious health outcome [which] may be manifested in pathophysiologic changes in target organs, psychiatric effects, or overt disease" (Chou et al. 1998). Interpret this definition to indicate that any effect that enhances the susceptibility of an organism to the deleterious effects of other chemical, physical, microbiological, or environmental influences is adverse.

Authors must identify all LOAELs in LSE tables and figures as "less serious" or "serious." In general, a dose that evokes failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death) is a serious LOAEL. For additional assistance regarding differences between less serious and serious effects, refer to Sections 2.2–2.19.

ATSDR acknowledges that a considerable amount of judgment is required in this process and that, in some cases; there will be insufficient data to decide whether an effect will lead to significant dysfunction. The chemical manager can help decide such questions by bringing knowledge of ATSDR's policies to the discussion. ATSDR feels that distinguishing between less serious and serious helps the users of the document see at what levels of exposure "major" effects begin to appear, and whether the less serious effects occur at approximately the same levels as serious effects or at substantially lower levels of exposure. ATSDR recognizes the difficulties in the use of LOAELs for this purpose, particularly when species and dosing regimens are different and displayed doses are administered doses rather than absorbed doses. Nonetheless, ATSDR believes that there is sufficient merit in the approach to warrant an attempt at distinguishing between less serious and serious effects. *The classification of an effect as less serious or serious is also important because the Agency's practice is not to derive MRLs from serious LOAELs.*

When assessing the relevance (to humans) of observed effects, be aware of lesions that may be species related. For example, nephropathy and renal tumors in the male rat associated with alpha$_{2u}$-globulin is not always considered relevant to humans (see Attachment F).

Refer to the classification scheme below for more definitive guidance in classifying effects as NOAELs, less serious LOAELs, or serious LOAELs.

***No Adverse Effects***

- Weight loss or decrease in body weight gain of <10% unless there are modifying circumstances (e.g., statistical significance, prolonged exposure). For pups, any percent >5% or statistical significant decreases are an adverse effect.
- Changes in organ weight of non-target organ tissues that are not associated with abnormal morphologic or biochemical changes (see guidance below on "Assessment of Organ Weight Change").
- Increased mortality over controls that is not significant ($p>0.05$).
- Some adaptive responses (see “Adaptive Response Consideration,” below).

***Less Serious Effects***

- Reversible cellular alterations at the ultrastructural level (e.g., dilated endoplasmic reticulum, loss of microvilli, myelin figures) and at the light-microscopy level (e.g., cloudy swelling, hydropic degeneration, fatty change).
- Necrosis (dependent upon location, distribution, and magnitude), metaplasia, or atrophy with no apparent decrement of organ function.
- Serum chemistry changes (e.g., moderate elevations of ALT, AST). Organ weight change in known target organ tissue that is not associated with morphologic or biochemical alterations (see "Assessment of Organ Weight Change," below).
- Weight loss or decrease in body weight gain of 10–19% (assuming normal food consumption).
- Some adaptive responses (see “Adaptive Response Consideration,” below).

***Transitional Effects (Between Less Serious and Serious)***

Some effects (such as necrosis, atrophy, metaplasia, and serum chemistry alterations) could be classified as less serious or serious based on their reversibility, the organ affected, or the degree of associated dysfunction.

***Serious Effects***

- Death
- Clinical effects of significant organ impairment (e.g., convulsions, icterus, cyanosis)
- Morphologic changes in organ tissues that could result in severe dysfunction (e.g., marked necrosis of hepatocytes or renal tubules)
- Weight loss or decrease in body weight gain of ≥20% (assuming normal food consumption)
- Serum chemistry changes (e.g., major elevations of ALT, AST, blood urea nitrogen [BUN])
- Major metabolic effects (e.g., ketosis, acidosis, alkalosis)
- Cancer effects

Sections 2.2–2.19 present details for 18 health effects categories and the sections provide specific guidance that encourages consistent MRL derivation. Classifications of "less serious" or "serious" LOAELs effects appear in Tables 2-A– 2-R.

Use scientific judgment to determine if the lowest dose is a LOAEL when an adverse effect occurs at all dose levels, but it is only statistically significant at the low dose (and not the high dose). If all dose levels significantly affect an endpoint, but there is no clear dose-response relationship, the lowest dose level might be a LOAEL. It is possible that a lower incidence of intensity of effects (such as cancer or other histopathological lesions) at the high dose is due to increased mortality at the high dose and more animals may have developed the lesion had they lived, or the maximum response was already achieved with the lowest dose level. It is also possible that the effect may be due to a saturated enzyme or pathway at the high dose, or the effect could be a spurious result.

## Assessment of Organ Weight Change

Organ weight change is considered an adverse effect if observed in a known target organ. Organ weight change in a known target tissue is considered a minimal LOAEL if the response is associated with no

other alterations (morphologic, biochemical); organ weight change in this case may be representative of early-stage adverse effects. Increased liver weight following exposure to known hepatotoxins is a good example of such an effect. Changes in the organ weight of nontarget organ tissues that are not associated with morphologic or biochemical alterations are not considered to be adverse effects.

Increased lung weight may be the result of pulmonary edema, so do not classify this as "minimal." Similarly, decreased organ weight may be associated with severe atrophy with resulting deterioration of organ function (e.g., testicular, ovarian, or thymic atrophy). In assessing the nature and significance of changes in organ weight, use data provided in the study along with sound scientific judgment.

Do not base LOAELs on changes in absolute organ weight in the absence of body weight information. If body weight information is provided and there are no body weight effects, either absolute or relative organ weight changes can be used as the basis for LOAELs.

If body weight is reduced, then a decrease in absolute organ weight could reflect a body weight reduction or it could be an effect all by itself. In the absence of relative organ weight data, do not base a LOAEL on absolute organ weight change unless there is more supporting data. Decreases in both relative organ weight and absolute organ weight may indicate an adverse effect and be identified as a LOAEL. Identify if a body weight is reduced. An increased absolute organ weight with a reduced body weight is likely adverse and may be identified as a LOAEL. If absolute organ weights do not differ, but there is dose-related decrease in body weight, then there would appear to be an increase in relative organ weight in the treated group; do not report a LOAEL for the increased relative weight. Assess all changes in body weight, absolute organ weight, and relative organ weight before deciding whether an organ weight change is real.

### Adaptive Response Consideration

The normal cell is constantly modifying its structure and function in response to changing demands and stresses. Until these stresses become too severe, the cell tends to maintain a relatively narrow range of structure and function—so-called homeostasis. If the cell encounters excessive physiologic stresses or certain pathologic stimuli, it can undergo adaptation, achieving an altered but steady state while preserving the health of the cell despite continued stress. Cellular adaptation is a state that lies intermediate between the normal, unstressed cell and the injured, overstressed cell.

Resultant to chemical exposure, adaptive responses within organisms may occur at subcellular (biochemical) and cellular (structural) levels. Examples of adaptive "biochemical effects" would include induction of the cytochrome P-450 mixed-function oxidase system in the liver and other organs, as well as glutathione depletion/synthesis in the liver. Examples of structural adaptive responses would include atrophy, hypertrophy, hyperplasia, and metaplasia.

Adaptive responses can enhance an organism's performance as a whole and/or its ability to withstand a challenge. However, in some instances, delineating the boundary between an adaptive and toxic response is difficult. Adaptive responses in effect may result in changes that are beneficial or potentially detrimental to the host. Hypertrophy of skeletal muscle in response to an increased workload is an example of an adaptive change that benefits the host. Thus, the classification of adaptive responses as adverse or not adverse is based on judgement and often controversial.

Sometimes metaplasia is an adaptive response, but the predictive value for lesion progression and secondary effects on other organs is not always clear. The morphologic term metaplasia does not give any information concerning the biological significance of such a change. If metaplasia occurs in the pancreas, for example, squamous metaplasia of pancreatic ducts associated with exposure to a test substance does not interfere with pancreatic function. However, if squamous metaplasia occurs in the tracheal epithelium, it may interfere with normal respiratory defense functions. Therefore, assess the biological significance of an adaptive response on a case-by-case basis in conjunction with the chemical manager.

Even though adaptive responses may be protective, the potential alteration in function or metabolic activity may render the host more susceptible to injury following subsequent toxic exposure. According to guidance, “any effect that enhances the susceptibility of an organism to deleterious effects of other chemical, physical, microbiological, or environmental influences is considered adverse.” ATSDR will typically consider adaptive responses to be a minimally adverse LOAEL.

### Mechanisms of Action

It is at the chemical manager’s discretion on whether a mechanism of action section (2.21) is included as a separate section of the Health Effects Chapter. General guidance is that if the mechanism of action spans more than one health effect, it may be best to have it as a separate section to reduce redundancy in

writing. However, if a mechanism of action is specific to only one health effect, then discuss it under the health endpoint.

When considering mechanism of action text, please refer to the following guidance.

1. Consider the known mechanisms of metabolism, absorption, distribution, and excretion; if necessary, provide an overview of these mechanisms.
2. Discuss any substance reactions or physiological processes that lead to or comprise the mechanism(s) of toxic effect. Identify the parent compound and active metabolites.
3. If general information is known about the substance (i.e., chemical class, structural similarities, physical/chemical properties, etc.), the author should use reasonable conjecture to discuss a possible mechanism of action (based on the scientific literature).

## 2.1 INTRODUCTION

The introduction to Chapter 2 consists of mostly boilerplate information as indicated below.

**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 [Substance x]. 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. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.** *Do not include the last sentence for profiles that have a separate Mechanism of Action section (Section 2.21).*

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

**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 by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).**

**As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in**

**humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to [Substance x], but may not be inclusive of the entire body of literature.** *When necessary include:* **A systematic review of the scientific evidence of the health effects associated with exposure to [Substance x] was also conducted; the results of this review are presented in Appendix C.**

*Some profiles will have epidemiology tables in individual sections or a single table at the beginning of the health effects section. When necessary, adapt the wording in this section for what is included in it. For instance, include the following on human studies when all are in one table.* **Summaries of the human observational studies are presented in Table 2-1.** *Alternatively, mention the sections that contain epidemiology studies.* **Animal inhalation studies are presented in Table 2-2 and Figure 2-2, and animal oral studies are presented in Table 2-3 and Figure 2-3; no dermal data were identified for [Substance x].** *Please note that the figure and table numbering will need to be adapted if there are no human studies.*

**Levels of significant exposure (LSEs) 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 endpoint 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 endpoints. 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.**

Make the following modifications to the next boilerplate paragraph as applicable:

- Delete the paragraph if there are no cancer studies.
- Where CELs are provided in all tables, do not provide specific table and figure numbers.
- Provide table and figure numbers when CELs are available in some health effects, but not all.

**Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of [Substance x] are indicated in Table 2-3 and Figure 2-3.** *Please note that the figure and table numbering will need to be adapted if there are no human studies.*

**A User's Guide has been provided at the end of this profile (see Appendix [C or D]). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.** *Please note that the User's Guide can be either Appendix C or Appendix D, depending on whether there was a systematic review completed.*

After the boilerplate, include a paragraph detailing the database of studies (epidemiological and animal) and a reference to Figure 2-1, which is an Overview of the Number of Studies Examining [Substance x] Health Effects (see Exhibit 10, Figure 2-1). When creating this figure use the Chapter 2 subsection health effects (Sections 2.2–2.19). Identify the most frequently studied health endpoints in the paragraph/subtitle. Follow this with any limitations to the database (e.g., few dermal or inhalation studies). Thereafter, have bullets that describe the health effect endpoints (using bold text, a period, indenting, and beginning the description on the first line) of the human and animal studies. Use as many bullets as necessary to discuss these. For example:

- **Hepatic Endpoints.** Hepatic effects are a presumed health effect for humans based on limited evidence in humans and strong evidence in mice following acute inhalation exposure and in rats and mice following acute, intermediate, and chronic oral exposure. The liver effects include increases in serum enzymes, increases in liver weight, hepatocellular degeneration, and bile duct damage.

- **Immune Endpoints.** Immunological effects are a suspected health effect for humans based on moderate evidence in rats following acute and intermediate oral exposure. Decreased immune responses to stimulants were observed in rats.

- **Other Endpoints.** Alterations in body weight and gastrointestinal, hematological, ocular, endocrine, and neurological effects have also been observed in inhalation and/or oral exposure studies in laboratory animals; however, these do not appear to be sensitive targets of [Substance x] toxicity.

If additional introductory material is necessary (e.g., to clarify the topic of discussion if the official title of the profile is potentially confusing), a brief discussion should be added. *Note: The decision as to what should be included in the text versus in the official title of the profile is the responsibility of ATSDR.* For profiles that only discuss some of the topics mentioned in the title (e.g., when the title covers more than one substance or form of a substance (such as isomers, mixtures, and compounds), clearly identify which forms will be discussed and why. Add information of this nature into Section 1.1, Overview and U.S. Exposures, as well. The introduction to this section should also:

- Differentiate between forms of the substance or compounds discussed in the text.
- Define any acronyms or abbreviations used to represent the substance(s) or compound(s).
- Discuss any important information that the reader should consider in the overall evaluation of the database.
- Provide a brief discussion of essentiality, if relevant. Discuss different forms or compounds in an order that is constant throughout the profile.

For profiles that include more than one form of a substance or compound in the text, but the LSE tables and figures only contain certain forms, provide a statement explaining the reason for this after the boilerplate (see PCBs, page 34). If the decision affected all of Chapter 2, then discuss this under the boilerplate in Section 2.1. If the substance was administered in different formulations (e.g., cis- and trans- mixtures) and quite a few different formulations were used, give consideration to adding a table of formulations and their composition to the introduction.

## 2.2 DEATH

The section under "death" addresses any observed increased mortality in human or animal studies related to the chemical exposure. It also discusses the cause of death. If death was from cancer in human studies, state that retrospective mortality studies associating exposure with cancer are in Section 2.19.

Always classify deaths as "serious LOAELs;" these do not have NOAELs. If an article does not provide data on the mortality of animals, do not discuss this article in the death section. If an article provides data regarding the mortality of animals and no animals died, write this as this "no deaths occurred." For studies that provide a range for an $LD_{50}$, use the lowest dose for the LSE table and figure LOAEL (do not put in ranges).

## 2.3 BODY WEIGHT

Body weight effects are changes in terminal body weight and changes in body weight gain for adult animals (in a non-reproductive study). Base the NOAEL and LOAEL on significant differences in terminal body weight between controls and treated animals. In a reproductive study, discussing changes in maternal body weight gain is appropriate. Weight loss or decreased body weight of 10–19% (assuming normal food consumption) is to be classified as a "less serious LOAEL" and decreases of ≥20% are to be classified as a "serious LOAEL;" a body weight decrease of <10% is not considered to be adverse effect. If a decrease in body weight is accompanied by decreased food consumption in a feeding study, then neither effect is considered an adverse effect.

Please consult the Table 2-A, below, for examples of body weight effect endpoints and their classification as "less serious" or "serious."

**Table 2-A. Body Weight Effect Endpoints[a]**

| Effect (compared with controls) | Less serious | Serious |
|---|---|---|
| Decreased body weight (10–19%) with normal food consumption | + | |
| Decreased body weight (>20%) with normal food consumption | | + |

[a]The MRL workgroup will consider exposure duration, animal functionality, and other factors in deciding the level of seriousness.

## 2.4 RESPIRATORY

Respiratory effects include any effects related to the respiratory system and its functioning. This includes effects to the lung, trachea, and nasal cavity. Examples of specific respiratory effect endpoints are listed and classified as less serious or serious in Table 2-B.

**Table 2-B. Respiratory Effect Endpoints[a]**

| Effect | Less serious | Serious |
|---|---|---|
| Respiratory distress symptoms<br>Tachypnea (rapid, shallow respiration)<br>Dyspnea (labored breathing)<br>Wheezing | + | + |
| Altered lung function (changes in respiratory volume, forced vital capacity, etc.) | + | |
| Pulmonary edema | | + |
| Lung congestion | + | |
| Hemothorax (blood in the pleural cavity) | | + |
| Bronchitis | + | + |
| Rales (abnormal respiratory sounds) | | + |
| Lung or nasal irritation | + | + |

[a]The MRL workgroup will consider exposure duration, animal functionality, and other factors in deciding the level of seriousness.

## Innovations

Computational fluid dynamics models of both human and laboratory animal respiratory systems provide insight into the equivalent doses species absorb at the same concentrations. These models can also predict aerosol deposition if the particulates are well characterized.

Differences in the gene expression of respiratory cells are being explored and may better explain effects, metabolism, and species differences when these systems are challenged with xenobiotics. In the future, biomarkers for damage (elicited macrophages, enzyme levels) may also provide more sensitive endpoints for risk assessment in the future.

### 2.5 CARDIOVASCULAR

Cardiovascular effects include any effects related to the heart and circulatory system and its functioning. Examples of specific cardiovascular effect endpoints are listed and classified as less serious or serious in Table 2-C.

**Table 2-C. Cardiovascular Effect Endpoints[a]**

| Effects/tests | Less serious | Serious |
|---|---|---|
| Altered blood pressure (increased or decreased) | + | + |
| Bradycardia (slowed heart rate) | + | + |
| Tachycardia (rapid resting heart rate) | + | + |
| Cardiac arrest | | + |
| Myocardial edema | | + |
| Myocarditis | | + |
| Long or short Q-T interval | | + |
| Palpitations | + | + |

[a]The MRL workgroup will consider exposure duration, animal functionality, and other factors in deciding the level of seriousness.

## 2.6 GASTROINTESTINAL

Gastrointestinal effects include any effects related to the digestive system. This includes effects of the esophagus, stomach, and small and large intestines. Pancreatic effects may be under gastrointestinal or endocrine. Examples of specific gastrointestinal effect endpoints are listed and classified as "less serious" or "serious" in Table 2-D.

**Table 2-D. Gastrointestinal Effect Endpoints[a]**

| Effect | Less serious | Serious |
|---|---|---|
| Diarrhea[b] | + | + |
| Emesis (vomiting)[b] | + | + |
| Ulceration; penetrates the muscularis mucosae | | + |
| Constipation | + | |
| Nausea | + | |

[a]The MRL workgroup will consider exposure duration, animal functionality, and other factors in deciding the level of seriousness.
[b]For diarrhea and emesis, these effects could be serious if they occur for a long length of time.

## Innovations

Though not completely understood, gut microbes play an important role in xenobiotic metabolism. Whether these microbes are biotransforming xenobiotics to active (harms DNA and other matter) or inactive metabolites, the body must deal with the consequences. Additionally, these bacteria may play a role in destroying mutagenic metabolites. The use of germ free rodents is valuable in studying how gut

microflora modulate toxicants. Current and future research in this field may continue to elucidate the differences between animals and humans and refine risk assessment in so doing.

## 2.7 HEMATOLOGICAL

Hematological effects include effects related to blood chemistry and hematology. Examples of specific hematological effect endpoints are listed and classified as "less serious" or "serious" in Table 2-E.

**Table 2-E. Hematological Effect Endpoints[a]**

| Effect | Less serious | Serious |
|---|---|---|
| Anemia | + | + |
| Cyanosis | + | |
| Erythrocytopenia (erythropenia) | + | + |
| Altered hemoglobin (increased or decreased) | + | |
| Altered hematocrit (increased or decreased) | + | |
| Leukopenia | | + |
| Thrombocytopenia | | + |
| Increased erythrocytes | + | + |
| Bone marrow hyper or hypoplasia | + | |
| Decreased bone marrow cellularity | + | |

[a]The MRL workgroup will consider exposure duration, animal functionality, and other factors in deciding the level of seriousness.

## 2.8 MUSCULOSKELETAL

Musculoskeletal effects are those related to the muscles and skeletal system and its functioning. Examples of specific musculoskeletal effect endpoints are listed and classified as "less serious" or "serious" in Table 2-F.

**Table 2-F. Musculoskeletal Effect Endpoints[a]**

| Effects/tests | Less Serious | Serious |
|---|---|---|
| Loss of muscle tone or strength | + | + |
| Muscular rigidity | | + |
| Muscular atrophy | | + |
| Arthritis | + | |
| Altered bone density | + | |
| Arthralgia (joint pain) | + | |

[a]The MRL workgroup will consider exposure duration, animal functionality, and other factors in deciding the level of seriousness.

## 2.9 HEPATIC

Hepatic effects are those related to the liver and gallbladder and their functioning.

In the liver, exposure to several substances may result in adaptive changes characterized by induction of the mixed function oxidase (MFO) enzyme system and proliferation of smooth endoplasmic reticulum; other changes that may include hepatocellular hypertrophy, cytoplasmic eosinophilia, increased organ weight, and liver enlargement. Modifications occurring in the MFO system because of the adaptive response may potentiate or inhibit toxic responses to other exogenous substances. Agents that induce chemical metabolizing enzyme systems generally tend to potentiate hepatic injury produced by compounds such as chloroform, carbon tetrachloride, or halothane. For ATSDR, this is an especially important concept to consider because, in addition to the specific chemical causing adaptive changes, there is the potential for exposure to many other substances at National Priority List (NPL) sites.

Sometimes, it is difficult to tell whether an effect is physiologically adaptive or toxic (e.g., functional impairment). The following guidance provides general direction for assessing hepatic adaptive responses; although this guidance will be appropriate in most cases, there may be exceptions. Use the following criteria for assessing the biological significance of adaptive responses in the liver. Consider biochemical changes characterized by MFO induction along with morphologic changes of hepatocellular hypertrophy and proliferation of smooth endoplasmic reticulum. Other supportive changes include increased organ weight, hepatic enlargement, and accentuated cytoplasmic eosinophilia. Please consult the section above that discusses ‘Adaptive Response Consideration’ for guidance on assessing the severity of adaptive changes in the liver.

Below is a list (Table 2-G) of specific hepatic effect endpoints with their classification of less serious or serious.

**Table 2-G. Hepatic Effect Endpoints[a]**

| Effect | Less serious | Serious |
|---|---|---|
| Altered liver enzymes | + | + |
| Hepatomegaly (enlargement of the liver) | + | |
| Porphyria (disturbance of porphyrin metabolism) | + | |
| Hepatocyte vacuolization | + | |
| Congestion of liver | + | + |
| Hepatic necrosis | | + |
| Cirrhosis | | + |
| Jaundice | + | + |
| Gall bladder effects | + | + |
| Fatty changes in liver | + | + |
| Hepatocellular degeneration | + | + |

[a]The MRL workgroup will consider exposure duration, animal functionality, and other factors in deciding the level of seriousness.

## 2.10 RENAL

Renal effects include any effects related to the kidneys and urinary bladder and their functioning.

Table 2-H lists specific renal effect endpoints into less serious or serious LOAEL classifications.

**Table 2-H. Renal Effect Endpoints[a]**

| Effect | Less Serious | Serious |
|---|---|---|
| Decreased urine volume (not associated with decreased water intake) | + | |
| Hematuria | | + |
| Hemoglobinuria | | + |
| Altered urinary creatinine | + | |
| Proteinuria (excess of serum proteins in urine) | + | |
| Decreased urine volume (not associated with decreased water intake) | + | |
| Urinary bladder effects | + | + |
| Altered BUN | + | |
| Renal tubular degeneration | + | + |
| Renal tubular casts | + | |

**Table 2-H. Renal Effect Endpoints[a]**

| Effect | Less Serious | Serious |
|---|---|---|
| Fatty degeneration of tubules | + | |
| Tubular necrosis | + | + |

[a]The MRL workgroup will consider exposure duration, animal functionality, and other factors in deciding the level of seriousness.

When considering renal effects, consult Attachment F as the male rat differs from humans (female rats, mice, or other species) in incidence of nephropathy and renal neoplasia.

## 2.11 DERMAL

Dermal effects include those related to the skin and its functioning. Address dermal sensitization, that could be considered both an immunological effect and a dermal effect, under "dermal effects" and cross-reference it to immunological effects. Discuss sensitization reactions and the mechanism of the allergic response.

Mention dermal effects that are not a true systematic effect (e.g., irritation resulting from substance). State that the effects were due to direct contact of the skin with the vapor. Present the data in the dermal LSE table and clearly state that the animal was exposed to the substance via air.

In the table below (Table 2-I) are specific dermal effect endpoints and their classification into serious or less serious LOAELs.

**Table 2-I. Dermal Effect Endpoints[a]**

| Effect | Less serious | Serious |
|---|---|---|
| Dermatitis | + | |
| Edema (swelling) | + | |
| Erythema (redness of skin) | + | |
| Hyperkeratosis (thinking of outer layer of skin) | + | |
| Ulceration | + | |
| Itching | + | |
| Rash | + | |
| Acne | + | |

**Table 2-I. Dermal Effect Endpoints[a]**

| Effect | Less serious | Serious |
|---|---|---|
| Necrosis of skin | + | + |
| Acanthosis (thickening of inner layer of skin, characterized by dark discoloration) | + | |

[a]The MRL workgroup will consider exposure duration, animal functionality, and other factors in deciding the level of seriousness.

## 2.12 OCULAR

Ocular effects are those related to the eyes and their functioning. See the example effects (Table 2-J) below and their classification into serious or less serious LOAELs.

**Table 2-J. Ocular Effect Endpoints[a]**

| Effect | Less serious | Serious |
|---|---|---|
| Blindness | | + |
| Cataracts | | + |
| Myopia (nearsightedness) | + | |
| Lacrimation/tearing | + | |
| Exophthalmia (protruding eyeballs) | | + |
| Conjunctivitis | + | |
| Irritation | + | |
| Discharge or exudate | + | |

[a]The MRL workgroup will consider exposure duration, animal functionality, and other factors in deciding the level of seriousness.

## 2.13 ENDOCRINE

Endocrine effects involve ductless hormone-secreting glands that includes the hypothalamus, pituitary gland, adrenal glands (including the adrenal cortex and medulla), thyroid glands, parathyroid glands, and the pancreatic islets. Examples of endocrine effects include adrenal cortical atrophy, pituitary hypoplasia, thyroid hyperplasia, and adrenal calcification. Functional changes involving hormonal deficiency or excess (e.g., deficiency of T3 and T4 [hypothyroidism], excess of cortisol [hyperadrenocorticism], and excess parathyroid hormone [hyperparathyroidism]) also fall in this category. Although the ovaries and testes have endocrine functions, for reasons of consistency always categorize effects involving these organs as reproductive effects.

The pancreas is both an endocrine and an exocrine organ. As an exocrine organ, it is considered to be part of the digestive system (secretion of digestive enzymes via the pancreatic duct into the duodenum), and when functioning in hormone secretion (e.g., insulin) it is an endocrine organ. Discuss the pancreas under "Endocrine Effects" unless the effect involves the external secretions of digestive enzymes in which case discuss this under "Gastrointestinal Effects."

Please consult Table 2-K for examples of endocrine effect endpoints classified as less serious or serious.

**Table 2-K. Endocrine Effect Endpoints[a]**

| Effect | Less serious | Serious |
|---|---|---|
| Alternations of serum adrenocorticotropic hormones | + | + |
| Decreased thyroid, pituitary or adrenal function | + | |
| Goiter (enlargement of thyroid gland) | + | |
| Thyroid hyperplasia | + | + |
| Pituitary hypoplasia | + | + |
| Adrenal calcification | + | + |
| Adrenal cortical atrophy | + | + |
| Pancreas effects | + | + |

[a]Evaluate endpoints based on clinical, biochemical, and morphologic alterations to determine severity.

## 2.14 IMMUNOLOGICAL

The immune system is a cellular complex that forms the basis of the body's defenses against both biological and chemical exogenous substances. Lymphoreticular effects are morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus. Cells that mature or reside in these tissues, such as various populations of lymphocytes and non-lymphoid cells (phagocytes), participate in immune responses. Nevertheless, lesions involving these tissues *may* or *may not* be associated with functional changes in the immune response.

Examples of lymphoreticular effects are lymphoid aplasia of the thymus, lymphoid hypoplasia of the lymph nodes, lymphoid hyperplasia of the spleen, hemosiderosis of the spleen, and lymph node histiocytosis. Immunological effects, in contrast, are functional changes in the immune response. These include a broad spectrum of effects, such as anaphylaxis, decreased cell-mediated immunity, autoimmunity, altered complement activity, altered T-cell activity, decreased mitogen response, and increased susceptibility to infection.

Provide sufficient supportive information identifying a substance as immunotoxic. Many substances impart an immunological effect for multiple routes and/or durations of exposure. Thoroughly assess the animal toxicological information to make comparisons with potential immunological effects in humans.

Less serious and serious LOAELs are listed for immunological and lymphoreticular effect endpoints, in Table 2-L.

**Table 2-L. Immunological and Lymphoreticular Effect Endpoints[a]**

| Effect | Less serious | Serious |
|---|---|---|
| Altered complement activity | + | |
| Altered macrophage activity | + | |
| Altered resistance to tumor susceptibility by sarcoma virus | + | |
| Altered T-cell activity | + | |
| Chronic urticaria | + | |
| Decrease in lymph node cortical lymphoid cells[b] | + | |
| Decreased mitogen response | + | |
| Decreased skin graft survival time | + | |
| Degeneration or necrosis in immunologic components | | + |
| Delayed rejection time of foreign skin graft (non-self) | + | |
| Enhanced natural killer cell (N.C.) activity | + | |
| Humoral or cell-mediated immune response to sheep red blood cells | + | |
| Increased susceptibility to infection | + | |
| Pemphigus vulgaris | | + |
| Reduced delayed-type hypersensitivity | + | |
| Reduced humoral antibody (I.G.) production | +[c] | + |
| Severe reduction in cell-mediated immune response | | + |
| Suppression of lymphoproliferative response to T-cell mitogen | + | |
| Thymus or spleen lymphoid atrophy[b] | | + |
| Lymphoid hyperplasia of lymph node or spleen[b] | + | |
| Histiocytosis of lymph node or spleen[b] | + | |
| Albumin/globulin (A/G) ratio | + | |

[a]The MRL workgroup will consider exposure duration, animal functionality, and other factors in deciding the level of seriousness.
[b]These changes are lymphoreticular effects because they represent morphological alterations in affected tissues; immunological effects imply a functional change in the immune response.
[c]The effect can be less serious or serious depending upon the degree.

Lymphoreticular effects may or may not be associated with functional changes in the immune response. Address potential functional alterations when morphological changes are present in lymphoreticular tissues.

## 2.15 NEUROLOGICAL

Neurological effects occur from a change in the structure or function of the central or peripheral nervous system by a biological, chemical, or physical agent. These effects may be permanent or reversible and produce neuropharmacological or neurodegenerative properties. They are the result of direct or indirect actions on the nervous system by a neurotoxicant. Categorize neurological effects as motor, mood and personality, sensory, cognitive, neurochemical, neurophysiologic, or neuropathologic. See Table 2-M, below, for examples of specific endpoints and their classification as less serious or serious.

### *Assessment of AChE Activity Inhibition*

Become familiar with Attachment G, Assessing Cholinesterase Activity Inhibition. Follow these guidelines in classifying the neurological health-effect endpoint of "inhibition of acetylcholinesterase activity" (in erythrocytes and/or brain).

- <20% enzyme inhibition is defined as a NOAEL
- 20–59% inhibition of enzyme activity is classified as a less serious LOAEL
- Enzyme activity inhibition of 60% or greater is classified as a serious LOAEL

However, in addition to these guidelines, consideration should be given to associated clinical symptoms (Tables 2-M and 2-N, below).

- Classify as a serious LOAEL any clinical effects that are consistent with moderate or severe poisoning, even if the degree of inhibition of acetylcholinesterase activity is <60%.

When there is <60% enzyme inhibition, specify the clinical effects that lead to this classification (as well as the percentage of enzyme inhibition) in Chapter 2 text and LSE tables.

Always classify inhibition of acetylcholinesterase activity of ≥60% as a serious effect. It is not appropriate to base LOAELs on the inhibition of pseudocholinesterase activity (plasma or serum

cholinesterase), but discuss the effect in the supplemental document study results. Discuss inhibition of erythrocyte acetylcholinesterase under neurological effects and not hematological effects.

Please reference the below tables for health effect endpoints resulting in serious or less serious classifications.

**Table 2-M. Neurological Effect Endpoints[a]**

| Category and symptoms | Less serious | Serious |
|---|---|---|
| Motor | | |
| Activity changes (sedation, anesthesia, somnolence, hyper-/ hypoactivity, decreased locomotor activity) | +[a] | + |
| Convulsions | | + |
| Lack of coordination (unsteadiness, intoxication, decreased swimming response ability, decreased psychomotor performance, ataxia) | +[a] | + |
| Paralysis | | + |
| Reflex abnormalities | +[a] | + |
| Tremor, twitching (muscular spasm) | | + |
| Weakness | + | |
| Behavioral changes | | |
| Excitability | + | |
| Delirium | | + |
| Depression | +[a] | +[a] |
| Hallucinations | | + |
| Irritability | + | |
| Nervousness, tension | + | |
| Restlessness | + | |
| Sleep disturbances | + | |
| Sensory | | |
| Auditory disorders | | + |
| Equilibrium changes | +[a] | + |
| Pain disorders | +[a] | + |
| Tactile disorders | +[a] | + |
| Vision disorders | | + |
| Cognitive | | |
| Confusion | | + |
| Learning impairment (decreased operant behavior) | +[a] | + |
| Memory problems | | + |
| Speech impairment | | + |

**Table 2-M. Neurological Effect Endpoints[a]**

| Category and symptoms | Less serious | Serious |
|---|---|---|
| General | | |
| Depression of neuronal activity | +[a] | + |
| Fatigue (lethargy) | + | |
| Loss of appetite | + | |
| Narcosis, stupor | | + |
| Nerve damage | | + |
| Prostration | | + |
| Other integrative effects (hand/eye coordination) | +[a] | + |
| Unconsciousness | | + |
| Neurochemistry | | |
| Changes in cAMP, cGMP, catecholamine, dopamine (decreased enzyme activity) | +[a] | + |
| Changes in GFA protein | | + |
| Decreased neuronal membrane lipids | +[a] | + |
| Decreased metabolism (glucose utilization) | +[a] | + |
| Neurophysiology | | |
| Altered EEG | +[b] | + |
| Salivation | + | |
| Neuropathology | | |
| Neuropathy, axonopathy demyelination, focal gliosis, cerebellar lesions, cerebellar degeneration, hemorrhage | | + |

[a]Classification considers symptom duration, reversibility, and severity; serious if >60% inhibition or accompanied by clinical effects.
[b]No other clinical effects.

Source: Anger 1986

**Table 2-N. Neurological Effects: Clinical Symptoms of Varying Severity of Organophosphorus Poisoning and Corresponding AChE[a] Value**

| Level of poisoning | Clinical symptoms |
|---|---|
| Mild<br><60% reduction of ACHE | Weakness, headache, dizziness, diminished vision, salivation, lacrimation, nausea, vomiting, lack of appetite, stomachache, restlessness, myosis, moderate bronchial spasm; convalescence in 1 day |
| Moderate<br>60–90% reduction of ACHE | Abruptly expressed general weakness; headache; visual disturbance; excess salivation; sweating; vomiting; diarrhea; bradycardia; hyperopia; stomachache; twitching of facial muscles; tremor of hand, head, and other body parts; increasing excitement, disturbed gait, and feeling of fear; meiosis nystagmus; chest pain; difficult respiration; cyanosis of the mucous membrane; chest crepitation; convalescence in 1–2 weeks |

**Table 2-N. Neurological Effects: Clinical Symptoms of Varying Severity of Organophosphorus Poisoning and Corresponding AChE[a] Value**

| Level of poisoning | Clinical symptoms |
|---|---|
| Severe | Abrupt tremor, generalized convulsions, psychic |
| 90–100% reduction of ACHE | Disturbances, intensive cyanosis of the mucous membrane, edema of the lung, coma; death from failure |

[a]Acetylcholinesterase activity.

Source: Kaloyanova and El Batawi 1991

## 2.16 REPRODUCTIVE

The reproductive effects sections cover effects resulting from exposures during the interval from the generation of parental germ cells to conception up through implantation of the offspring.

ATSDR defines reproductive toxicity as a dysfunction induced by a chemical, physical, and/or environmental agent that affects the process of gametogenesis from its earliest stage to implantation of the conceptus in the endometrium.

Discuss maternal resorptions, decreased fecundity and fertility under this section. Discuss other maternal effects under the health effect that occurred in the dam (e.g., neurological, dermal, ocular).

Table 2-O details examples of reproductive endpoints and classifies them as "less serious" or "serious" LOAELs.

**Table 2-O. Reproductive Effect Endpoints[a]**

| Effects | Less serious | Serious |
|---|---|---|
| Abnormal sperm[b] (morphology, count, motility) | | + |
| Abortions | | + |
| Atrophy | | + |
| Decreased fertility[c] | + | + |
| Decreased litters | | + |
| Decreased spermatogenesis[b] | + | + |
| Degeneration of epididymides[c] | + | + |
| Disrupted spermatogenesis | | + |
| Females: no reproduction | | + |
| Maternal toxicity | | + |

**Table 2-O. Reproductive Effect Endpoints[a]**

| Effects | Less serious | Serious |
|---|---|---|
| Increased estrus | | + |
| Irreversible histological change in testes | | + |
| Ovarian dysfunction | | + |
| Ovary weight change | + | |
| Postimplantation loss | | + |
| 50% reduction in number of offspring | | + |
| Sterility | | + |
| Testicular atrophy | | + |
| Testicular degeneration | + | + |
| Granuloma epididymides[d] | + | + |
| Tubular degeneration | | + |
| Tubule edema | + | |
| Vaginal bleeding | + | |

[a]The MRL workgroup will consider exposure duration, animal functionality, and other factors in deciding the level of seriousness.
[b]There is a certain degree of variability between normal/less serious/serious; e.g., a normal human semen specimen has a volume of 3–4 mL, a sperm count of $30x10^6$, and 80% morpho-logically normal and motile spermatozoa.
[c]The effect can be less serious or serious depending upon the degree.
[d]This condition can be considered serious because it can lead to progressive fibrosis.

Include reproductive effects that are also considered developmental effects in the LSE table with the other developmental effects. Present changes in maternal body weight and body weight in males when the study is reproductive. Testicular effects can often be secondary to decreased body weight gain.

## 2.17 DEVELOPMENTAL

The developmental effects section will include developmental health effects *on the offspring* resulting from exposures to *parental germ cells* (formed when the parents were *in utero*), *the conceptus through the pre-implantation blastocyst stage*, and all subsequent developmental stages up through 18 years of age in humans or sexual maturity in animals. It will discuss exposures that might result in developmental health effects *to the embryo or later stages of life*.

Deaths at any stage before implantation will still only be discussed in the reproductive section because such outcomes do not affect the health of the embryo, fetus, infant, child, adolescent, adult, or adult's offspring.

This changed happened because animals show that exposure of parental germ cells or pre-implantation conceptuses to certain mutagens can result in structural malformations in late-stage fetuses and neonates. These malformations commonly cause an expectedly high death rate in littermates (Generoso et al. 1990; Rutledge et al. 1992, 1997; Spielmann and Vogel 1989). In theory, either genetic or non-genetic damage (such as changes in DNA methylation or disruption of the expression of key developmental regulatory molecules) to the parental germ cells or the pre-implantation conceptus could result in functional or structural defects in the offspring. Mutations and childhood cancer are also theoretical outcomes of pre-implantation damage. However, the most susceptible period for the induction of structural malformations is organogenesis.

Determining whether exposure causes developmental effects or just causes health effects during childhood is sometimes difficult. If this determination is unclear, discuss the effect in the developmental effects section or the appropriate other section, and include a cross-reference.

*Discuss topics, below, when information is available.*

- What observed health effects, if any, are in children? Are there health effects observed in adults that are also of potential concern in children? Do adults exposed as children have any health effects? What are the observed health effects in immature animals from embryos up through maturity? Do children and immature animals exhibit the same types of health effects as adults? Do the doses that cause effects in children and adults or in immature and adult animals consistently differ? How? Consider any epidemiologic studies that focus on the consequences of exposures before age 18 years, even if the effects are not evident until adulthood.
- Does the toxicant alter the developmental process? Discuss data on children and animals. Remember that the reproductive, immune, and nervous systems especially continue to develop after birth. Developmental problems may include functional neurological development, such as learning deficits and deficits in social behavior. Consider endocrine disruption of developmental processes and any epidemiologic studies that focus on the consequences of exposures before age 18 years, even if the effects are not evident until adulthood. How does the effective dose or range of effective doses in children compare with that in adults when there is an alteration in the developmental process caused by a toxicant? Discuss developmental effects only occurring near maternally toxic doses. For more assistance in interpreting developmental, see Attachment H. Age at Weaning and Sexual Maturity for Common Laboratory Species and Humans and Attachment I. Historical Background Rates for Various Developmental Outcomes.

- Are there any studies linking pre-conception exposure of either parent to germ line mutations, developmental defects, childhood cancer, or other health effects?

Developmental toxicity is any adverse effect on the developing organism from implantation, through prenatal development, or postnatally to the time of sexual maturation. These effects can result from exposure of either parent prior to implantation or exposure during prenatal and postnatal development. Health effects seen in the developing organism prior to sexual maturity are secondary to adverse developmental effects.

To distinguish between developmental and reproductive effect evaluate the conceptus after implantation. ATSDR defines reproductive toxicity as dysfunction induced by a chemical, physical, or biologic agent that affects the processes of gametogenesis from its earliest stage to implantation of the conceptus in the endometrium.

Categorize developmental effects as structural abnormalities, altered growth, functional deficiencies, and death of the developing organism. Examples of specific endpoints within these effect categories are listed and classified as serious or less serious in Table 2-P.

**Table 2-P. Developmental Effect Endpoints[a]**

| Effect | Less serious | Serious |
|---|---|---|
| **Structural abnormality** | | |
| Delayed ossification | + | |
| Skeletal anomalies (Spina bifida, cleft palate, fused ribs, webbed feet) | | + |
| Skeletal anomalies (ring tail[b], supernumerary ribs, wavy ribs) | + | |
| Visceral anomalies (heart defects) | | + |
| Ultrastructural changes[c] | + | |
| **Altered growth** | | |
| Alteration in offspring organ weight[d] | + | |
| Alteration in offspring body weight[d] | + | |
| Change in crown-rump length[d] | + | |

**Table 2-P. Developmental Effect Endpoints[a]**

| Effect | Less serious | Serious |
|---|---|---|
| **Functional deficiency** | | |
| Immunosuppression in offspring (see Table 2-L) | | + |
| Systemic effects | | + |
| Behavioral abnormalities (see Table 2-M) | | + |

*The MRL workgroup will consider exposure duration, animal functionality, and other factors in deciding the level of seriousness.
[b]Ringtail: a disease of obscure etiology in suckling rats in which one or more fine constricting ring occurs at some place along the length of the tail.
[c]Changes in cellular structure (cellular organelles).
[c]Could be considered serious, depending on the degree of severity.

Structural abnormalities include malformations and variations such as anomalies, deformations, or aberrations. Use the term "teratogenicity" to describe permanent structural abnormalities that may adversely affect survival, development, or function. The induction of altered growth occurs at any stage of development and may be reversible, or may result in permanent change. Changes in the mother (dam or doe) can influence or confound interpretation of altered growth in the fetus or neonate. In general, altered growth seen in conjunction with adverse effects in dams, such as decreased weight gain is an adverse effect in the fetus or neonate.

Adverse effects observed in offspring (exposed *in utero*) prior to sexual maturity are developmental effects. At 21–28 days, weaning occurs in mice and rats and follows with a juvenile phase. Full sexual maturity occurs several weeks after weaning (at about 60–70 days). Effects noted in the juvenile phase after pre- and postnatal exposure is a developmental effect. For example, if animals are exposed *in utero* and grow up into young adulthood (45–60 days), a change in grip strength is an adverse effect from prenatal exposure and a developmental effect. Furthermore, a change in grip strength on day 300 (after sexual maturity) for these animals is an, adverse developmental effect because exposure occurred *in utero*.

However, a different categorization occurs when considering continuous exposure experiments (multi-generation studies or when offspring exposed postnatal after sexual maturity), developmental effects and other effects at the point of sexual maturity. In a multi-generation study, at the time when the F1 animals mate and produce the next generation, ATSDR uses the other health effect category (e.g., reproductive, neurological, etc.).

Please consult Table 2-P, for examples of developmental effect endpoints and their classification as "less serious" or "serious". The list in Table 2-P is by no means all-inclusive.

ATSDR defines functional deficiency as alterations or delays in functional competence of the organism or organ system following exposure to an agent during critical periods of development pre- and postnatal. Examples are:

- **Immunosuppression (suppression of natural immune responses) in offspring.** Immune dysfunction leading to increased risk of infectious diseases or to development of neoplasia, autoimmune disorders, or allergies. Any of the immunological endpoints listed in Table 2-L apply.
- **Behavioral tests in offspring**. Use tests listed in Table 2-M to assess newborns' behavioral abnormalities. Measuring swimming behavior is a common technique for the evaluation of neuromotor development. The neuromotor system is the system commonly tested when assessing functional development. Take care in this assessment as testing conditions and motivational state of the animals commonly influences performance of certain responses. Accurate toxicity assessment requires a test battery using multiple behavioral endpoints.

***Maternal Toxicity***. *Findings of developmental toxicity in the presence of maternal toxicity (i.e., when adverse developmental effects are produced only at maternally toxic doses) are still considered to represent developmental toxicity and should not be discounted as being secondary to maternal toxicity*. Maternal toxicity (even in the absence of developmental toxicity) is an important endpoint to evaluate in the context of all available toxicity data. The following are some examples of maternal toxicity endpoints:

- Mortality
- Gestation length (when allowed to deliver pups)
- Body weight
- Body weight change
- Organ weights (in cases of suspected organ toxicity and when supported by adverse histopathology findings)
- Food and water consumption (where relevant)

- Clinical evaluations, including types and incidence of clinical signs, enzyme markers, and clinical chemistries
- Gross necropsy and histopathology

Body weight and changes in body weight are indicators of maternal toxicity for most species. These endpoints may not be as useful in rabbits, because body weight changes in rabbits are not good indicators of pregnancy status. Changes in maternal body weight corrected for gravid uterine weight at sacrifice may indicate whether the effect is primarily maternal or fetal. For example, there may be a significant reduction in weight gain and in gravid uterine weight throughout gestation but no change in corrected maternal weight gain that would generally indicate an intrauterine effect. Conversely, a change in corrected weight gain and no change in gravid uterine weight generally suggest maternal toxicity and little or no intrauterine effect.

Because maternal animal and not the conceptus is usually treated during gestation, developmental toxicity data may be presented as incidence per litter or as number and percent of litters with particular endpoints, as in the following examples.

## Litters with Implants

- Number of implantation sites/dam
- Number and percentage of live offspring/litter
- Number and percentage of resorption/litter
- Number and percentage of litters with resorption
- Number and percentage of late fetal deaths/litter
- Number and percentage of litters with nonlive (late fetal deaths + resorption) implants/litter
- Number and percentage of affected (nonlive + malformed) implants/litter
- Number and percentage of stillbirths/litter

### Litters with Live Offspring

- Number and percentage of litters with live offspring
- Number and percentage of live offspring/litter
- Mean offspring body weight/litter
- Number and percentage of externally malformed offspring/litter
- Number and percentage of viscerally malformed offspring/litter
- Number and percentage of skeletally malformed offspring/litter
- Number and percentage of malformed offspring/litter
- Number and percentage of litters with malformed offspring
- Number and percentage of litters having offspring with variations
- Individual offspring and their malformations and variations (grouped according to litter and dose)
- Clinical signs (measured at intervals until study termination) Gross necropsy and histopathology

Under developmental effects, discuss maternal toxicity if the effects were manifest at the same levels. Do not dismiss developmental effects in favor of maternal toxicity.

Mention reproductive effects that are developmental in the developmental section too. Include these in the LSE table with the other developmental effects.

## 2.18 OTHER NONCANCER

This category includes a wide variety of effects that do not fit into the other categories listed previously. Metabolic effects are within this purview. A listing with classification of serious or less serious, of some of these effects are in Table 2-Q.

**Table 2-Q. Other Effect Endpoints**

| Effect | Less serious | Serious |
|---|---|---|
| Altered water consumption | + | |
| Altered food consumption | + | |
| Animal fur discolorations | + | |
| Alopecia | + | |
| Hirsutism (abnormal hairiness) | + | |

## Metabolic Effects

Metabolic effects include disturbances in acid-base balance. Distinguish the type and cause of the abnormality as it correlates to the laboratory results, clinical signs, and morphologic changes presented in the study. Essential to the interpretation of metabolic effects is sound knowledge of the biomedical field. Discuss these other states as metabolic effects: water depletion and water excess, hyper- and hyponatremia, hyper- and hypo-kalemia, hyper- and hypocalcemia, hyper- and hypomagnesemia, hyper- and hypophosphatemia, ketosis, hyperglycemia, hyperuricemia, increased osmolal gap, and so on.

The equilibrium between $H^+$ and $HCO_3^-$ levels in the body creates the pH of extracellular fluid (ECF). In healthy individuals, the pH range is between 7.35 and 7.45. Metabolic acidosis represents a primary fall in ECF bicarbonate concentration with a reduction in both blood pH and $HCO_3^-$ levels. Respiratory acidosis involves a primary increase in arterial carbon dioxide pressure; blood pH decreases and $HCO_3^-$ concentrations increase if renal function is intact.

ATSDR defines metabolic alkalosis as a primary increase in blood bicarbonate levels; blood pH and $HCO_3^-$ levels are both elevated. Respiratory alkalosis involves a primary decrease in carbon dioxide pressure; blood pH rises and $HCO_3^-$ levels fall.

Table 2-R provides examples of specific metabolic effect endpoints and their classification into less serious or serious effects.

**Table 2-R. Metabolic Effect Endpoints***

| Effect | Less serious | Serious |
|---|---|---|
| Acidosis or alkalosis | | + |
| Altered body temperature (hyper or hypothermia) | + | + |
| Altered perspiration | + | + |
| Ketosis, hyper or hypo: glycemia, uricemia, atremia, kalemia, calcemia, magnesemia, phosphatemia, etc. | | + |
| Increased osmolal gap | | + |
| Altered oxygen consumption | + | |
| Altered metabolic rate | + | + |

[a]Seriousness dependent on severity and health effect duration.

## 2.19 CANCER

The cancer category consists of human and animal studies that consider tumor incidence as an endpoint.

Writing this section is dependent upon whether:

- a comprehensive single epidemiological table is reported at the beginning of the health effects section
- there are at least five studies for a single cancer endpoint in this case, a forest plot may be developed,
- the author can clump cancers into a type (e.g., soft tissue) then report a table
- multiple epidemiological tables are developed for each type of cancer
- meta-analyses or pooled analyses for cancer are available
- there are not enough studies to warrant an epidemiological cancer table, in which case this is a text only section

When available, begin this section with meta-analyses and pooled analyses. Follow with epidemiological studies by summarizing the cancer information in humans, the most relevant exposure activities, and the most frequent exposure route.

The author, in consultation with the chemical manager, may use forest plots (Exhibit 10, Figure 2-2) to present cancer risk ratios. If this is the case, ATSDR recommends that:

- Only the highest level of exposure, select exposure (e.g., tetrachloroethylene-only exposure versus any solvent exposure), longest duration, or dose-response data are in the exposure analysis column. Speak with your chemical manager if you think it is important to list multiple analyses.
- Identify in the exposure analysis column the type of central tendency estimate (e.g., OR, RR) and quartile when a quartile is given.
- Alphabetize the references.
- Plot meta-analyses, pooled epi references, and epidemiological studies in one figure. However, identify these analyses differently (color, line, footnote) than the other epi studies.
- A forest plot may be more than a page long. Let the study data decide page breaks. You might also like to clump the forest plots by occupation or occupational exposure.

If possible, summarize the cancer information available for animals. Inform the reader of the species, exposure levels, exposure duration, and results for each study. Refer the reader back to the LSE table and figure.

This section must include a discussion of possible cancer mechanisms. If possible, discuss whether genotoxicity, mechanistic, and epidemiological data support cancerous health effects.

Lastly, report EPA, Department of Health and Human Services (via the National Toxicology Program [NTP]), and International Agency for Research on Cancer (IARC) classifications for cancer. Do not complete a weight of the evidence analysis for cancer studies. Toxicological profiles do not use the terms "moderate or suggestive evidence" for cancer endpoints.

## Considerations

- In 2016, the American Statistical Society (ASA) released a statement on the use of the p-value in evaluating research studies (http://amstat.tandfonline.com/doi/pdf/10.1080/00031305.2016.1154108). Some epidemiologists have adopted the ASA's interpretation and because of this, the terms "not elevated/elevated" or "no association/association" may no longer be discerned from using the p-value cutoff ($p<0.05$ or $p>0.05$).
- Tables and text may not use the terms negative or positive to indicate whether an effect was observed. Use those terms only to describe the direction of the change; for example, a negative association describes a relationship in which one variable (such as dose) increases as the other variable (such as serum cholesterol) decreases.
- NOAELs for cancer are not included, and cancer effects are always "serious LOAELs." Indicate (particularly on LSE tables and figures) CELs where applicable.
- Discuss mechanisms for cancer effects and address the following issues, if possible. Is this xenobiotic a complete carcinogen? An initiator? A promoter? Can DNA adducts be isolated? Have mutation spectra (a unique pattern of transitions, transversions, and basepair deletions or additions characteristic of exposure to a particular mutagen) been identified? Are electrophiles (potential DNA attackers) identifiable in the metabolic scheme? If so, explicitly identify the electrophiles on the metabolic diagram in Section 3.4.3 (Metabolism). Is the xenobiotic an intercalator? A clastogen?

## 2.20 GENOTOXICITY

Genotoxicity studies consist of *in vitro* and *in vivo* studies investigating the mutagenicity of the compound. Genotoxicity studies presented in this section should define the exposure conditions (level, duration, species, and strain) and effects observed. Refer to Exhibit 10, Tables 2-4 and 2-5 for example genotoxicity tables.

Report genotoxicity studies that define the exposure conditions (level, duration, species, and strain) and effects observed. Describe *in vitro* genotoxicity and *in vivo* genotoxicity test results in separate tables. Place emphasis on describing the exposure conditions, because genotoxicity studies conditions vary greatly and are not in LSE tables or figures. For *in vivo* studies, make a weight of the evidence conclusion regarding the compound's genotoxicity.

### Innovations

There is increasing evidence that exposure to some chemicals may result in heritable changes to gene function. These alterations are known as epigenetic and can result from histone modifications, DNA methylation (without nucleotide sequence changes), nucleosome remodeling, or alterations to micro-RNA (miRNA) expression. Epigenetic changes are reversible as opposed to genetic ones. Health endpoints that have been associated with epigenetic changes include cancer, diabetes, and autoimmune diseases. Although limited, there is evidence that suggests epigenetic alterations may occur in tandem with DNA damage (Ren et al. 2017). In the future, it may be wise to include an epigenetic table in this section.

For more details on genotoxicity and epigenetics, consider the following reviews: Baccarelli and Bollati 2009; Bakulski and Fallin 2014; Becker and Workman 2013; Chatterjee and Walker 2017; Kanwal et al. 2015; and Ren et al. 2017.

## 2.21 MECHANISM OF ACTION *(AS NEEDED)*

Use this section when a mechanism of action spans many health effects and with at the discretion of the ATSDR chemical team and contractor. For mechanisms of action that have only one health effect, include the details of the mechanism of action within the health effect section.

The narrative for this section presents a brief overview of any known mechanisms of metabolism, absorption, distribution, and excretion, and then discusses any substance reactions or physiological processes that lead to or comprise the mechanism(s) of toxic effect. Briefly discuss the parent compound, active metabolites, and any significant environmental breakdown products (identified in Chapter 5).

Use reasonable conjecture to discuss a possible mechanism of action (based on the scientific literature) for a substance (i.e., chemical class, structural similarities, physical/chemical properties, etc.). If there is an unknown mechanism, use structure-activity relationships to identify potential areas of concern.

## Target Organ Toxicity

What is the mechanism by which the chemical initiates organ toxicity? Present the evidence for individual steps in the toxic sequence, indicating what is established and hypothesized. (A diagram may be helpful here.) Indicate alternate hypotheses where there are adequate supportive data. Link discussions to Chapter 6 (Adequacy of the Database).

Are there human diseases or metabolic conditions that predispose the target organ to damage? (This may require a separate search on target organ impairment.) If needed, link this discussion to Section 3.2 (Children and Other Populations That Are Unusually Susceptible).

Are there species and/or strain features or special metabolic conditions in the laboratory animals used in toxicity testing that may influence their responsiveness (due to enhanced or decreased susceptibility) as compared with humans? Are physiological or anatomical differences of concern in the extrapolation of the animal data to humans (i.e., likely to cause under- or overestimation of dose-response relationships)?

## Effect of Dose, Metabolites, Duration, and Route on Toxicity

Is the dose-response curve unusually steep, or is there other reason to expect that toxicity seen at high doses may not extrapolate linearly to low doses? Is there reason to expect that capacity limitation of pathways of metabolism and/or excretion may influence dose-response relationships for toxicity? Can such phenomena indicate that a threshold in the dose-response relationship for toxicity is to be expected? Is there a specific concentration of chemical in a target organ that causes the toxicity? Is toxicity reversible and related to excretion of the chemical? Can the organism adapt to xenobiotic exposure, such

as by induction of metabolic or DNA repair enzymes? If there is any question about whether a particular endpoint represents an adaptive response, this is the appropriate place to discuss the issue. Is there evidence to suggest that chronic exposure may lead to depletion of essential co-substrates for metabolic elimination such that dose-response relationships may change as a function of time of exposure? If so, is this likely to be different in situations of high- and low-dose exposure?

In cases where toxicity results from the action of toxic active/reactive metabolite(s) and where the toxicity assessment is from epidemiological data gathered from occupational exposure, clearly state how the dose/exposure level was calculated and the confidence you have in this assessment. Indicate whether the available evidence distinguishes between causality of the chronic low-level exposure and the possibility of occasional accidental higher-level exposure.

# CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

The subsections within this chapter are:

3.1 Toxicokinetics
- 3.1.1 Absorption
- 3.1.2 Distribution
- 3.1.3 Metabolism
- 3.1.4 Excretion
- 3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models
- 3.1.6 Animal-to- Human Extrapolation

3.2 Children and Other Susceptible Populations

3.3 Biomarkers of Exposure and Effect
- 3.3.1 Biomarkers of Exposure
- 3.3.2 Biomarkers of Effect

3.4 Interactions with Other Chemicals

At the discretion of the chemical manager, this chapter may contain tables for toxicokinetic parameters or other topics within this chapter. For instance, it may contain a table for distribution and excretion of a radiolabeled substance. When available, it shall contain figures for metabolic pathway(s) and PBPK model(s). See Exhibit 11, Chapter 3 Figures and Tables for (non-exhaustive) examples of tables and figures.

The discussion in this chapter is likely to overlap with Chapter 2, Health Effects as it relates to the mechanisms of action.

## 3.1 TOXICOKINETICS

The toxicokinetics section shall have the following subsections.

3.1.1 Absorption

3.1.2 Distribution

3.1.3 Metabolism

3.1.4 Excretion

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

3.1.6 Animal-to-Human Extrapolation

The beginning of Section 3.1 shall have bullets that summarize the information in the four subsections (absorption, distribution, metabolism, and excretion) and discuss the information available for the principal routes of exposure (inhalation, oral, and dermal). When there is evidence of dose-dependent kinetics, discuss it. When applicable, a statement about the relevance of each of these topics is to be included in each bullet. The following information about the chemical shall be bulleted and in the order specified:

- Absorption: Provide a synopsis and absorption percentages if there is data.
- Distribution: Describe distribution methods, parameters, and identify where (fat, organ names) the highest concentrations are stored.
- Metabolism: Discuss the predominant metabolism pathway(s) and include how (e.g., reduction, oxidation, conjugation).
- Excretion: Detail excretion and indicate half-life (when available) number, major route of excretion, identify the excretion product(s), and lesser routes of excretion.

This section, like all preceding sections, provides a synthesis and weight-of-evidence analysis, with a description and discussion of key studies. In both the text and tables, give special attention to providing quantitative data such as the rate coefficients/constants for absorption, distribution, metabolism, excretion, and elimination. Metabolic parameters, such as the maximum velocity ($V_{max}$) and the Michaelis-Menten coefficient ($K_m$), for specific enzyme-catalyzed metabolic pathways are quite useful, because these may aid in the identification of dose-response thresholds. Pay attention to changes in kinetic parameters with dose (e.g., transition from linear to nonlinear kinetics), which may be demonstrable only in studies in which pharmacokinetics have been studied over a sufficiently wide range of doses. This section will include pharmacokinetic mechanisms.

Presently, pharmacokinetic parameters are available for a limited number of chemicals. However, search the literature for qualitative and quantitative data on biochemical (e.g., $V_{max}$, $K_m$) and physiological (e.g., blood:air and blood:tissue partition coefficients. Sex and species differences (especially between humans and animals) and route of administration differences (including those that may indicate a significant first-pass effect or enterohepatic recycling input) are also important. Have the profiles reflect how well

authors defined what they are measuring in kinetic studies. For example, when following elimination after administration of a radiolabeled chemical, assessing the researchers' methodology for separating the parent compound from its metabolites is essential. Use total radioactivity measurements with caution as these provide information on radiolabel retention but contributes little to the understanding of the relationships between dose, body burdens, elimination rates, and toxicodynamics.

The following sections discuss toxicokinetic data by major headings (absorption, distribution, metabolism, and excretion). As in the discussion of toxicity, organize the discussions by human versus animal studies and, within these divisions, by duration of exposure where possible (especially for the duration of exposure in inhalation studies and the dose-time interval for repeated dosing/exposure studies).

## Toxicokinetic Considerations

How does the substance move within the body? Is there a mechanism from the gut, lungs, and skin to the blood (or site of toxic action, if the blood is not involved in transport)? For example, are the mechanisms passive or active? Is a specific facilitated or active transport mechanism involved? Does absorption involve an intermediary (e.g., metallothionein)? Is the absorption process saturable or capacity limited? Does the parent compound have the ability to ionize? If so, what section of the gastrointestinal tract is likely to absorb it, based on its $pK_a$? If the compound is lipophilic, is it of a small enough molecular weight to diffuse passively across the cell membranes of gut, dermal, or pulmonary epithelial cells? Does metabolism occur by gut microflora or enzymes of the intestinal mucosa? If so, how does this affect absorption? Is it absorbed primarily into the lymphatics or the blood? Does diet or micronutrients affect absorption? Is information available on the influence of different vehicles or diluents on dermal or oral absorption? Is pulmonary absorption perfusion or ventilation limited?

### 3.1.1 Absorption

The discussion of absorption should explain the process by which the substance crosses biological membranes, and the site(s) of uptake where the substance enters the systemic circulation. Differentiate between exposure and dose. For example, specify administered dose, systemically absorbed dose, or target organ or tissue dose. Also, specify and distinguish between the rate and the extent of systemic absorption, especially "peak" values and steady-state concentrations. Focus on data that provide quantitative estimates of the amount absorbed and the absorption rate coefficient for each route of exposure. When known, give levels (percentages) of the substance absorbed following inhalation, oral, or

dermal administration. Be particularly careful to mention the dosage vehicle for oral or dermal dosing, especially when citing more than one study. Identify and discuss any other factors that are important determinants of absorption, such as changes in the rate and amount of absorption over a range of doses, effects due to chemical form or method of presentation (e.g., in water versus food), the nutritional status of the dosed animals, and so on.

Because some compounds may have only limited water solubility, and because the presence of food (especially fatty food) in the gastrointestinal tract may significantly affect the rate and extent of absorption of lipid-soluble environmental chemicals (often decreasing the rate but increasing the extent), describe how the chemical was administered to test animals. Indicate whether this is likely to be the same mode of exposure in humans. An absorption rate or extent in an animal study that incorporates the chemical into the food may have little predictive value for the rate and extent of absorption of the chemical by humans from drinking water. If the mode of oral administration in the study is unclear, or if the general relevance of the animal study for likely routes of human exposure is unclear, state this in the text and identify the absence of reliable and relevant data as a data need. Overall, if reliable data are not available or are questionable, say so.

## Guidance Specific to Children's Health

- Is absorption known or suspected to be different in children?
- Are there nutritional deficiencies that enhance the absorption of [Substance x] in children or animal models (e.g., influence of calcium and iron deficiencies and fasting on absorption of lead)?

### 3.1.2 Distribution

The discussion of systemic distribution includes the extent (i.e., concentrations or amounts) of distribution to major organs and tissues. Use a table to report concentrations in various tissues; see Exhibit 11, Table 3-1 for an example. A comparison of concentrations or amounts of the chemical distributed to different tissues is more important than data for single tissues. If available, present autopsy data in this section. If total radioactivity studies are included in the distribution section, caveats need to be included in the text that no distinction can be made between parent compound, metabolites, or "recycled" carbon incorporated into normal body macro-molecules. Partition coefficients (blood:tissue) should be provided here, if known, as these are important parameters for PBPK modeling.

Focus on bioaccumulation in repetitive dosing studies; for example, describe data showing that repeated doses result in a steady state. Discuss any known mechanisms involved in the translocation of the substance(s) to tissues (e.g., passive diffusion, facilitated/active transport), including whether one or more depots for the sequestration of chemicals (e.g., the fat for lipophilic substances) are involved. If depots are involved, mention whether sequestered material is readily available for subsequent redistribution. State whether the parent compound or metabolites bind to plasma or tissue proteins. Does binding to plasma proteins restrict hepatic metabolism during a first pass or otherwise contribute to delivery-limited elimination by the liver or other organs? Are there differences in distribution and/or rate depending on the route of administration? Where data are available, discuss bioaccumulation in target organs. When data are available, include discussions of maternal-fetal transfer and maternal-infant transfer.

## Guidance Specific to Children's Health

- Is distribution known or suspected to be different in children?
- Are there nutritional deficiencies that change the distribution of [Substance x] in children or animal models?
- Do [Substance x] or its active metabolites reach (this may depend on the route of exposure to the mother: inhalation, oral, or dermal exposure) and cross the placenta or placental precursors? Do [Substance x] or its metabolites preferentially accumulate on the fetal side of the placenta? If possible, indicate quantitatively how much of [Substance x] crosses the placenta. Does the placenta itself trap and accumulate [Substance x]? *If there are intraperitoneal (i.p.) or intravenous (i.v.) data about the permeability of the placenta, discuss them in with caveats about extrapolating to inhalation, oral, or dermal exposure of the mother.* Point out that human exposures are unlikely by i.p. or i.v. routes, but that any positive data show that [Substance x] could cross the placenta if exposure was great enough to achieve comparable maternal blood levels. Of course, negative i.v. data could be interpreted to mean that [Substance x] (at least the parent compound) would be unlikely to cross the placenta regardless of the exposure circumstances. *[Note that the scientist selecting literature must acquire the data about i.p. and i.v. exposure and placental transfer.]*

It is particularly important to discuss access to the placenta in cases where the active form of [Substance x] could not possibly reach the placenta. An example of this would be inhaled formaldehyde. Although formaldehyde itself can cross link DNA and protein, inhaled formaldehyde converts to formic

acid on the surface of the upper respiratory tract, and inhaled formaldehyde itself never reaches the systemic circulation. Thus, inhaled formaldehyde would never reach the placenta, and small amounts of formic acid in the maternal blood are likely to be innocuous.

- Can [Substance x] or its metabolites reach (this may depend on the route of exposure to the mother: inhalation, oral, or dermal exposure) and get into breast milk? Are [Substance x] or its metabolites preferentially accumulated in breast milk? If possible, indicate quantitatively how much of [Substance x] is transferred to the breast milk. *If there are relevant i.p. or i.v. data about transfer into breast milk, discuss this in Other Routes of Exposure subsections with caveats about extrapolating to inhalation, oral, or dermal exposure of the mother.* Point out that humans are unlikely to be exposed to [Substance x] by i.p. or i.v. routes, but that any positive data show that [Substance x] could be transferred to breast milk if exposure was great enough to achieve comparable maternal blood levels. Of course, negative i.v. data could be interpreted to mean that [Substance x], at least the parent compound, would be unlikely to transfer to breast milk regardless of the exposure circumstances. *[Note that the scientist selecting literature must acquire the data about i.p. and i.v. exposure and transfer into breast milk.]*

It is particularly important to discuss access to the breast milk in cases where the active form of [Substance x] could not possibly reach the breast. An example of this would be inhaled formaldehyde. See the previous discussion about the placenta.

- Is [Substance x] stored in maternal tissues during *pre-conception exposure*, and if so, are the stores mobile during pregnancy or lactation? Will this process result in exposure to the embryo/fetus or neonate?
- Discuss the pharmacokinetic plausibility of the active form of [Substance x] actually reaching parental germ cells. It is particularly important to discuss this issue in cases where the active form of [Substance x] could not possibly reach the germ cells. An example of this would be inhaled formaldehyde. See the previous discussion about the placenta.

*Remember that the formation of parental germ cells occurred when the parents themselves were in utero, so relevant exposure times could range from the parental gestation period to the time of conception. Damage to germ cells could either be genetic or, theoretically, epigenetic (e.g., changes in DNA methylation).*

*This issue is particularly important if [Substance x] has been shown to be genotoxic in test systems or if the results of any studies link pre-conception exposure of either parent with germ line mutations, developmental defects, childhood cancer, or other health effects. If this is the case, make cross-references to the appropriate sections. If there are no data on the ability of [Substance x] to reach the germ cells, say so.*

## Toxicokinetic Considerations

What is the pattern of tissue distribution of the substance and its metabolites? Is distribution dose-related? What is the mechanism of transport for the chemical to go from the site of absorption to the site(s) of deposition? Is there an intermediary, such as a binding protein? Is there a first-pass effect in the liver, if the compound is absorbed from the gastrointestinal tract into the blood? If the compound is volatile, does it disappear during first-pass pulmonary circulation?

Are there mechanisms for storage, e.g., binding to particular cellular macromolecules such as metallothionein, lipophilic partitioning into adipose tissue, or sequestration to "deep" depots such as bone?

### 3.1.3 Metabolism

The discussion of metabolism includes information on metabolic pathways (involving catabolic and anabolic reactions) that either convert the substance to a form that is less toxic and/or can readily be excreted or that produces a biologically active intermediate that is responsible for the toxic action (i.e., metabolic activation). Discuss pathways of detoxification that are capacity limited and at what levels “saturation” occurs, if available in the literature.

Mention specific organs or tissues, qualitatively or quantitatively, and describe major or minor metabolic pathways. *In vitro* studies may be important not only because they provide important information on intermediates and pathways but also because, for enzyme-catalyzed reactions, they allow the determination of $V_{max}$ and $K_m$. Discuss qualitative and/or quantitative strain or species differences in metabolic pathways. Stress dose dependency in discussions of physiological, metabolic, and toxicologic thresholds. Evaluate the doses that cause one or more metabolic pathways to be “saturated;” if these constants are known or if they are clearly identified by nonlinearity of the applied dose and evoked toxic response.

Include a diagram of the metabolic pathway whenever there are adequate data (see Exhibit 11, Figure 3-1). Identify the pathway(s) leading to the toxic metabolite(s). Phase II metabolism often contributes to the generation of potentially reactive metabolites and/or provides a means of transport from the site of formation (e.g., the liver) to target tissues. Hence, include phase II reactions in the diagram as a rule and give equal consideration in the text unless it is well known that these pathways do not contribute to toxification/detoxification processes. Label diagrams if metabolism differs with the route of exposure. Indicate above the arrows, enzyme systems involved in the metabolism of the substance. Identify major and minor pathways as such. The diagram should serve as an illustration for the text and not a replacement. Where relevant, indicate activation and detoxification pathways, including those involving both phase I and phase II reactions.

## Guidance Specific to Children's Health

See Attachment J from Leeder and Kearns (1997) and text in NRC (1993; Pesticides in the diets of infants and children) on developmental patterns of various enzymes. See Attachment K for alternative names for enzymes in Attachment J. Note that in humans CYP2E1 expression begins several hours after birth and continues to increase during the first year of life (Vieira et al. 1996). The intention of the attached tables is to aid in outlining this section, and the information in them is only a starting point for it. New additions occur daily. Metabolism of [Substance x] by a certain enzyme spurs a thorough literature search that includes the enzyme and child-specific search terms, and perhaps age. If there is developmental variation (such as noted for CYP2E1 above) for a relevant enzyme, then retrieve the original papers documenting this variation and reference it in the toxicological profile. Is metabolism of [Substance x] established or suspected to be different in children? If the enzymes that metabolize [Substance x] are known, does their expression or activity differ in children in general? What is known about placental metabolism? Discuss relevant animal studies. Are there nutritional deficiencies that change the metabolism of [Substance x] in children or in animal models?

## Toxicokinetic Considerations

Is toxicity associated with the parent chemical, its metabolite(s), or a combination(s) of parent compound and metabolites? Is toxicity initiated by the action of a chemically reactive metabolite of the parent compound and/or by reactive oxygen species generated by redox cycling of one or more of its

metabolites? In what organs are these metabolic enzymes present? Do the pathways of metabolism become capacity limited or saturated within the dose ranges used in animal testing or in the expected range of human exposure? Discuss methods, if available, to identify the doses at which capacity limitation of pathways may influence dose-response relationships. Can we use the ratios of various metabolites or increases in the absolute levels of metabolites or of metabolite-derived products as biomarkers of effect and/or susceptibility? Which pathways have the lowest capacities and are these capacities related to possible exposure levels?

What is the relationships between target tissue dose and applied dose? In the case of toxicity resulting from an active or reactive metabolite, what information is available that relates production of the ultimate toxic metabolite to metabolic parameters and metabolic distribution to applied dose? Are ultimate toxic metabolites formed in the target tissue or transported after formation elsewhere, as such or as proximate metabolites? Are there estimations for the rate of delivery of proximate or ultimate toxic metabolites to target tissues from PBPK models?

Do relevant human polymorphisms (e.g., allelic variation for key metabolic pathways) exist? If so, are they likely to influence susceptibility? (Find polymorphisms data by doing a search on the identified enzymes as substance – specific searches do not identify this information.) Link discussions with both Section 3.2 (Children and Other Populations That Are Unusually Susceptible) and Section 6.2 (Identification of Data Needs).

### 3.1.4 Excretion

Include where available, quantitative data for the principal excretory routes: urine, feces (including biliary excretion), and exhaled breath. If substantive, discuss the other elimination routes such as hair, nails, milk, sweat, and saliva. Discuss differences in excretion patterns between humans and animals, as well as between different species, strains, and sexes. If available and applicable, include any equations for elimination/clearance/excretion in this subsection.

Compound elimination is the temporal relationship of blood concentrations post-administration. This process involves distribution, metabolism, and excretion of the compound. In the text, distinguish between excretion of the compound (i.e., removal from the body) and elimination (i.e., loss of the compound from systemic circulation). The kinetics may be of a simple first-order nature but more frequently require a multi-exponential equation to describe them. Do not assume first-order half-life

kinetics for persistent compounds. When available, report the half-life of the substance in different tissues. Elimination also includes the half-life of the substance in different tissues. The terminal elimination rate is, perhaps, the most important parameter with respect to understanding the persistence of the substance in the systemic circulation. Initial elimination rates, observed following intravenous administration or after a peak blood concentration following administration by other routes, are often useful indicators of rates of distribution.

## Decay constants

In general, the compound excretion equation is $C(t) = C(0) \times \{e^{-\alpha t} + e^{-\beta t} + e^{-\delta t} + ...\}$. C (t) is concentration at time t and C (0) is the initial concentration or concentration at time 0 in a given body compartment, usually the blood. A, β, δ, etc. may be called decay constants. The equations are generally written so that α>β>δ>, etc.; α is called the first order decay constant; β is called the second order decay constant; and δ is called the third order decay constant. In general, the bigger the α, β, δ, etc. constants are, the faster the compound disappears from the body, if the equation is describing excretion. A special case is when β=δ=0, then $0.693/\alpha$ is called the half-life or $t_{1/2}$ and in one half-life, the amount of compound present decays to half the original amount.

## Guidance Specific to Children's Health

- Is excretion known or suspected to be different in children?
- Are there nutritional deficiencies that change the excretion of [Substance x] in children or in animal models?

## Toxicokinetic Considerations

How does elimination of the chemical occur from the body? What contribution is made by excretion of the parent compound or metabolites via renal, pulmonary, biliary, and other routes? Are these excretion mechanisms active or passive? Do they show evidence of capacity limitation (saturation) within the dose range of animal testing and/or expected human exposure? Concerning metabolic elimination, is there a significant contribution by tissues other than the liver? If more than one pathway of metabolism is involved in the metabolic elimination of the compound, do the various pathways show differences in capacity limitation that might cause the metabolic profile of the substrate to change as a function of dose?

Is there a "futile cycle" in the metabolism of the chemical? An example is the N-acetylation of dapsone in humans; the N-acetyldapsone formed cannot be excreted into urine but must be first deacetylated back to dapsone before elimination can occur, either by urinary excretion of dapsone or metabolism to N-hydroxydapsone, which can be excreted. Are there mechanisms for reabsorption, such as cleavage of conjugates in the urine or enterohepatic circulation?

### 3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

**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 endpoints.**

Divide the section into subsections if there are several PBPK models to discuss. Name the subsections after the reference and modeled animal(s). For instance, **3.1.5.1. Loccisano et al. (2011, 2013) Monkey and Human Models.**

The PBPK section discusses available PBPK/PD modeling. Include in it basic pharmacokinetic studies, PBPK models, and biologically based dose-response (BBDR) models. Differentiate between broad-based and other models in the discussion of mathematical models. Address the objectives and value of PBPK/PD models in species-to-species, high-to-low dose, and route-to-route extrapolations and in risk assessments for the profiled substance. Present information (in brief) on all available models, with more detailed discussion of the model(s) that can be used for MRL derivation. Discuss whether researchers have validated the models by comparing simulation with experimental data. State, in text, if a PBPK model has not yet been developed and validated for a chemical.

When using a model for MRL derivation, include a summary table of model input data, including physiological and anatomical parameters and a figure illustrating the structure of the PBPK model. See Exhibit 11, Table 3-2 for an example of parameter tables and Exhibit 11, Figure 3-2 for a PBPK model figure. Provide an evaluation and assessment of the most appropriate values to use if many values exist

for a given parameter. Provide the rationale for selecting specific parameters in the text. Outline the uncertainties in the interpretation of the pharmacokinetic and toxicological information considered for use in a PBPK/PD model. Include pharmacokinetics following intravenous administration, because this route avoids complications arising from interactions at the site of uptake and the sometimes complex factors involved in delivery of the dose to the systemic circulation (e.g., first-pass effect). Discuss whether the kinetic data suggest mechanistic considerations (such as capacity-limited metabolic processes) for low-dose extrapolation applicable to the risk assessment process.

### 3.1.6 Animal-to-Human Extrapolations

Discuss qualitative and quantitative differences in the metabolic pathways for relevant comparison species (i.e., species for which the majority of the toxicity data are available, or were key in derivation). Do these metabolic differences explain interspecies variance in toxicity? Are certain animal models inappropriate to use for extrapolation to humans?

If toxicity differs between species, what is the most appropriate animal model for human health effects? Is there a biologically plausible mechanism for explaining positive epidemiological studies? Have animal models demonstrated certain health effects that are unlikely to occur in humans (e.g., male rat-specific nephropathy caused by α2u-globulin accumulation)? Does the mechanism suggest potential endpoints to examine in future epidemiological studies? Discuss relevant *in vitro* data and models.

## 3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

*Use the following boilerplate to introduce this section.*

**This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. 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. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.**

**This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include**

**genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.**

**Populations at greater exposure risk to unusually high exposure levels to [Substance x] are discussed in Section 5.7, Populations with Potentially High Exposures.**

Separate the discussions in this section under bold and italicized sub-headings only when applicable and data are available. Examples of subheadings include but are not limited to ***Age-Related Exposure and Pharmacokinetic Differences, Age-Related Differences in Susceptibility, Transgenerational Effects, Genetic Polymorphisms Altering Susceptibility.*** Follow the headings by a period and two spaces with the discussion beginning on the same line.

When there is information on the class of compounds (such as organophosphates) in which [Substance x] is included, it may be appropriate to discuss these data and state that "…extrapolating to [Substance x] would suggest the following…." It may be necessary to do a limited search on the class of compounds and child-health-specific terms to see if such data exist. Authors of Chapters 2 and 3 should be familiar with both the main literature search strategy and any supplemental search strategies used for [Substance x] and be able to determine whether these searches are likely to have missed any relevant resources. *Authors of Section 3.2 are responsible for instigating any necessary supplemental literature searches [see Literature Search]*. The need for a supplemental literature search may become obvious at any time during development of the toxicological profile.

Present, in profile text, if there is evidence of a particularly susceptible population identified in the boilerplate.

Use scientific evidence to base children's susceptibility statements. Be warned that review articles may speculate on children's vulnerability.

Address the following questions in the relevant sections of the toxicological profile (noted in bold in parentheses) *and* in this section. When necessary for clarity, define the specific stages of growth and development to which the discussion applies. *Section 3.2 (Children and Other Populations that are Unusually Susceptible) is to be a STAND-ALONE section of the profile. Section 3.2 should be an ANALYTICAL SYNOPSIS of information discussed elsewhere in the profile, not a word-for-word*

*regurgitation of the information discussed in other sections.* Within each topic, please discuss human data first, then relevant animal data.

Begin this section with child exposures then discuss child susceptibility followed by other susceptible populations.

- Are children exposed? Discuss any exposure or body burden measurements made on children.
- Have measurements been made of [Substance x] or its metabolite levels in amniotic fluid, meconium, cord blood, neonatal blood, or other tissues that indicate prenatal exposure? It may be necessary to skim the epidemiology studies in Chapter 2, or consult with the Chapter 2 author to answer this question. Alternately, Chapter 5 may contain National Health and Nutrition Examination Survey (NHANES) data and may shed light on whether measurements have been made.
- Have measurements been made of [Substance x] or its active metabolites in breast milk? If so, discuss these measurements here (it is now unnecessary to have this discussion in Section 5.6. General Population Exposure). This should address exposure both from normal background and from other exposure scenarios.

Consult with the Chapter 3 author (Section 3.1 Toxicokinetics, 3.1.2. Distribution) and note whether animal pharmacokinetics experiments have demonstrated that [Substance x] or its metabolites are transferred to breast milk, and if so, in what quantities (zero, trace, significant, large). In other words, is it expected that milk of exposed women will have significant quantities of [Substance x] or its active metabolites based on results with animal studies? Does PBPK modeling (Section 3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models) suggest that significant quantities of [Substance x] or its active metabolites should be expected in the milk of exposed women? Mention the measurements of [Substance x] in human breast milk in Section 3.3 Toxicokinetics and Section 3.1.2 Distribution also.

- Does the susceptibility of children to the health effects from [Substance x] differ from that of adults? How? Why? Are there any specific theoretical reasons for thinking that embryos, fetuses, infants, children, and adolescents would differ in their vulnerability from adults? Such reasons might include whether the metabolic enzymes activating or detoxifying [Substance x] have age-dependent expression. State and discuss evidence if children are less susceptible, or have the same susceptibility as that of adults. Discuss relevant animal models.

- What health effects occur in children from exposure to [Substance x]? Example, childhood asthma discussed in the respiratory effects sections. Are there health effects observed in adults that are also of potential concern in children? What health effects occur in adults exposed as children? What health effects have been observed in immature animals from embryos up through maturity? Do children and immature animals exhibit the same types of health effects as adults? Do the doses that cause effects in children and adults or in immature and adult animals consistently differ? How? Consider any epidemiologic studies that focus on the consequences of exposures before age 18 years, even if the effects are not evident until adulthood. If there are little or no data on children, state that "The effects of [Substance x] have not been (thoroughly?) studied in children, but they would likely experience the same health effects seen in adults exposed to [Substance x]."
- Does the susceptibility of children to the health effects from [Substance x] differ from that of adults? How? Why? Are there any specific theoretical reasons for thinking that embryos, fetuses, infants, children, and adolescents would differ from adults in their vulnerability? Such reasons might include whether the metabolic enzymes, activating or detoxifying [Substance x] have age-dependent expression. State and discuss evidence if children are less susceptible, or have the same susceptibility as that of adults. Discuss relevant animal models.
- Does the toxicant alter the he developmental process? Discuss data on children, animals, and *in vitro* developmental models. Remember that the reproductive, immune, and nervous systems especially continue to develop after birth. Developmental problems may include functional neurological development, such as learning deficits and deficits in social behavior. Consider endocrine disruption of developmental processes and any epidemiologic studies that focus on the consequences of exposures before age 18 years, even if the effects are not evident until adulthood. If the developmental process is altered by the toxicant, how does the effective dose or range of effective doses in children compare with that in adults? If developmental effects only occur near maternally toxic doses, this point should be discussed.
- Are pharmacokinetics known or suspected to be different in children? Are there nutritional deficiencies that change the pharmacokinetics of [Substance x] in children or animal models (e.g., influence of calcium and iron deficiencies and fasting on absorption of lead)?
- Is metabolism of [Substance x] known or suspected to be different in children? If the enzymes that metabolize [Substance x] are known, does their expression or activity differ in children in general? What is known about placental metabolism? Are there nutritional deficiencies that change the metabolism of [Substance x] in children or in animal models? Discuss relevant animal studies.

- Are there PBPK models for children, fetuses/pregnant women, infants/lactating women, or humans at any other appropriate age? Discussion of general models for these stages may be appropriate.
- Is the mechanism of action known or suspected to be different in children? Discuss the evidence.
- Does [Substance x] or its metabolites indirectly affect the fetus? Examples include interference with blood flow, oxygen, or nutrient transport to the placenta or with waste transport from the placenta
- Can parental exposure affect children (i.e., are there any transgenerational effects)? How? Remember that parental germ cells form when the parents themselves were *in utero*, so relevant exposure times could range from the parental gestation period to the time of conception. Damage to germ cells could either be genetic or, theoretically, epigenetic (e.g., changes in DNA methylation). Are there any studies linking pre-conception exposure of either parent to germ line mutations, developmental defects, childhood cancer, or other health effects? Is [Substance x] known to be genotoxic in test systems? Discuss the pharmacokinetic plausibility of the active form of [Substance x] actually reaching the germ cells. It is particularly important to discuss this issue in cases where the active form of [Substance x] could not possibly reach the germ cells. An example of this would be inhaled formaldehyde. If there are no data on the ability of [Substance x] to reach the germ cells, say so. Discuss any issues related to childhood cancer and either prenatal or postnatal exposures to [Substance x].

Use appropriate care when extrapolating from juvenile and newborn animals to humans (see pages 25 and 51 of NRC 1993):

> *"For example, the newborn mouse or rat more nearly resembles the human fetus in the third trimester of gestation than the human infant at birth. On the other hand, the rate of maturation and growth of the mouse or rat after birth is relatively more rapid than the human. Thus, cross-species comparisons of potential toxicity for pesticides [or other chemicals] in the very young animal, although helpful, cannot be used in the same manner that cross-species comparisons are used with adult animals because of differences in developmental patterns…."*
>
> *"Newborn mice and rats are among the most immature of commonly used test species, so it is not surprising that they often differ markedly from adult animals in sensitivity to*

*chemicals…. Maturation in rodents is very rapid, so that even a few days of age can result in a marked disparity in test results….”*

See Attachment H: Age at Weaning and Sexual Maturity for Common Laboratory Species and Humans for assistance with interpreting the literature and knowing to which ages of humans the results of animal studies may apply.

When there is information on the class of compounds (such as organophosphates) in which [Substance x] is included, it may be appropriate to discuss these data and state that “…extrapolating to [Substance x] would suggest the following….” *It may be necessary to do a limited search on the class of compounds and child health specific terms to see if such data exist.*

Lastly, for this section, identify known or potential populations that may be unusually susceptible. There may be some overlap between this section and Section 3.4 (Interactions with Other Chemicals). Write this text from the perspective of a senior scientist discussing individuals who are likely to be more severely affected than the “average” individual by exposure to the profile substance. Discuss all reported and potential susceptible populations. Do not rely solely on published reports of susceptible individuals specifically linked to the profiled substance. Rather, start from the known toxicokinetics of the profiled substance, present reasonable conjecture concerning individuals that are likely to be more sensitive, and support the conjecture with cited literature where possible. This supporting literature may come from standard literature concerning toxicokinetics, specific studies of the profiled substance, or studies of similar substances.

Some examples of populations to consider are:

- Individuals in who target organs of the profiled substance are already compromised or damaged; for example, consider individuals with certain types of anemia when discussing sodium nitrite, which can cause methemoglobinemia. Other possible compromised populations include individuals with impaired pulmonary function (e.g., asthma, emphysema, bronchitis, cystic fibrosis), cardiovascular function (e.g., angina, congestive heart failure, cardiomyopathy), impaired kidney or liver function, immune problems (human immunodeficiency virus infection), and hypertension.

- Individuals exposed to a substance that is known to interact adversely with the profiled substance, for example, alcoholics and carbon tetrachloride exposure. (Interactions between alcohol and carbon tetrachloride potentiates liver and kidney damage.)
- Populations known to be susceptible to a closely related substance or its class.
- The fetus or neonate, especially for substances that cause developmental effects. Neonates or young children are generally more susceptible than older children are. The organ systems that are immature during the first few months of life (e.g., nervous, endocrine, reproductive, immune systems; teeth) are most susceptible to injury by chemicals.
- The elderly.

Although children and the elderly are frequently listed as populations at greater risk of chemical injury, both age groups may be more *or* less susceptible than the general population.

*Note: Do not identify populations who are at risk because of unusually high exposure. (This is in Section 5.7.)*

## 3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Begin this section with the following boilerplate.

**Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).**

*Include the following boilerplate only if the profile contains NHANES data:*

**The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for [Substance x] from this report are discussed in Section 5.6, General Population Exposure.**

*After this or when there is no NHANES data continue with:*

**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. Biomarkers of exposure to [Substance x] are discussed in Section 3.3.1.**

**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 effect caused by [substance x] are discussed in Section 3.3.2.**

**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 3.2, Children and Other Populations that are Unusually Susceptible.**

The author of Section 3.3 must link discussion of this section to other parts of the profile. Section 6.2 (Identification of Data Needs) may refer to biomarkers and needs to be entirely consistent with information discussed in Section 3.3.

Chapter 1 of Biological Markers in Reproductive Toxicology (NAS/NRC 1989) provides a good general discussion of this topic. The contractor will receive a copy of this reference from ATSDR.

## Guidance Specific to Children's Health

- Have any biomarkers of exposure or effect been validated in children or adults who were exposed to [Substance x] during childhood? Are there any biomarkers of exposure or effect that are unique to children? Are there biomarkers in adults that identify previous childhood exposure?

### 3.3.1 Biomarkers of Exposure

This section shall contain information that will help the reader identify biomarkers to determine whether exposure to the substance has occurred. These biomarkers do not necessarily need to be unique for exposure to the substance. Biomarkers discussed in this section can be those that are "shared" by other substances (e.g., phenols are "shared" biomarkers of exposure to benzene, lindane, dichlorophenols, and other aromatic compounds). Biomarkers of exposure can include the substance itself or a metabolite.

The profiled substance or any known measurable metabolite(s) is a primary biomarker of exposure. As such, data on the excretion of the substance or its metabolites should be one of the first places to look for potential biomarkers. Indicate when the measured substance is not specific for exposure to one substance (e.g., urinary phenol can result from benzene or phenol exposure). Indicate substance or class confounders or other factors that might influence interpretation of the observed results, such as biological half-life and sequestering (e.g., in bone or fat).

When background levels of a biomarker exist in human tissue or fluid (e.g., for essential mineral nutrients or for phenols or other compounds that might occur in the body through means other than exposure to the substance), state the normal ranges for that biomarker. Also, state whether the levels of such biomarkers could rise significantly above their normal ranges due to exposures that might occur. Refer readers to Section 3.1 (Toxicokinetics).

### 3.3.2 Biomarkers of Effect

Indicate the most sensitive organs and/or tissues. Focus first on biomarkers of effects, such as DNA adducts, enzyme levels, damaged or dead cells, and organ dysfunction that are in studies involving the profiled substance. Indicate effect biomarkers (i.e., cholinesterase activity) or panels of biomarkers (e.g., FEP levels, accumulation of Zn protoporphyrin, anemia) that can implicate exposure to the substance or its class. These biomarkers may or may not be symptoms of exposure that are specific to the substance. Discuss interpretation of these biomarkers for characterizing effects caused by the substance. Also, discuss limitations and confounders associated with relating biomarkers of effects to exposure to this substance, and indicate that other substances can cause the same effects as those caused by the profiled substance.

State the dose or dose range at which these biomarkers of effect appear in humans, if known. Discuss interpretation of these biomarkers for identifying and/or quantifying exposure to the substance. Also, discuss limitations and confounders associated with the use of a battery of biomarkers as indicators of exposure (e.g., other substances that can cause the same physiological effects). Check to see if there is an American Conference of Governmental Industrial Hygienists (ACGIH) Biological Exposure Index (BEI) for the chemical.

In general, do not discuss non-specific symptoms of exposure such as headache, tremor, and cough in this section. However, do discuss any symptom or combination of symptoms that are specific for the substance or its class. For example, the combination of constriction of the pupils of the eyes and tremor are indicators of exposure to anticholinesterases.

Additional biomarkers of effects caused by the profiled substance may exist but might not have been located during the substance-specific literature review. Do not discuss these additional biomarkers, but refer the reader to appropriate sources that cover biomarkers of the appropriate organ system(s). ATSDR has developed reports that cover biomarkers for effects on the immune, renal, hepatic (ATSDR 1990) and neurological (OTA 1990) systems. ATSDR will provide complete references for these reports. Also, refer the reader to Chapter 2 for a more detailed discussion of the effects caused by the profiled substance.

## 3.4 INTERACTIONS WITH OTHER CHEMICALS

Discuss mechanism of interaction, if known. Discuss the influence of other substances on the toxicity of the profile substance. Other substances include pharmaceuticals, hazardous substances, and substances not designated as hazardous substances. As discussed in Chapter 2, address limitations of all studies, and discuss human studies before animal studies. If interactions have only been demonstrated in animals or in vitro, speculate about whether such an interaction is likely to occur in humans, and give the basis for that speculation. Discuss effects that are potentiative, synergistic, antagonistic, inhibitory, or masking (see below). Also, discuss the relative timing of the exposures producing the interaction (i.e., whether the interaction only occurs with simultaneous exposures or when one chemical precedes the other). Discuss the mechanism of the interaction, if known. Several types of interactions may occur. For example:

- Compounds may directly interact with one another, causing a chemical change in one or more of the compounds.

- One compound may affect the pharmacokinetics/metabolism of another such that it alters the quantity of the biologically active moiety reaching the target organ.
- One compound may modify the biological actions of the second by exerting biological effects that enhance or counteract the actions of the second compound
- A compound may cause alterations in the target or receptor(s) for the second compound.

## Definitions for Interactions

The definitions presented in the table below are oriented toward their use in risk assessment. For example, the definition of a mixture actually describes mixed exposures. From a toxicological standpoint, however, the joint exposures are similar to the single exposure (perhaps time-varying) that would result if the chemicals were physically combined into a true chemical mixture. The following definitions are generally consistent with those found in the literature.

**Table 3-A. Definitions of Terms Used to Characterize Mixtures and Interactions**

| Term | Definition |
|---|---|
| Mixture | Any set of two or more chemical substances, regardless of their sources, that may jointly contribute to toxicity in the target population. |
| Simple mixture | A mixture containing two or more identifiable components, but few enough that a person can characterize the mixture toxicity by a combination of the component toxicities. |
| Complex mixture | A mixture containing so many components that any estimation of its toxicity based on its component toxicities contains too much uncertainty and error to be useful. The chemical composition may vary unpredictably over time or with different conditions occurring during production. Complex mixture components may be generated simultaneously as by-products from a single source or process, may be intentionally produced commercial products, or may coexist because of disposal practices. Risk assessments of complex mixtures is based on toxicity and exposure data for the complete mixture. Gasoline is an example. |
| Similar mixtures | Mixtures having the same components in slightly different ratios or having most components in nearly the same ratios with only a few different (more or fewer) components and displaying similar types and degrees of toxicity. Diesel exhausts from different engines are an example of similar mixtures. |
| Interaction | The circumstance in which exposure to two or more chemicals results in a qualitatively or quantitatively altered biological response relative to that predicted from the additive actions of the components administered separately. The multiple chemical exposures may be simultaneous or sequential in time, and the altered response may be greater or smaller in magnitude (adapted from NRC 1980). For quantitative evaluations, the “no-interaction” prediction is based on dose or response addition, as appropriate. |
| Synergism | A response to a mixture of toxic chemicals that is greater than that suggested by the component toxicities. |

**Table 3-A. Definitions of Terms Used to Characterize Mixtures and Interactions**

| Term | Definition |
|---|---|
| Antagonism | A response to a mixture of toxic chemicals that is less than that suggested by the component toxicities. |
| Potentiation | A special case of synergism in which a substance that does not have a toxic effect on a certain organ or system on its own increases another chemical's toxicity when added to it. |
| Inhibition | A special case of antagonism in which a substance that does not have a toxic effect on a certain organ or system on its own lessens another chemical's toxicity when added to it. |
| Masking | A situation in which the toxic effect of one chemical is not displayed because of functionally competing effects from another chemical. The most striking example is when the carcinogenic activity of a mixture is not observed at experimental doses because of more obvious toxic signs, particularly mortality, induced by other toxic components. |

Source: EPA 1988b; NRC 1988

**Table 3-B. Selected Examples of Types of Interactions between Toxic Compounds**

| Pair of toxic, genotoxic exposures | Kind of interaction | Effect |
|---|---|---|
| Benzene + radiation | Additive or synergistic | Leukemia |
| Cigarette smoking + β-naphthylamine | Additive or synergistic | Bladder cancer |
| Benzene + toluene | Antagonistic | Chromosomal damage |
| Carbon tetrachloride + ethyl or isopropyl alcohol | Synergistic | Hepatic and renal damage[a] |
| Carbon monoxide + methylene chloride | Synergistic | Cardiac damage[a] |
| Cigarette smoking + asbestos | Synergistic | Lung cancer[a] |
| Cigarette smoking + carbon monoxide | Synergistic | Cardiac damage[a] |
| Cigarette smoking + uranium (radon) | Synergistic | Lung cancer[a] |
| Sulfur oxides + air particulate | Synergistic | Chronic obstructive pulmonary disease[a] |

[a]Indicates interaction is well established (Krishnan and Brodeur 1991; Trieff et al. 1990).

## Guidance Specific to Children's Health

- Have any interactions with other chemicals been observed in children? Are there any interactions with other chemicals that are unique to children? Are adult interactions likely to occur in children?

# CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

ATSDR's mandate is to produce toxicological profiles for substances found at NPL sites. This chapter acts as a reference for all compounds discussed in the profile. As such, the tables must include all forms of the compounds discussed therein.

Before substantial work begins, undertake a search for all relevant compounds to capture and include them in Chapter 4. Occasionally, it might even make a difference in the emphasis of the profile.

There are typically two sections in this chapter:
4.1 Chemical Identity
4.2 Physical and Chemical Properties

Usually some text for Sections 4.1 and 4.2 appears first, followed by their tables. This introductory text must include a discussion/interpretation of pertinent information provided in the tables that follow. This will summarize the expected forms of the substance (relative to production and usage) and behavior of the chemical in biological or environmental media based on its chemical/physical properties. For example, a high $K_{oc}$ indicates that the chemical tends to bioaccumulate; high water solubility indicates that the chemical might find its way into groundwater. From the known chemical/physical properties, what can you say about the chemical in terms of its behavior upon release to the environment, or after assimilation into an organism?

Ordinarily, this section needs only a few relevant observations. However, provide additional text for complicated substances. Have the text provide insights into the importance of its forms or properties? Explain:

- A complicated family of substances such as PCBs or PAHs; e.g., What are the naming conventions for these compounds?
- A substance with multiple forms or properties that have important physical, chemical, or toxicological differences, such as inorganic versus organic mercury.
- A technical or complex mixture. Provide the technical formulations and percent active ingredients of pesticides and herbicides. List the typical or expected ingredients for fuels, formulations, and other complex mixtures.

Chapter 4 is a reference on important *forms* of the substance. To maintain a more concise profile, refer readers to other parts of the profile that may overlap this section, especially if the overlapping information is lengthy. Make sure to direct the reader clearly, by using wording such as "for additional important information regarding [Substance x] see [Section XY]."

*Note: Chapter 4 and Chapter 5 inter-relate in that they pull data from the Substance Priority List (SPL). We recommend pulling the data from the SPL for these two chapters at the same time.*

Include in tables, the chemical forms (e.g., ionic species, complex) reported at NPL sites and forms important in the fate and transport of the substance in the environment. This includes:

- All compounds discussed in Chapter 2 (including LSE tables). *Note: Discuss with the chemical manager any substances for which only lethality data are available.*
- All MRL substances shall have a corresponding substance in Chapter 4, including ionic forms, (e.g., Chemical Abstracts Service Registry Number [CASRN] 18540-29-9 for hexavalent chromium ion).

Generally, "HZ" CASRN contaminants will not be included in Chapter 4 (they are process wastes and other poorly identified substances). However, sometimes they can give insight to forms found at hazardous waste sites and this could influence compounds discussed in Chapter 5.

Metabolites are not included in Chapter 4, unless these forms are part of the toxic dose or are environmental contaminants with analytical results. Refer to Chapters 2 and 5 to compile a list of relevant forms to report in the tables. An illustration of the standard tabular form is in the Exhibit 12, Chapter 4 Figures and Tables. If there is no information, use either "No data" or "ND" in the table; define "ND" as "No data" in the footnote. If the information is not relevant, use "NA" and define it in the footnote as "Not applicable." Do not use "No data" if the information is not relevant.

In profiles containing information that cannot fit onto one portrait-style (vertical) page, use a landscape (horizontal) page to present the data.

Most profiles will only have Sections 4.1 and 4.2. Particularly complicated substances can have more. For example, the hydraulic fluids profile had multiple sections because it covered multiple types of fluids. PCBs has a list of all 209 congeners. Radioactive compounds contain figures of their isotope chains in Chapter 4. Lastly, the total petroleum hydrocarbons profile did not contain a Chapter 4 for chemical and

physical information and instead had a very extensive overview of the identity and analysis of hydrocarbons.

## 4.1 CHEMICAL IDENTITY

Write an introductory paragraph for the chemical indicating whether it is natural or manmade, any common meanings (e.g., DDT refers to p,p'-DDT), the primary previous and current chemical use, and brief formulation information. A sentence stating, **"Table 4-1 lists common synonyms, trade names, and other pertinent identification information for [Substance x]"** will follow this.

After the paragraph, place a table. The table shall contain the chemical name, synonyms and registered trade names, chemical formula, chemical structure, and CASRN. Restrict synonyms and trade names to a reasonable number in the table (Exhibit 12, Table 4-1). This does not need to be an exhaustive list of names. Select those that are most common or most distinctive. If there are multiple chemical forms, there will be multiple entries for these in the table.

Highly related substances, such as hydrated and un-hydrated forms, may be place into the same column of Table 4-1. When doing this, be sure to clarify which CASRN applies to which form. For example: 1327-41-9 (anhydrous), 123-45-6 (trihydrate).

## 4.2 PHYSICAL AND CHEMICAL PROPERTIES

Write an introductory paragraph for the chemical detailing the substances' state (solid, liquid, gas), its relative (high, medium, low) vapor pressure, and relative water solubility. Follow this with **"Table 4-2 lists important physical and chemical properties of [Substance x]."**

Authors may cite more than one value for any of the properties listed in this table (Exhibit 12, Table 4-2) with reference(s). For inorganic compounds, include valence state in the table. National Fire Prevention Association (NFPA) classifications for flammability and reactivity may be very useful. If they are used, give the numbers and provide a definition in the footnote. Use NFPA (2010; Fire Protection Guide to Hazardous Materials) or most recent edition.

Table 4-2 may include reactivities/incompatibilities with other substances and other class- or substance-specific information where appropriate. For example, in the "Other" category, note if the substance reacts violently with water.

Table 4-2 displays the required minimum information to be include in this table. Other chemical identifiers and properties also may be included in this table. Add information that may affect toxicity/ environmental fate from abiotic transformation processes. Include quantitative or qualitative distinctions between chemical forms, as the form is a prime determinant of tits ultimate toxicological behavior. Tailor this information to the substance being profiled; that is, the information for a metal such as chromium may be quite different from that for an organic pesticide such as parathion. For example, the following identifiers and properties might be in Table 4-2 for chromium.

- Possible environmental oxidation states and associated species.
- Associated redox potentials.

In comparison, the following might be in Table 4-2 for parathion.

- Abiotic hydrolysis rate constants and products of such hydrolyses.
- Oxidation reactions, products, and rate constants under environmental conditions.
- Soil and sediment binding parameters.

In a recent (2017) review of profiles, ATSDR notes the following extra information in this table.

- Valence state
- Boiling point of pure versus technical grade
- Multiple melting points
- Vapor density
- Taste
- Solubility in multiple liquids (water, organic and inorganic solvents)
- Rate constants
- Incompatibilities and reactivity

As a result, the contents of Table 4-2 will likely differ from profile to profile. Include only information that assists in assessing human health risk from environmental exposure.

# CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

This chapter contains seven subsections and nine additional headings. These headings are:

5.1 Overview
5.2 Production, Import/Export, Use, And Disposal
    5.2.1 Production
    5.2.2 Import/Export
    5.2.3 Use
    5.2.4 Disposal
5.3 Releases To The Environment
    5.3.1 Air
    5.3.2 Water
    5.3.3 Soil
5.4 Environmental Fate
    5.4.1 Transport and Partitioning
    5.4.2 Transformation and Degradation
5.5 Levels In The Environment
5.6 General Population Exposure
5.7 Populations With Potentially High Exposures

The chapter has one required figure and many tables. Some tables are required while others are optional. Number the tables sequentially from the beginning of the chapter. *Note: There is a different outline in exhibits to provide flexibility in table selection. The exhibits for this chapter are mostly by section with required tables first and optional tables after.* See Exhibit 13, Chapter 5 Figures and Tables for Chapter 5 figures (only one required) and tables (required and optional).

Throughout Chapter 5, cite primary references where possible, and avoid citing the Hazardous Substances Data Bank (HSDB). However, use HSDB if primary references on historic releases to the environment cannot be located. Express air concentrations in the same units as those used in the Chapter 2 inhalation LSE table. There should be consistency regarding units used for reporting data in a given environmental medium throughout this document.

Use one consistent unit for results. Express results for a given medium in the same units (for example, mg/L, mg/kg, $mg/m^3$; that is weight-to-volume or weight-to weight) whenever possible. Use of a single standard unit for a given medium may result in unwieldy numbers because of the wide range of concentrations that often occurs when comparing samples from the same medium collected at pristine versus contaminated sites. For comparability of units within a given medium, use conversions with the same units in the denominator (e.g., L, kg) and units in the numerator (e.g., ng or mg) that result in the smallest numerical value. As an example, for sediments, concentrations such as 100–11,000 ng/g may occur in ambient sediment, while 1,900 mg/kg concentrations occur at a hazardous waste site. Converting the concentrations at the hazardous waste site to the units used for the ambient range (ng/g) would yield 1,900,000 (or $1.90x10^6$) ng/g. It would be better to use the same units in the denominator, while using the unit resulting in the smallest number of digits in the numerator. For example, 100–11,000 ng/g would become 0.1–11 μg/g and 1,900 mg/kg would become 1.9 mg/g.

When using values for ppm and ppb as the standard units for a given medium, include w/v (weight per volume) or v/v (volume per volume) in parentheses for comparability with inhalation LSE tables. In addition, because not all of the chemical(s) present in a certain matrix may be bioavailable to act as toxicants, include a statement (especially for contaminated soils, subsoils, and sediments) such as: *It should be noted that the amount of [profile chemical name or names] detected by chemical analysis is not necessarily the amount that is bioavailable.*

## 5.1 OVERVIEW

The boilerplate, as written below, shall appear first in this section of the profile.

**Substance x [has/have] been identified in at least [###] of the [#,###] hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR [20xx]). However, the number of sites evaluated for [Substance x] is not known. The number of sites in each state is shown in Figure 5-1.** *When identifying non-continental (territorial) U.S. NPL sites (with the exception of Hawaii and Alaska), add the following to the boilerplate.* **Of these sites, [###] are located within the United States, [#] are located in the Virgin Islands, and [#] are located in Puerto Rico (not shown).**

In addition to sites in the Commonwealth of Puerto Rico, identify sites in any of the other three U.S. territories (American Samoa, Guam, and the U.S. Virgin Islands) as appropriate. Place the phrase "not shown" at the end of the sentence, if maps for NPL sites in any of the U.S. territories are not available.

After the boilerplate, insert the map of the Unites States with NPL hazardous waste sites contaminated with the substance identified (see Exhibit 13, Figure 5-1). The contractor will construct the map using the SPL database. When creating the map, use the most recent database available. Always add the map reference, in parenthesis, underneath the figure key. For example, the current database is 2017 so reference this as "Source: ATSDR 2017".

Modification of the boilerplate will be required for profiles with more than one map. Additionally, the contractor and ATSDR will work together to determine the best method of presenting NPL information (maps and text) in profiles that cover more than one chemical form, compound, or radioactive isotope.

After the map, include bulleted summaries for:

- The most likely route of exposure for the general public
- The media (air, water, etc.) that the chemical is most often found
- Fate processes

Include paragraphs relating to the bullets. Indicate the concentrations of the substance in air, water, and sediment/soil. Create sentences about bioaccumulation potential, degradation, and half-life/lives. Reiterate the most likely exposure route(s). Indicate whether industrial emissions, workplace exposure, and waste sites are a cause of concern.

Restrict discussion to major use categories, import quantities, and domestic production processes and quantities unless other topics substantially affect human exposure and health. Present information in narrative form; avoid extensive listing of tabular information. Use citations to appropriate primary or secondary reference sources for quantitative information on production, import/export, use, and disposal volumes. Citations to HSDB are appropriate and may be the only source of information on historic production, import/export, or use volumes for some chemicals.

### Retrieving Data from the Full SPL Spreadsheet

- Always use version 1 as it is the official SPL and has only NPL sites in it. Version 2 is experimental and includes non-SPL sites. Do not use version 2.
- The most recent total number of NPL sites is found in the "Summary Statistics" tab, "NPL Sites Only" Line 1, "Number of Sites/Events". For 2017, this count is 1,854 NPL sites. Example: benzene is at 972 of 1,854 NPL sites.
- Search for all CASRNs and names that are in Chapter 4.
- If querying "Contaminants," be sure the column "NPL" is set to Y (Yes) so that only NPL sites are included in results.
    - A profile with multiple substances requires the use of the "Contaminants" worksheet to avoid duplication. Sum the total site count for the profiled substances.
    - Use the "Contaminants" worksheet to search for all substance forms (e.g., CASRN and name variant searches). For arsenic, one might search on "arsen" and "arson." Often you can get a feel for name variants from existing Chapter 4 synonyms. When relevant, include these in Chapter 4.
    - For substances that have no other chemical forms, the site count is appropriate for the frequency. For example, the 2017 SPL V1 count for benzene is 972 NPL sites.

## 5.2 PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

This section includes the following headings:

5.2.1 Production
5.2.2 Import/Export
5.2.3 Use
5.2.4 Disposal

Keep the level of detail in Section 5.2 appropriate to an overview. Write text that summarizes the most pertinent information within two to three pages. Figures for production, use, import, export, and disposal should be the most recent. Possible sources for this information include:

- U.S. International Trade Commission (USITC)

- Stanford Research Institute, Incorporated (SRI) (Menlo Park, California; Directory of Chemical Producers of the United States)
- Chemical Marketing Reporter (CMR) (Schnell Publishing: New York, New York)
- Chemical and Engineering News (C&EN) (Facts and Figures for the Chemical Industry and Top 50 Chemical Products)
- U.S. Department of Interior, Bureau of Mines (Mineral Commodity Annual Summaries and Mineral Yearbooks)
- U.S. Department of Commerce (DOC) (U.S. general imports for consumption)

### 5.2.1 Production

Begin this section by describing the chemical family of the profiled substance. Indicate the reaction that creates the chemical. Is it available and if so, in what formulations?

Relay the annual production for the chemical in the United States. Who manufactures it? Does the EPA Chemical Data Reporting (CDR) list the chemical's production volume? If not, list why. If it does, report the volume.

Detail the following production aspects for the profiled chemical in this section:

- Production methods (general, few details)
- Production volumes (past, present, and/or trends)
- Information on production and processing facilities as shown in Exhibit 13, Table 5-1A and Exhibit 13, Table 5-1B

Display the most recent year's data from the Toxics Release Inventory (TRI) in Table 5-1. Sort facilities by state. If the TRI listings for individual production and processing facilities are longer than half a page, then present the summary information on facilities alphabetically by state. Include the information below in the two versions of Table 5-1.

Information on individual production and processing facilities as shown in Table 5-1A includes:

- Facility name
- Location (city, state)
- Range of maximum amounts on site in pounds
- Activities and uses

Information on the summary table by state for processing facilities as shown in Table 5-1B includes:

- State postal abbreviation
- Number of facilities in each state
- Range of maximum amounts on site in thousands of pounds
- Activities and uses

If the profile chemical is not required to be reported to TRI, use the following boilerplate.

**No information is available in the TRI database on facilities that manufacture or process [Substance x] because this chemical is not required to be reported under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986) (EPA 2005).**

Cite the most recent version of the report entitled *Toxic Chemical Release Inventory Reporting Form R and Instructions*. U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, D.C. EPA 745-K-97-001.

Check for historical data in the EPA Inventory Update Rule (IUR). If there is information for a chemical, then report it in tabular form with dates and pounds produced.

When available, report the chemical reactions that create the profiled chemical(s).

Focus this section on the production of the profile chemical from manufacturing or mining processes and/or any similar anthropogenic sources. Do not include reference to inadvertent production of a chemical (i.e., as a byproduct in the production of another chemical or as a product of some anthropogenic

process such a combustion of fossil fuels or natural process such as volcanic activity, forest fires, or sources such as the unintentional production of ammonia by livestock). Address the other sources in the appropriate sections of Chapter 5 (Potential for Human Exposure), particularly in Section 5.3 (Releases to the Environment).

### 5.2.2 Import/Export

Cover the following aspects of U.S. trade in this section:

- Import volumes (past, present, and/or trends)
- Export volumes (past, present, and/or trends)

When data are available, report it in tabular form.

### 5.2.3 Use

Address the following aspects of industrial, commercial, and consumer uses for the profile chemical:

- Past uses
- Present uses
- Approximate amounts by use or percentage of production by use

### 5.2.4 Disposal

Report the following:

- Rules and regulations regarding disposal practices
- Typical methods of disposal (including biological waste treatment and other new treatment and disposal technologies) (refer to Section 5.3 Releases to the Environment)
- Amounts of substance disposed of by each means
- Past disposal, present disposal, and/or disposal trends (refer to Section 5.3 Releases to the Environment)
- Information on recycling and/or reuse of the substance

## 5.3 RELEASES TO THE ENVIRONMENT

This section includes the following headings:

5.3.1 Air
5.3.2 Water
5.3.3 Soil

To evaluate the potential for ambient exposure to a substance, it is necessary to trace the substance from its point of release to the environment until it reaches the receptor population. These subsections summarize information pertaining to environmental releases from sources such as natural occurrence (e.g., volcanic activity or other natural chemical/biological processes, chemical production processes, and end-user use and disposal, as well as diffuse sources such as fossil fuel combustion from electric generating facilities, auto emissions, household product use, and storm drain and agricultural runoff. Search TRI (the latest year reported) and the published literature. Use information from the ATSDR SPL database on the media (air, surface water, groundwater, soil, and sediment) in which the pollutant has been detected at NPL hazardous waste sites.

Discuss releases to all media (air, water, and soil). When presenting information, use the reported values and place comparable units in parentheses. Note any differences in estimates reported and the facilities, point/nonpoint sources, etc., that were included in the estimates. *Note: Do not include data from a contract laboratory statistical database.*

Employ the following boilerplate in Sections 5.3.1, 5.3.2, and/of 5.3.3 only if information is available on publicly-owned treatment works (POTW) treatment removal efficiencies for the profile chemical:

**The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ ≥10 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 2005).**

If no TRI information is available for the profile chemical, use the following statement for Sections 5.3.1, 5.3.2, and/or 5.3.3.

**There is no information on releases of [Substance x] to the [atmosphere, water, soil] from manufacturing and processing facilities because these releases are not required to be reported (EPA 2005).**

## TRI Tables

Display data from the most recent TRI as shown in Exhibit 13, Table 5-2. The TRI data format differs from that used in previous ATSDR profiles. The column headings should present information in the following order: state, reporting facility, facility, and reported amounts released in pounds per year for air, water, underground injection, land, total environment, POTW transfer, and off-site waste transfer.

Look carefully at the data displayed in Table 5-2 for all media (air, water, soil, underground injection) as well as the amount that is transferred to POTWs and the amount that is transferred off-site. Report any trends that may be present in the narrative text for the respective medium (air, water, soil). For example, if the largest percentages of environmental releases are to the air, then assess the potential for exposure via inhalation to these releases in Sections 5.6 and 5.7 (General Population Exposure and Populations with Potentially High Exposures). In addition, if certain facilities released large amounts via underground injection, discuss this under disposal (Section 5.2.4).

When presenting TRI data (summation of all states) in the narrative text for total releases for air, water, soil, and underground injection, include the total amount released for each and the relative amount (%) of the total environmental release that each contributed. See examples below for air, water, and soil and use similar material in the discussions for Sections 5.3.1, 5.3.2, and 5.3.3 respectively.

### 5.3.1 Air

This section will begin with boilerplate, as selected from below.

If there is TRI information, then use the following boilerplate:

**Estimated releases of [##] pounds (~[##] metric tons) of [Substance x] to the atmosphere from [insert total number of facilities reporting to TRI] domestic manufacturing and processing facilities in [current release year], accounted for about [##]% of the estimated total environmental releases from facilities required to report to the TRI (TRI[## ####]). These releases are summarized in Table 5-[#].**

*Note: This chapter has required and optional tables and due to that we are not able to provide you the exact table number. Please number tables accordingly.*

If there is no TRI information, use the following instead of the above:

**There is no information on releases of [Substance x] to the atmosphere from manufacturing and processing facilities because these releases are not required to be reported (EPA 2005).**

When data are available, a table of environmental releases from production, process, or use facilities for the chemical shall be included in this section.

### 5.3.2 Water

The water section shall begin with boilerplate selected from below.

If there is TRI information, then use the following boilerplate:

**Estimated releases of [##] pounds (~[##] metric tons) of [Substance x] to surface water from [insert total number of facilities reporting to TRI] domestic manufacturing and processing facilities in [current release year], accounted for about [##]% of the estimated total environmental releases from facilities required to report to the TRI (TRI[## ####]). An additional [###] million pounds (~[##] metric tons) were released to publicly owned treatment works (POTWs) (TRI[## ####]).**

**These releases are summarized in Table 5-[#].** *Note: This chapter has required and optional tables and due to that we are not able to provide you the exact table number. Please number tables accordingly.*

If no TRI information is available, use the following instead of the previous boilerplate:

**There is no information on releases of [Substance x] to water from manufacturing and processing facilities because these releases are not required to be reported (EPA 2005).**

Use the following sentence only if information is available on POTW treatment removal efficiencies for the profile chemical:

**As a result of secondary treatment processes in POTWs, only a small % ([##]%) of [Substance x] that enters POTWs is subsequently released to surface water. This information is available for some chemicals in the open literature.**

*Note: if no TRI information is available for the profile chemical, use the following statement:*

**There is no information on releases of [Substance x] to water from manufacturing and processing facilities because these releases are not required to be reported (EPA 2005).**

When data are available, a table of environmental releases from production, process, or use facilities for the chemical shall be included in this section.

### 5.3.3 Soil

The last section on soil for releases to the environment shall begin with boilerplate selected from below.

If there is TRI information, then use the following boilerplate:

**Estimated releases of ## million pounds (~[##] metric tons) of substance x to soils from [insert total number of facilities reporting to TRI] domestic manufacturing and processing facilities in [current release year], accounted for about [##]% of the estimated total environmental releases from facilities required to report to the TRI (TRI[## ####]). An additional [##] million pounds**

**(~[##] metric tons), constituting about [##]% of the total environmental emissions, were released via underground injection (TRI[## ####]). These releases are summarized in Table 5-[#].**

*Note: This chapter has required and optional tables and due to that we are not able to provide you the exact table number. Please number tables accordingly.*

If no TRI information is available for the profile chemical, use the following statement:

**There is no information on releases of [Substance x] to soil from manufacturing and processing facilities because these releases are not required to be reported (EPA 2005).**

The amount of the chemical waste that is transferred off-site should also be noted (cross-reference this to Section 5.4, Disposal).

When data are available, a table of environmental releases from production, process, or use facilities for the chemical shall be included in this section.

## 5.4 ENVIRONMENTAL FATE

Divide this section into two subsections.

5.4.1 Transport and Partitioning
5.4.2 Transformation and Degradation

### 5.4.1 Transport and Partitioning

Divide this section into four subsections that are written in bold font followed by a period and with the description beginning on the first line.

Air
Water
Sediment and Soil
Other Media

Delete any of the above, unnumbered sections if there are no data for a particular media or if there is no need to separate them out for the discussion. When possible, include data of long-range transport in tabular form.

The purpose of this section is to describe how the substance or its metabolite(s) moves in the environment after its initial release. Is it a metal? Is it metallic, inorganic, or organic? What is the valence state? Include a discussion of mobility in each medium (air, water, and soil), as well as any tendency to partition from one medium to another (e.g., from water to sediment). Key properties and factors for transport and partitioning include particle size range for particulate pollutants, groundwater retardation factors, log octanol-water coefficient (log $K_{ow}$), water solubility, log adsorption coefficient relative to organic carbon (log $K_{oc}$), vapor pressure, and Henry's Law constant. Discuss the form in which the substance exists in air (e.g., in the vapor phase, as a particulate), residence time and transport information, and factors that control its removal (e.g., dry deposition and wet deposition). Information about factors that control removal of the substance from the air is important for assessing bioavailability.

Discussion should include but not be limited to volatilization, sorption, bioconcentration, biotransformation, and bioavailability. Consider all processes that would affect transport and partitioning between air, water, sediment, and soil, and within each compartment (e.g., the potential to leach into groundwater will depend on factors such as $K_{oc}$, soil type, organic matter, rainfall, depth of groundwater, and extent of degradation). Are there major reservoirs or sinks for the substance? If this information is unknown but data exist on properties of the substance that predicts transport and partitioning, make such predictions. However, those predictions should be clearly and specifically designated as estimations based on some data. Do not speculate. Identify data needs where data are lacking. If applicable, consider a discussion of kinetics, including whether the assumption of first-order kinetics, half-life values (kinetics of disappearance), and persistence (residence time) would affect transport and partitioning between air, water, sediment, and soil, and within each compartment.

Discuss the potential importance of bioconcentration and increases in concentration or appearance in various plants and animals resulting from food-chain magnification. Indicate if these are significant reservoirs or sinks for the chemical. When discussing terrestrial plants, consider the major pathway of vegetation contamination (e.g., air-to-leaf transfer, root uptake, and translocation to aboveground parts of edible plants). The description of bioconcentration potential includes both bioconcentration and bioaccumulation within a single trophic level (i.e., within an organism) and biomagnification (i.e., the potential for a substance to move up the food chain through several trophic levels).

Add information on experimentally measured bioconcentration factors (BCF) when available. Provide the species in which the BCF was actually measured and experimental parameters (e.g., duration of exposure, age of organism etc.) for the analysis. If measured BCF values are unavailable, estimate BCF values (derived from log $K_{ow}$ or by analogies to structurally related compounds) and clearly identify these as "estimates."

Present the available information or estimates if a chemical is subject to bioconcentration in aquatic organisms, or bioaccumulation by terrestrial plants, or if biomagnification is a potentially important route of human exposure.

### 5.4.2 Transformation and Degradation

The four subsections are similar to the last and are unnumbered. Like the previous, these are to be in bold font with a period at the end, two spaces and then begin writing on the same line.

Air
Water
Sediment and Soil
Other Media

Delete any unnumbered sections if there are no data for a particular media or if there is no need to separate them out for the discussion.

Each subsection includes descriptions of abiotic and biological transformation processes, rates, and products. Information on biodegradation (aerobic and anaerobic), abiotic degradation in surface and subsurface soil and in surface and groundwater, and photochemical and other abiotic degradation in air and water should be included, if available. Do not provide speculations if this information is not available. Indicate in text that the information is not available. Differentiate clearly between field and laboratory findings. Indicate when the information on biodegradation comes from studies on individual species or mixtures of microorganisms in culture media. Highlight and discuss the rates of biodegradation and abiotic degradation in environmental media.

Exercise caution when working with half-lives found in the published literature. The disappearance of the more persistent compounds in nature often does not follow half-life kinetics, especially when only a small percentage of the original compound remains or after some time has elapsed. The values predicted by half-life kinetics often differ more and more from the actual values in nature as time progresses. The published values for half-lives are often misleading because they assume first-order kinetics. If half-lives are given in the profile, the text should indicate whether first-order kinetics have really been shown; that is, a half, quarter, eighth, sixteenth, etc., of the substance remains after one, two, three, four, etc., half-lives.

Be wary that incorrect conclusions on persistence occur from misuse of a single datum or an incomplete set of data to calculate half-lives. Provide half-lives only when the published information contains data showing that the rate of disappearance is indeed first-order. If the second through $\infty$ half-lives differ from first-order kinetics, cite the half-life with the qualifying statement that the half-life represents the calculated time for loss of the first 50% of the substance. Continue with a statement that the remaining half-lives may be substantially longer and that the rate of disappearance may decline further as time progresses.

Emphasize biodegradation or abiotic transformation products when these products may be more toxic, more persistent, and/or more mobile in nature than the parent substance. In this case, identify the product(s), the environmental medium in which they were found should be specified, and their persistence in that medium (to the extent known) should be stated. If the product(s) are toxic to humans or other mammals, state this fact (together with appropriate references). Use the product's chemical and physical properties (e.g., mobility, persistence) or compare it to structurally related compounds when no data exist.

When possible, provide figures for environmental transformation showing the chemical structures and possible pathways for their formation and interconversion (for example, see Exhibit 13, Figure 5-2). In some cases, a single figure may be adequate to describe a chemical's transformation and degradation; however, for complex processes, one figure may be required to illustrate atmospheric processes while a second figure demonstrates abiotic and biotic processes occurring in water and sediment. This portion of the text on environmental transformation may overlap with the metabolism discussion in Chapter 3; cross-references between the two chapters are helpful. Thoroughly discuss degradation, transport and fate when degradation products represent a more serious hazard than the original substance.

Include environmental conditions that affect biodegradation or abiotic degradation (for example, dissolved oxygen, pH, organic matter, clay content, temperature, and inorganic nutrients). Discuss known conditions. Consider these other properties for inclusion:

- Complex equilibria (e.g., with humic substances, ligand formation with ammonia, cyanide)
- Abiotic hydrolysis rate constants and products of such hydrolyses
- Oxidation reactions, products, and rate constants under environmental conditions
- Soil and sediment binding parameters
- Groundwater retardation constants
- Complex equilibria with common metals such as Ca2+

If data are not available on photochemical degradation, write about chemical properties (for example, absorption wavelength) to predict relative degradation rates. Definitely indicate when these are estimates based only on chemical properties.

## 5.5 LEVELS IN THE ENVIRONMENT

As an introduction to this section, including the following boilerplate to indicate that the amount of a chemical detected by analysis is not necessarily the same amount that is toxicologically available.

**Reliable evaluation of the potential for human exposure to [Substance x] depends, in part, on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of [Substance x] 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 [Substance x] 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.**

After the boilerplate, insert the lowest limits of detection table and a couple of sentences like this: Table 5-[#] shows the limit of detections typically achieved by analytical analysis in environmental media. Presented in Table 5-[#] is a summary of the range of concentrations detected in environmental media (see Exhibit 13, Tables 5-3 and 5-4). *Note: This chapter has required and optional tables and due to that, we are not able to provide the exact table number. Please number tables accordingly.*

Divide this section into the four subsections listed below:

5.5.1 Air
5.5.2 Water
5.5.3 Sediment and Soil
5.5.4 Other Media

These major subsections contain general statements regarding the concentrations of the substance measured in the medium under discussion and potential variations by geographic location. When available, include monitoring data obtained in the United States. Provide ranges of concentrations, where possible, as well as means or medians. Express air concentrations in the *same units* as those used in the Chapter 2 inhalation LSE table. As indicated in Section 5.1, use the same units whenever possible. If necessary, show conversion factors to relay information to the lay public or to the news media. Consistently use the same units in an environmental medium.

Data presented shall be in tabular form. The tables include those for ambient levels and ranges for indoor and outdoor air (remote, rural, urban, and industrial) as seen in Section 5.5.1 (see Exhibit 13, Tables 5-5–5-7). Report drinking water, surface water, and groundwater levels in Section 5.5.2 (see Exhibit 13, Tables 5-8–5-10). Continue creating tables for soil and sediments in Section 5.5.3 (see Exhibit 13, Table 5-11) and other media (aquatic organisms and plants, terrestrial animals and plants, birds, food and beverages, cigarettes, mainstream and side stream smoke, consumer products, etc. in Section 5.5.4 (see Exhibit 13, Table 5-12). Please remember to also view the optional tables in the aforementioned sections. Additional sources for this information include the National Oceanic Atmospheric Administration (NOAA), FDA, EPA, the U.S. Department of Agriculture (USDA), U.S. Fish and Wildlife Service (FWS), and the U.S. Geological Survey (USGS), as well as the World Health Organization (WHO), IARC, the International Programme for Chemical Safety (IPCS), and British Health and Safety Executive Reviews (secondary sources).

Present ambient or typical background levels first, followed by levels from media with known contamination. Include monitoring data, where available, from epidemiological studies conducted on environmentally exposed populations discussed in Chapter 2. It is particularly important to include concentrations measured near industrial sources and disposal sites.

Give the specific form of the substance being measured, to the extent that this information exists ("form" refers to the oxidation state of cations or anions, non-protonated or protonated or ionic species, chelates, chemical sorbed to environmental surfaces, chemical present in non-aqueous-phase liquids, etc.). Specifying the form is particularly important because different forms of the same substance often have distinctly different toxicities or environmental transport and fate.

Describe site-specific or generic information on bioavailability if available. Major deficiencies in toxicological evaluations exist because of the lack of information on chemical bioavailability in environmental media. Site concentrations do not necessarily mean that the chemical is available for absorption. The availability may vary from zero to 100%. If sorption, presence in a non-aqueous-phase liquid, or sequestering in environmental matrices is known or is likely to occur for the substance but no data exist on bioavailability, state that the toxicologically significant concentration may be somewhat or substantially less than the concentrations found in soils, subsoils, or sediments.

Identify those data collected by the most reliable and advanced sampling and analytical methods. When necessary, state (with citation) if there are limitations to the data or reasons to suspect that data may be erroneous due to questionable sampling or analytical methods. Indicate when analytical methods are not refined enough to allow for detection in specific media. Stating such information will aid in assessing data gaps/needs in Section 6.2 (e.g., methods are not sensitive enough to measure background levels in air).

Summarize large amounts of environmental monitoring data in tables that total no more than three pages for each medium. Keep ambient data separate from contaminated site data by using different tables. Focus the text on the interpretation of existing data rather than the presentation of monitoring data from various sites. If there are modeling studies, then cite and identify (e.g., modeling study) them.

## 5.6 GENERAL POPULATION EXPOSURE

This section discusses sources and pathways of exposure for the general population. Express air concentrations in the same units as those used in the Chapter 2 inhalation LSE table.

Report estimates of potential daily intake of the chemical from all routes of exposure, if available. Relate whether the indoor and outdoor background exposure levels (reported in Section 5.5.1) are a health hazard. For ambient levels of indoor and outdoor air, drinking water and surface water, soil, and food,

consider a table showing percentage exposure from each source for an infant/child, an adult nonsmoker, and an adult smoker (where applicable) might be useful.

When available, use EPA Total Exposure Assessment Model (TEAM) studies to show exposure sources and estimated levels for the general population. Where exposures are unquantified, describe them qualitatively. For example, physical/chemical properties and speciation, if available, must be included in the assessment of the relative importance of each exposure route and by each source. Concentrations measured in human tissues and fluids (e.g., blood, serum, urine, feces, organs, breast milk, hair, nails), where available, should also be included in this section. Present levels in human tissues and fluids in a table. Body burden data give reliable evidence of human exposure, although it is often difficult to identify the exposure concentrations. Refer the reader to Chapter 2 if autopsy information from case studies is there. Include information on levels in human tissues and fluids in order to identify data gaps/needs in Section 6.2.

*Exposure from eating animal products:* ONLY discuss data from Chapter 5. Each state, Native American tribe, or U.S. territory chooses its own criteria (e.g., measurement methods for contaminants, and risk assessment models and assumptions) for issuing fish and wildlife advisories. Do not base any Chapter 5 conclusions on an existing fish or game advisory. ATSDR has not investigated the methodologies used by each state, so the agency might or might not agree with specific fish and game advisories.

*Exposure from consumption of produce:* Consider the potential for residues on or in imported produce.

This section shall contain tables of NHANES data (see Exhibit 13, Tables 5-13 and 5-14) for the contaminant(s) in the general population, when available.

## Guidance Specific to Children's Health

- Are unique exposure pathways for children known or possible? If applicable, include discussions of pica (eating paint chips or other inappropriate substances); hand-to-mouth activity; putting foreign objects in their mouths; tendencies not to wash hands; propensity to accidental poisonings by ingesting household products, pharmaceuticals, cosmetics, folk remedies, and other swallowable items; sniffing household or commercial products; use of childhood-specific medications (e.g., head lice treatments); use of playground equipment; tendencies to play in surface water, such as creeks; tendencies to trespass on posted property; and habitation of

microenvironments (such as close to the floor) that might contain different levels of [Substance x]. Do not forget other factors mentioned in the boilerplate. Any quantitative estimates of exposure should be discussed. This should address exposure from both normal background and other exposure scenarios.

- The tendency of young children to ingest soil, either intentionally through pica or unintentionally through hand-to-mouth activity, is well documented. Document through discussion whether childhood exposures to [Substance x] occurs through soil ingestion. Please discuss the likelihood of such exposures, using information from other sections of Chapter 5, even if significant exposure of children to [Substance x] has not been studied; i.e., has [Substance x] been measured in soil? Do significant quantities of [Substance x] sorb to soil? Does [Substance x] biodegrade quickly or slowly in soil? Does [Substance x] volatilize quickly from soil, so that little is likely to be found in surface soil? Is [Substance x] bioavailable from soil for ingestion? Does Chapter 3 indicate anything about the efficiency (high, moderate, low?) of oral absorption of [Substance x] in children or adults if it were to be significantly bioavailable from soil?
- Young children often play close to the ground and frequently play in dirt, which increases both their dermal exposure to toxicants in dust and soil, as well as inhalation exposure to toxicants in airborne particulate matter. Please discuss the likelihood of such dermal and inhalation exposures, using information from the remainder of Chapter 5, even if significant exposure of children to [Substance x] has not been studied (see previous bullet for questions involved in such an analysis). Is [Substance x] bioavailable from soil or dust for dermal and inhalation exposures? Does Chapter 3 indicate anything about the efficiency (high, moderate, low?) of dermal or pulmonary absorption of [Substance x] in children or adults if it were to be significantly bioavailable from soil?

Where appropriate, you may summarize the above points in text such as “Children may potentially be exposed to [Substance x] from oral/inhalation/dermal exposure(s) if they play in the soil of contaminated areas such as hazardous waste sites.”

- If [Substance x] is significantly heavier than air (ask Chapter 4 author), point out: “[Substance x] vapors are heavier than air and since young children are closer to the ground or floor because of their height, they may be exposed to more [Substance x] than nearby adults during accidental exposures.”
- Mention exposures discussed in Section 5.4. Other Media (includes foods) and Section 5.6. General Population Exposure that apply to children, and discuss whether these might

disproportionately affect children (e.g., because of factors mentioned in the boilerplate). Refer the reader to Section 5.4 or 5.6 for other information.

- Are significant dietary exposures likely? Remember that a child's diet often differs substantially from that of adults. Is the dietary [Substance x] exposure different for children then from adults on an mg/kg/day basis? Sometimes the results of FDA market basket surveys and analyses (such as Total Diet Studies) may be helpful.

  *Exposure from eating animal products:* ONLY discuss data from Chapter 5. Because each state, Native American tribe, or U.S. territory chooses its own criteria (e.g., measurement methods for contaminants, and risk assessment models and assumptions) for issuing fish and wildlife advisories, no conclusions in Chapter 5 should be based on an existing fish or game advisory. ATSDR has not investigated the methodologies used by each state, so the agency might or might not agree with specific fish and game advisories.

  *Exposure from consumption of produce:* Consider the potential for residues on or in imported produce.

- Are structural materials of the home (e.g., lead from plumbing, and radioactivity from certain construction materials made of certain mining slags) likely to release [Substance x]?
- Are children likely to be exposed to significant amounts of [Substance x] while household products and pesticides are being used by adults? Are children likely to be exposed to pesticides by premature re-entry into treated areas? For example, there have been multiple papers about measurement of chlorpyrifos residues on children's toys and the floor and carpet where they may crawl after waiting the specified re-entry time.
- Are children likely to be exposed to [Substance x] because of its use on pets?
- Are parents' lifestyles or cultural practices (e.g., use of mercury in occult practices such as Santeria, Voodoo, and Espiritismo; use in folk remedies for stomach disorders in Indian and Asian populations; or use of lead in certain folk remedies and cosmetics) likely to be a source of exposure to children?
- Are children more or less exposed than adults to [Substance x]? Have measurements and calculations been done to determine whether children are different in their weight-adjusted intake of the toxicant?
- Are the parents' working clothes, skin, hair, tools, or other objects removed from the workplace likely to be a source of exposure to children? Please be very clear in distinguishing actual

observations of take-home exposure from any discussions of theoretical reasons why take-home exposure might or might not occur.

Take-home or secondary exposure from parental jobs is particularly likely to be a problem with lead and asbestos. See *Report to Congress on Workers' Home Contamination Study Conducted Under the Workers' Family Protection Act* (NIOSH 1995) for a good review of the literature and examples of other chemicals likely to be taken home inadvertently.

- Is the exhaled breath of occupationally exposed parents likely to be a source of exposure for children? To determine if it is an exposure source, compare to MRLs or background levels but do not discuss the MRLs in this chapter.

  An example of this is tetrachloroethylene exposure discussed in Section 6.5 of the toxicological profile. The following text appears in the Tetrachloroethylene Toxicological Profile. "Indoor air of apartments where dry cleaners lived was about 0.04 ppm compared to 0.003 ppm in the apartments of the controls (Aggazzotti et al. 1994a), indicating that dry cleaners serve as a source of exposure for their families. Breath concentrations of tetrachloroethylene in dry cleaners, family members, and controls were 0.65, 0.05, and 0.001 ppm, respectively (Aggazzotti et al. 1994b). A study which combines PBPK modeling with a single compartment model for a "typical" home (Thompson and Evans 1993) suggests that tetrachloroethylene levels in a home with a worker exposed to a TWA of 50 ppm for 8 hours as the only source of tetrachloroethylene could result in concentrations of 0.004-0.01 ppm. The air exchange rate in the house made a larger difference in the house air concentrations than the choice of metabolic data used in the PBPK model." It should be noted that the chronic inhalation MRL is 0.04 ppm.

- Could adolescents, or even younger children, be exposed occupationally? Keep in mind the children of migrant farm workers.
- Are particular incidents (e.g., children playing with mercury) likely to be sources of exposure to children?
- Is inappropriate home use or improper application of pesticides, such as banned and restricted-use pesticides, likely to be a source of exposure to children? An example is the series of methyl parathion misuse cases in homes.
- Are children likely to be exposed to [Substance x] at school? Through arts and crafts?

- If screening of children "as a public health practice" for exposure to [Substance x] is appropriate, explain when. What have ATSDR and CDC recommended? Relevant recommendations by EPA, WHO, and other agencies may be discussed if appropriate. If possible, provide a brief explanation of the recommended screening test. How is the sample obtained (e.g., by drawing blood or collecting urine)? What is being detected in the test (e.g., [Substance x], its metabolites, or some other biomarker)? Are there any unique issues relevant to the test? For example, for blood lead screening, contamination by lead external to the body is an issue. The initial screening is done with capillary blood obtained from a finger prick, and it is particularly important that the finger be adequately cleaned to prevent contamination of the blood sample with environmental lead. A positive initial test is followed up with a venous blood draw – usually from the arm, which is more difficult and time-consuming to do than a finger prick, but less likely to be confounded by contamination with environmental lead from the skin surface of the hand. Refer the reader to Section 3.3 Biomarkers of Exposure and Effect for further details about the test.
- Are there any childhood-specific means to decrease exposure?

## 5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Describe populations that have potentially high exposures, including the potential for exposure (route, pathway, and medium) for populations living around hazardous waste sites contaminated with the substance, manufacturing and processing facilities, or disposal operations (including underground injection). Consider locations of NPL waste sites contaminated with the substance (see Exhibit 13, Figure 5-1), along with the location of production and user facilities (see Exhibit 13, Tables 5-1A and 5-1B and Exhibit 13, Table 5-2). Discuss these populations and their potential for high exposure first. Consider other populations, groups, or individuals with potentially high exposures. Such populations may be highly exposed through occupation, special habits (e.g., smoking), behavior (e.g., eating soil), diet (e.g., high fish and wildlife consumption by recreational or subsistence fishers and hunters including Native American populations), activities, religious practices and beliefs, geographic location, use of particular consumer products, medical treatments, etc. Consider passive exposures as well as exposures during active use.

Search for occupational exposure data that reflect current exposure levels in the United States. Occupational exposure data are available from the National Institute for Occupational Safety and Health (NIOSH) (Surveillance Initiatives; National Occupational Hazard Survey [NOES], 1972–1974; National Occupational Exposure Survey [NOES], 1981–1983; and Health Hazard Evaluations) and the

Occupational Safety and Health Administration (OSHA) Medical Screening and Surveillance activity. Try to locate data from more recent years. Address what the NIOSH surveys covered (e.g., NOES provides estimates of the number of workers potentially exposed to substances in the workplace). In addition, identify which occupations are included in the NOES surveys (see Fifth Set Profile for Benzene). Access the NOES database directly or through TOMES CD-ROM (latest edition) by accessing the Registry of Toxic Effects Chemical Substances (RTECS) file containing the NOES 1983–1986 data.

Reference the book, Poisoning and Drug Overdose, by Kent Olson. If possible, include tables from an area with pollution with contaminant measured in serum or urine; see Exhibit 13, Table 5-15 and 5-16 for examples.

# CHAPTER 6. ADEQUACY OF THE DATABASE

There are three sections in this chapter.

6.1 Existing Information on Health Effects
6.2 Identification of Data Needs
6.3 Ongoing Studies

This section begins with the following boilerplate.

**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 [Substance x] 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 adverse health effects (and techniques for developing methods to determine such health effects) of [Substance x].**

**Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk 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.**

## 6.1 EXISTING INFORMATION ON HEALTH EFFECTS

This section shall begin with the following boilerplate.

**Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to [Substance x] that are discussed in Chapter 2 are summarized in Figure 6-1. The purpose of this figure is to illustrate the information concerning the health effects of [Substance x]. The number of human and animal studies examining each endpoint is indicated regardless of whether an effect was found and the quality of the study or studies.**

Insert a summary of health effect studies figure using a portrait page (see Exhibit 14, Figure 6-1). Write a paragraph about the figure and the findings. Are there more inhalation or oral studies? What was the endpoint most studied?

Note to authors: Any human study (including but not limited to case studies) discussed in Chapter 2 is to be counted in this figure. The chemical manager will be evaluating whether the Number of Studies Figure in Chapter 2, matches with the content of Chapter 2 (by endpoint) and also matches the Existing Health Effects Studies in Chapter 6.

## 6.2 IDENTIFICATION OF DATA NEEDS

This section begins with the following boilerplate

**Missing information in Figure 6-1 should not 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* (ATSDR 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.**

Divide this section into the following unnumbered subsections.

Acute-Duration MRL
Intermediate-Duration MRL
Chronic-Duration MRL
Health Effects (use these subsections as needed)
- Respiratory
- Cardiovascular
- Gastrointestinal
- Musculoskeletal
- Hepatic
- Renal
- Dermal
- Ocular
- Endocrine

Immunological
Neurological
Reproductive
Developmental
Other Noncancer
Cancer
Genotoxicity
Mechanisms of Action

Epidemiology and Human Dosimetry Studies
Biomarkers of Exposure and Effect
Absorption, Distribution, Metabolism, and Excretion
Comparative Toxicokinetics
Children's Susceptibility
Physical and Chemical Properties
Production, Import/Export, Use, Release, and Disposal
Environmental Fate
Bioavailability from Environmental Media
Food Chain Bioaccumulation
Exposure Levels in Environmental Media
Exposure Levels in Humans
Exposures of Children

Write the subheading in bold followed by a period, two spaces, and then text. Write short sentences or bullets about the category. If there is no data need then do not include it in text.

Do not cite references for general statements, e.g., a number of chronic studies have examined the renal toxicity of [Substance x]. Only use the word "adequate" for describing MRL needs; not others. Do not convey agency judgement of priority for filling data needs lease do not prioritize data needs.

In stating what information currently exists, be specific, and pay particular attention to routes of exposure and threshold effect levels, when appropriate. Draw conclusions based on the information identified. For example, "Analytical methods are available for measuring carbon tetrachloride in air, water, soil, and solid waste, and most of these methods have good sensitivity and specificity." If there does not appear to be a need for additional information at this time, state this. If there are no data, give reasons why there

may be a shortage of data in that area. For example, particular routes of exposure may not be relevant or there may not exist a known exposed population necessary for an exposure registry. If appropriate, state what additional information would be useful and why.

Justify the need for additional research by relating how the information will aid in assessing potential toxicity or human exposure, with particular focus on the exposure conditions of concern at or near hazardous waste sites. Clearly state the need for all additional research. Consider supplementing all data needs with related substance information as appropriate.

Present human data (inhalation, oral, and dermal) before animal data (inhalation, oral, and dermal). For each of the three exposure routes (inhalation, oral, and dermal) identify insufficient data and propose additional studies.

### Acute-Duration MRLs

Were data sufficient to derive inhalation and oral MRLs? If not, state what information is lacking- either inadequate identification of target organs or levels of exposure (LOAELs or NOAELs) that cause the effect.

Example data need.

> **Acute-Duration MRLs.** The available acute inhalation database was inadequate for deriving an MRL. Limitations include the lack of examination of the respiratory tract, lack of reporting incidence data for the liver and kidney lesions, and lack of developmental toxicity studies, particularly since developmental toxicity is a sensitive endpoint following oral exposure. There is a need for additional inhalation toxicity studies that investigated the suspected sensitive targets including the respiratory tract, kidney, and liver. Additional developmental toxicity studies would help to determine whether this is a more sensitive endpoint than liver or kidney toxicity. The data base provided enough data to derive an acute-duration oral MRL.

### Intermediate-Duration MRLs

Same as Acute-Duration MRL section.

### Chronic-Duration MRLs

Same as Acute-Duration MRL section.

### Health Effects

Present the data needs associated with the health effects in Sections 2.3 through 2.20. Do not discuss MRL issues in this section.

Consider the following information:

- Is there sufficient information in humans (or several animal species) to identify target organs following exposure via all three routes?
- In the absence of route-specific toxicity data, state whether pharmacokinetic data are available that may support the identification of target organs across routes of exposure. The end result may be that qualitatively we would expect similar endpoints, but the levels (that cause the effects) may or may not be possible to predict.

Follow this general discussion with endpoint-specific data needs; only include endpoints in which there are data gaps. Present the endpoint specific-data needs in the same order as Chapter 2.

For example:

> **Health Effects.** Toxicokinetic studies (Backer et al. 2000; Kenyon et al. 2015; Nuckols et al. 2005) provide evidence that inhalation and dermal exposure to bromodichloromethane are significant contributors to the blood bromodichloromethane levels. However, Torti et al. (2001) is the only available inhalation study in laboratory animals and no dermal exposure studies were identified. There are needs for inhalation and dermal exposure studies examining a wide range of potential endpoints to identify whether the critical targets of toxicity for these routes differ from oral exposure targets and establish dose-response relationships. Oral toxicity studies in laboratory animals have administered bromodichloromethane via drinking water, gavage in oil, and feed. In humans, exposure via drinking water would be prominent oral exposure route. Studies investigating possible differences between various oral exposure subroutes would provide

insight into the applicability of dietary and gavage administration studies for assessing potential human toxicity of bromodichloromethane.

***Hepatic.*** Oral exposure studies in laboratory animals have found considerable overlap in NOAEL and LOAEL values across studies, which are likely due to differences in oral route of exposure (i.e., gavage, drinking water, feed) and the vehicle used. Another need is studies that evaluate the relevance of each of these routes to humans exposed to bromodichloromethane in tap water.

***Renal.*** Available oral exposure studies in laboratory animals suggest a higher toxicity associated with gavage administration than drinking water or feed exposure. Additional studies are needed to explain these differences and evaluate whether the results of gavage studies are applicable to humans.

Endpoint-specific data needs to consider:

### Immunological

- Is there reason to believe that the immune system is a target for this substance, either from empirical data or from references from related substances? For example, were there any effects on lymphoid tissue or blood components (peripheral lymphocytes) in the 90-day study? If the answer is a resounding "no," it may be possible to conclude that there are no additional needs at this time.
- If the answer above is "yes" (please refer to Section 2.14 where immunological and lymphoreticular effects are discussed), has a battery of immune function tests been performed?
- Is there any reason to suspect the effects may be route- or species-specific?

### Neurological

- Is there reason to believe that the nervous system is a target for this substance, either from empirical data or from inferences from related substances? Specifically, is there behavioral, histopathological, neurochemical, or neurophysiological information? If available, conclude that there are no needs.
- Is there any reason to suspect the effects may be route- or species-specific or age-dependent?

- If a substance is an adult neurotoxin, ask for developmental neurotoxicity studies.

### Reproductive

- When developing this discussion, remember that the Agency places extreme importance on the acquisition of reproductive toxicity data; in fact, it is desirable to have such data from inhalation and oral routes prior to developing MRLs.
- State whether there is sufficient information in humans (or several animal species) to indicate that the substance affects reproductive health following exposure via all three routes. Do the animal data support the human data? Remember that the emphasis is on human health significance.
- In the absence of route-specific data, state whether pharmacokinetic data may support the substance's potential to affect reproduction across routes of exposure. The result may be that qualitatively we would expect similar health outcomes, but the levels that cause reproductive effects may or may not be possible to predict.
- If there is a need for intermediate-duration (90-day) studies, include discussion of this data need, i.e., examine reproductive organ pathology in a 90-day study.
- When data supports the reproductive system as a possible target organ then recommend multigenerational studies.

### Developmental

- Similar to reproductive health outcomes, the Agency places importance on assessment of developmental toxicity; it is desirable to have such data from inhalation and oral routes prior to developing MRLs.
- State whether there is sufficient information in humans (or in several animal species) to indicate that the substance affects development following exposure via all three routes. Do the animal data support the human data? Remember that the emphasis is on human health significance.
- In the absence of route-specific data, state whether pharmacokinetic data may support the substance's potential to affect development across routes of exposure. The result may be that qualitatively similar health outcomes are expected, but the levels (that cause the effects) may or may not be possible to predict.

## Cancer

- Focus discussion on the qualitative evaluation of carcinogenic potential across routes of exposure and the mechanism(s) of action.
- Regarding mechanism(s) of action draw needs from peculiarities noted in the data, i.e., bolus versus non-bolus effects, vehicle effects, initiation versus promotion, route-specificity, etc.
- In the absence of route-specific data, do pharmacokinetic data may support the carcinogenic potential of the substance across routes of exposure.
- Because the Agency has not formally adopted a non-threshold policy for carcinogens or the use of modeling to derive low-level risks, it is not appropriate to request additional studies for purposes of generating data necessary for modeling.

## Genotoxicity

- Do human data indicate whether the substance may act by a genotoxic mechanism?
- Do *in vivo* animal data (and/or *in vitro* studies) lend support to the substance's genotoxic potential?
- In the absence of genotoxicity data, are there "structural alerts" (e.g., electrophilic centers) that suggest the substance is genotoxic?
- What additional *in vivo* and *in vitro* studies would be important to either confirm or refute the substance's genotoxic potential? If either the Salmonella mutagenicity test or an *in vitro* test for chromosome aberrations is positive, consider requesting *in vivo* tests of chromosome aberrations in exposed humans or animals.
- Consider suggesting lower dose values when genotoxicity testing occurs only at the maximum tolerated dose (MTD).

## Mechanisms of Action

- When conclusive evidence for a specific mechanism is not known, identify potential areas of concern through structure-activity relationships.

## Epidemiological and Human Dosimetry Studies

- Describe any human studies that are currently available and their limitations.
- Is there likely to be an identifiable subpopulation in the general populace and/or in the workplace potentially exposed to the substance?
- Discuss the type of study that might be proposed, and highlight endpoints for which there is information from animal studies or from case studies suggesting that those endpoints may be of concern.
- Relate how this information will be useful for establishing cause/effect relationships and future monitoring of individuals living near hazardous waste sites.

## Biomarkers of Exposure and Effect

Chapter 1 of NAS/NRC (1989; Biological Markers in Reproductive Toxicology) provides a good general discussion of this topic. ATSDR provides this reference to each contractor. This data need should contain the following two subheadings.

**Exposure.** A biomarker of exposure is the measured exogenous substance, or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule or cell (e.g., measurement of the parent compound or its metabolite(s), DNA adducts, etc.).

- Identify known substance-specific exposure biomarker(s), and state the biological materials to monitor for (a) short-term exposure, (b) intermediate- term exposure, and (c) long-term exposure.
- State whether the identified biomarkers are specific for the substance (e.g., metabolites).
- If the parent compound(s) or its metabolite(s) are the only known biomarkers, discuss the usefulness of developing alternative biomarkers to complement this analysis
- Keep in mind that the purpose for developing a biomarker is often to facilitate future medical surveillance, which can lead to early detection and possible treatment.
- Are existing methods sensitive enough to measure (a) background levels in the population and (b) levels at which biological effects occur?
- Identify the data needs and why they are needed.

**Effect.** For the purpose of this data need, a biomarker of effect is a recognized and established or potential health impairment or disease. It is a measurable biochemical, physiological, or other alteration within an organism.

- Identify known biomarker(s) of effect (i.e., enzyme levels, lymphocytes, aberrations) for the substance, and state what biological materials to monitor that will determine effects resulting from (a) short-term exposure, (b) intermediate-term exposure, and (c) long-term exposure. State whether the biomarkers are useful for dosimetry or if it is only indicative of effect.
- Are existing methods sensitive enough to measure (a) background levels in the population and (b) levels at which biological effects occur?
- Identify the data needs and why there is a need.

### Absorption, Distribution, Metabolism, and Excretion

This data need should discuss these parameters by route and duration of exposure; the subsequent data need should describe toxicokinetics across species.

- Is information available to assess relative rates and extent of ADME regarding the three routes of exposure?
- Are there differences in ADME regarding time or dose, i.e., do saturation phenomena come into play?

### Comparative Toxicokinetics

This data need should examine toxicokinetics across species; the preceding data need (ADME) should describe route- and duration-specific pharmacokinetic needs.

- Are there available human and animal data and do they indicate similar target organs?
- Are there toxicokinetic studies in both humans and animals? What do these studies show, i.e., are rats a good model?
- Are there toxicokinetic studies in multiple species? If so, are results similar, and would it be reasonable to expect humans to handle the substance similarly (and have similar target organs)?

For example:

> **Comparative Toxicokinetics.** There are limited data available that allow for a comparison of the toxicokinetic properties across species. Since metabolites are responsible for the toxicity of bromodichloromethane, studies comparing metabolism in different animal species and humans could provide valuable information in extrapolating animal toxicity data to humans.

### Children's Susceptibility

The discussion in this subsection should be closely coordinated with the text in the Developmental Toxicity Data Needs subsection. The following questions should be addressed, with explanations where necessary.

- Have children or immature animals that have been exposed to [Substance X] been adequately studied for health effects? Is there a need for such data? Is there a need to determine if health effects due to [Substance X] can be observed in adults exposed as children?
- Are there any specific theoretical reasons for thinking that children would differ from adults in their vulnerability? Are data adequate to know whether the susceptibility of children to the health effects from [Substance X] actually differs from that of adults? Is there a data need to investigate the susceptibility of children to health effects caused by [Substance X]? Explain why or why not.
- Refer the reader to the Developmental Toxicity Data Needs subsection, with the boilerplate statement: ***Data needs related to both prenatal and childhood exposures, and developmental effects expressed whether prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.*** Sometimes studies designed to observe developmental effects may also identify nondevelopmental health effects during childhood, and vice versa. Where both purposes can be served by a single study, this should be noted in the text.
- Is experimental evidence adequate to evaluate whether pharmacokinetics are different in children, or is this a data need? Are data adequate on whether [Substance X] or its active metabolites can cross the placenta or be excreted in breast milk? Are data adequate to know whether [Substance X] is stored in maternal tissues during pre-conception exposure, and whether any of these stores can be mobilized during pregnancy or lactation? Are there adequate animal data on any of these issues? Are there PBPK models for children, embryos/fetuses/pregnant women, infants/lactating women, or adolescents? Is there a need for this type of model?

- Is experimental evidence adequate to evaluate whether metabolism is different in children or the developing fetus than in adults? Is this a data need? Have any studies in animals been done that might suggest that metabolism might be different in children or the developing fetus than in adults? If the key enzymes metabolizing [Substance X] have been identified, is their expression generally known to differ in children or fetuses compared with adults? Is there a need for information on the specific metabolism of [Substance X] in children or fetuses compared with adults? Are there any data needs related to placental metabolism?
- Is evidence adequate to evaluate whether the mechanisms of action are different in children, developing embryos and fetuses, or immature animals? Is this a data need?
- Is there any reason to suspect that parental exposure might affect children? Are data adequate to determine if this is the case? Explanations may be necessary to describe pharmacokinetics and metabolism in relation to parental germ cells, the genotoxic potential of [substance X] or its metabolites, the ability of [Substance X] or its active metabolites to cross the placenta or accumulate in breast milk, or the ability of [Substance X] to indirectly affect the fetus during maternal exposure.
- Discuss any issues related to childhood cancer and either prenatal or postnatal exposures to [Substance X].
- Have any biomarkers of exposure or effect been validated in children or adult who were exposed to [Substance X] during childhood? Is this a data need? If there are no biomarkers in adults, it may be appropriate to suggest that the development of biomarkers for the general population should take precedence over developing or validating biomarkers in children.
- Are data sufficient to determine whether there are interactions with other chemicals that are unique to children or whether interactions observed in adults occur in children? Is this a data need? Have interactions been observed in immature animals?

## Physical and Chemical Properties

- Do we know enough about the chemical and physical properties (i.e., log $K_{ow}$, log $K_{oc}$, Henry's law constant, vapor pressure, etc.) of the substance to permit estimation of its environmental fate?
- When using toxicokinetic, physical, or chemical information to predict the fate of a substance, indicate the need for confirmation

## Production, Import/Export, Use, and Disposal

In the absence of information on the number of people potentially exposed to the substance near waste sites and other sources, use this data need as a surrogate for evaluating human exposure potential. Include an introductory statement based on the information that supports the potential for human exposure to the substance. For example, if the production volume of the substance is high and its usage is widespread in the home, in the environment, and in industry, then the risk for human exposure may be substantial.

***Production.*** Do we know whether the substance is currently produced and, if so, in what quantity? Do we know if this amount is larger or smaller than in the past? Do we know what production might be in the future?

***Use.*** Do we know whether the substance is widely used in the home, environment, or workplace? Do we know if it is a food contaminant?

***Release.*** Considering typical releases of the substance in the home, environment, and workplace, which environmental media has the most quantities of the substance?

***Disposal.*** Are current disposal methods efficient, and is there a need to improve them? Is there information on the amounts of the substance disposed of by each method? Do we know if there are rules and regulations governing disposal of the substance?

***Regulatory Information***. Do we know if there are rules and regulations governing disposal of the substance?

## Environmental Fate

- Do we know whether the substance partitions in the environment? If so, in what media? Is the substance mobile? Does the substance have a characterization profile?
- Does the substance undergo transport in any environmental medium? Consider a data need for half-life if there is no information on the half-life of the substance. To determine the half-life of a substance in water, soil, and sediment, was field testing or microcosms used? Or is the information from controlled lab experiments? How relevant are the data to real-life situations?

- Is the substance degraded or transformed in each environmental medium? Does it persist in some media? Include the fate of degradation products

### Bioavailability from Environmental Media

- State whether the substance is absorbed following inhalation, oral, or dermal contact.
- State whether there is any information on absorption (bioavailability) of the substance from contaminated air, water, soil, or plant material. If not, can predictions be made? For example, can we predict bioavailability following contaminated soil ingestion if a substance is poorly absorbed from the gut and it has a very large $K_{oc}$ value?

### Food Chain Bioaccumulation

- Do we know whether the substance is bioconcentrated in plants, aquatic organisms, or animals (i.e., elevated tissue levels indicating storage in the organism resulting from exposure to contaminated media)?
- Do we know whether the substance is biomagnified (increased levels in predators resulting from consumption of contaminated prey organisms)?

### Exposure Levels in Environmental Media

- Has the substance been detected in air, water, soil, plant materials, or foodstuffs? *Remember that the focus is on media surrounding hazardous waste sites.* If not, what environmental monitoring studies have happened? If so, are the data current (within 3 years)?
- Have any estimates been made for human intake of the substance from various environmental media?

### Exposure Levels in Humans

- Has the substance been detected in human tissues such as blood, urine, fat, or breast milk? Remember that the focus is on populations surrounding hazardous waste sites. If not, what biological monitoring studies have happened? If so, are the data current (within 3 years)?

### Exposures of Children

This section shall speak to exposures from conception to 18 years in humans. Address the following questions.

- Are children exposed? Is there a need for exposure and body burden studies on children? Are there unique exposure pathways for children? Is there a need for studies to explore this issue?
- It may be appropriate to e relate this issue to data needs about bioavailability from soil and dust for oral, dermal, and inhalation exposure from play activities on the ground and soil pica.
- Are children different in their weight-adjusted intake of [Substance x]? In other words, are children more or less exposed to [Substance x] than adults? Does this issue need studies?
- Are there any childhood-specific means to decrease exposure? Is this a data need?

## 6.3 ONGOING STUDIES

Describe ongoing studies related to filling these data gaps. Narrative text like the following may be used.

> A search of the most recent Federal Research in Progress (FEDRIP [####]) identified numerous research studies that are currently being conducted that may fill some of the data needs discussed in Section 6.2.

Indicate the following when there are no studies.

> No ongoing studies were identified for [Substance x].

# CHAPTER 7. REGULATIONS AND GUIDELINES

This chapter shall begin with the following boilerplate.

**Pertinent international and national regulations, advisories, and guidelines regarding [Substance x] in air, water, and other media are summarized in Table 7-1. This table is not an exhaustive list, and current regulations should be verified by the appropriate regulatory agency.**

**ATSDR develops MRLs, which are substance-specific guidelines intended to serve as screening levels by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites. See Section 1.3 and Appendix A for detailed information on the MRLs for [Substance x].**

There will be *no MRL discussion in Chapter 7*. The boilerplate of Chapter 7 indicates where to find the MRLs.

Organize Table 7-1 by air, water, and food, cancer, occupational, and emergency criteria. Columns include agency, description, information, and reference. Include the agencies as shown in Exhibit 15, Table 7-1. Within each criteria, alphabetize the agencies and organizations. The references will be hyperlinked to the website where the information is stored in the final draft. Include only international guidance from WHO and IARC. Do not include other international organization regulations. *Proposed international standards shall be included on a case-by-case basis.*

Where available, state the EPA, NTP, and IARC cancer classifications. *Do not include the ACGIH cancer classification.* Each carcinogen classifications shall have a footnote that briefly defines it on the last page of the table. Refer to the Code of Federal Regulations or the most recent Federal Register notice for citations.

Units shown in Table 7-1 for international and federal regulations and guidelines are those presented in the cited references.

Describe information that is relevant but does not fit conveniently into the tabular format in a brief paragraph in the introductory text of Chapter 7. If the profiled substance is banned for reasons other than human health risks, explain why.

*Make sure to include any regulations and advisories that have separate values for children.*

Include relevant CDC, NIOSH, FDA, OSHA, or other governmental recommendations about [Substance x] in Table 7-1. Report ACGIH Threshold Limit Values (TLVs) *only when* there is no information for OSHA or NIOSH. *Some of this type of information may not be able to be as presentable in tabular form and should therefore be included in the introductory text of Chapter 7.*

Provide available occupational regulations and guidelines including the current OSHA Permissible Exposure Limit (PEL) for the chemical for an 8-hour workday. Indicate the recommended exposure limit (REL) for time weighted average (TWA) occupational exposures set by NIOSH for the chemical based on a 10-hour average workday and a 40-hour workweek.

*Include applicable national regulations and guidelines whether a number is associated with them or not.* Example, if there is no EPA reference dose (RfD), put "No data" under the information column. For EPA's reference concentration (RfC) and RfD values include a footnote describing the basis of the value. For example:

> The RfD is based on a LOAEL of 17.9 mg/kg/day for renal cytomegaly in chronically exposed mice (NTP 1986).

Do not include descriptions of those regulations and guidelines that are inappropriate for the substance (e.g., pesticide regulations for substances that are not pesticides). Do not use immediately dangerous to life and health (IDLH) information for NIOSH guidelines; use RELs.

# CHAPTER 8. REFERENCES

The intent of this chapter is to provide interested readers with a list of references concerning the toxicology of the substance and environmental fate and exposure information. Every citation in the text, tables, or figures of the profile shall appear with an asterisk in the reference chapter. Citations in the supplemental document shall appear with a plus sign in the reference chapter. The following shall be included on the bottom of the first page of this chapter:

_____
* Cited in text
+ Cited in supplemental document

The contractor must provide copies of the references to the ATSDR chemical managers.

Do not cite secondary sources except when the facts are entirely non-controversial (as in the case of chemical property values such as molecular weight or boiling point). The inability to find or review a primary reference is not cause for citing the secondary reference. In such a case, the primary source should be referenced "as cited in" the secondary reference. In addition, the ATSDR chemical manager must approve any abstract included in the profile.

In general, journal citations shall contain the last names of the authors with the first letter of first. In the case of three or more authors, use commas between author's names and et al. after the third name. Follow the last author with a period and two spaces, and then four-digit year for the publication date. Follow this with a period, two spaces, and title of the article. Capitalize only the first word of the article title unless it includes proper nouns. The title is followed by a period, two spaces, and the name of the journal. Journal names are abbreviated with no periods. The issue and volume number are next. If there is an issue number place that in parentheses. Follow with a colon and the page number range a period on the end. If there is a digital object identifier (DOI) number provide it only once, either as the http hyperlink (preferred) or as the number. Do not have the DOI number appear twice in the reference. A period ends the reference.

To ease the burden of finding references, ATSDR asks that the DOI number be included in the reference. Provide the DOI only once in the citation, either as the http hyperlink (preferred) or as the number. In the examples provided below, the first is the preferred option.

Chan MS, Artichoke BW, Chen Z, et al. 2017. A reference for everything. Hum Reprod 30(11):2645-2657. http://doi.org/10.1093/humrep/dev219.

Chan MS, Artichoke BW, Chen Z, et al. 2017. A reference for everything. Hum Reprod 30(11):2645-2657. 10.1093/humrep/dev219.

Many journals are now providing authors the ability to upload supplemental materials, which may be, but are not limited to, more comprehensive methods, data tables, or other materials. It may be important for the reader to have knowledge of the supplemental document, as such, please label the supplementary material the next letter in the alphabet, for the year of publication. Clarify that it is supplementary material in the title name. See the example, below, and note that the supplemental materials is "b" and has the word supplementary in the title name.

Bloom MS, Whitcomb BW, Chen Z, et al. 2015a. Associations between urinary phthalate concentrations and semen quality parameters in a general population. Hum Reprod 30(11):2645-2657. http://doi.org/10.1093/humrep/dev219.

*Bloom MS, Whitcomb BW, Chen Z, et al. 2015b. Supplemental material: Associations between urinary phthalate concentrations and semen quality parameters in a general population. Hum Reprod 30(11):2645-2657. http://doi.org/10.1093/humrep/dev219.

If citing an article containing an erratum or comment, the related document may have its own separate title, or may just reference the original article. If it does not have its own title, use the original article title followed by the appropriate notes, as shown below. Errata typically have the same author and titles as the original article, so if none are listed, use those listed for the original.

***Errata***

Pellizzari E, Lioy P, Quackenboss J, et al. 1995. Population-based exposure measurements in EPA region 5: A phase 1 field study in support of the National Human Exposure Assessment Survey. (Erratum in: J Expo Anal Environ Epidemiol 5(4):583). J Expo Anal Environ Epidemiol 5(3):327-358.

Pellizzari E, Lioy P, Quackenboss J, et al. 1995. Population-based exposure measurements in EPA region 5: A phase 1 field study in support of the National Human Exposure Assessment Survey. (Erratum to: J Expo Anal Environ Epidemiol 5(3):327-358). J Expo Anal Environ Epidemiol 5(4):583.

***Comment***

Cavaliere MJ, Puga FR, Calore EE, et al. 1998. Protective effect of pralidoxime on muscle fiber necrosis induced by organophosphate compounds. (Comment in: J Toxicol Clin Toxicol 37(3):347). J Toxicol Clin Toxicol 36(4):295-300.

Dilone L, Hack JB. 1999. Organophosphate poisoning: No clear etiological origin. (Comment on: J Toxicol Clin Toxicol 36(4):295-300). J Toxicol Clin Toxicol 37(3):347.

Examples of other common types of reference citations follow.

***Book Chapter***

Krishnan K, Andersen ME, Clewell HJ, et al. 1994. Physiologically based pharmacokinetic modeling of chemical mixtures. In: Yang RSH, ed. Toxicology of chemical mixtures: Case studies, mechanisms, and novel approaches. San Diego, CA: Academic Press, 399-437.

Kroschwitz JI, Howe-Grant M. 1994. Perfluorooctanoic. Kirk-Othmer encyclopedia of chemical toxicology. 4th ed. Vol. 11. New York, NY: John Wiley & Sons, Inc., 551.

***Government Document***

EPA. 1988. Recommendations for and documentation of biological values for use in risk assessment. Washington, DC: U.S. Environmental Protection Agency. PB88179874.

ATSDR. 2008. Public health Assessment for perfluorochemical contamination in Lake Elmo and Oakdale, Washington County, Minnesota. EPA facility ID: MND980704738 and MND980609515. Agency for Toxic Substances and Disease Registry. http://www.health.state.mn.us/divs/eh/hazardous/sites/washington/lakeelmo/phaelmooakdale.pdf. November 13, 2008.

***Database***

ChemIDplus. 2008. Perfluoroalkyls. ChemIDplus. Bethesda, MD: U.S. National Library of Medicine. http://sis.nlm.nih.gov/chemical.html. July 10, 2008.

***TSCATS Microfiche***

3M. 1983. Two year oral (diet) toxicity/carcinogenicity study of fluorochemical FC-143 in rats. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8E. OTS0204926-1.

Miller ME, Temple GW. 1980. Letter from International Minerals and Chemical Corporation to USEPA submitting additional information on 2-nitropropane with attachments. Albany Medical College. Submitted to the U.S. Environmental Protection Agency by International Minerals and Chemical Corporation. OTS0200504. Doc #88-8000156. 8EHQ-1280-0170. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0200504.xhtml. April 5, 2018

***Foreign Language***

Klingmüller D, Alléra A. 2011. [Endocrine disruptors: hormone-active chemicals from the environment: a risk to humans?]. Dtsch Med Wochenschr 136(18):967-972. http://doi.org/10.1055/s-0031-1275832. (German)

If the title was not translated keep the natural language (no need to indicate language):

Mahieu S, Calvo ML, Millen N, et al. 1998. Crecimiento y metabolismo del calcio en ratas sometidas a intoxicacion cronica con hidroxido de aluminio. Acta Physiol Pharmacol Ther Latinoam 48:32-40.

The reference list is single spaced and the second line has a hanging indent of 0.3." References are alphabetized by the first word, typically the agency or the first author's last name. Organize studies as

follows: single authors studies first, followed by double-author studies, and then studies with three or more authors. Alphabetization continues with first author's initials, then second author's last name and initials, and then third author's last name and initials. Further organize by year (oldest studies first) and then article title. Add letters after the year when necessary. If a new reference must be added after letters have been assigned, the new reference should be added at the end of the list instead of its proper alphabetical order to avoid having to re-letter established references.

An example reference list follows.

Abedin Z, Cook RC Jr, Milberg RM. 1980. Cardiac toxicity of perchloroethylene (a dry cleaning agent). South Med J 73:1081-1083.

Abbott BD. 2009. Review of the expression of peroxisome proliferator-activated receptors alpha (PPARα), beta (PPARβa), and gamma (PPARγ) in rodent and human development. Reprod Toxicol 27(3-4, Sp. Iss. SI):246-257. 10.1016/j.reprotox.2008.10.001.

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Abbott BD, Wolf CJ, Schmid JE, et al. 2007. Perfluorooctanoic acid (PFOA)-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator activated receptor-alpha. Toxicol Sci 98(2):571-581.

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# LIST OF ATTACHMENTS FOR GUIDANCE

| Attachment Letter | Description |
|---|---|
| A | Evaluating the Quality of a Toxicological Study |
| B | Evaluating the Quality of an Epidemiological Study |
| C | Checklist for PDF-Ready Copies |
| D | Guidance on Preparing the Supplemental Document |
| E | Guidance on Preparing the LSE Tables and Figures |
| F | Interpreting Renal Pathology in the Male Rat |
| G | Assessing Cholinesterase Activity Inhibition |
| H | Age at Weaning and Sexual Maturity for Common Laboratory Species and Humans |
| I | Historical Background Rates for Various Developmental Outcomes |
| J | Metabolic Enzymes whose Expression or Activity Varies Developmentally |
| K | Alternate Names for Enzymes |

## ATTACHMENT A. EVALUATING THE QUALITY OF A TOXICOLOGICAL STUDY

I. Test material

1. Was it purchased or synthesized in-house?
2. Was the same lot used for all experiments?
3. Were any impurities present? If so, were the impurities removed?
4. Is the test material stable under experimental conditions? If not, were any adjustments made?
5. Was a vehicle used for administration?
6. Were the doses reported in the study?

II. Animal selection

1. What is the rationale for the species selection?
2. Were the animals disease-free?
3. Is the model appropriate for the end-point effects studied?
4. Optimal criteria at specific intervals:

| | Acute | Intermediate | Chronic |
|---|---|---|---|
| Number of treatment groups | ≥3 | ≥3 | ≥3 |
| Animals/group | 6–10 | 10–20 | 50/sex |
| Animal age | >6 weeks | Young adult | Young adult |
| Control groups | Required | Required | Required |

5. Are the species, strain, sex, age, treatment schedule, and vehicle the same for control as for treated animals?

III. Study design

1. Are the route(s) expected for human exposures or other (inhalation, oral [diet, drinking water gavage, other], dermal [intact, abraded, occluded])?
2. Is the exposure regimen daily, continuous, or intermittent (e.g., 6 hours/day, 5 days/week)?
3. Is the mortality loss for a chronic study no more than 5–10%?
4. Optimal criteria at specific intervals:

| | Acute | Intermediate | Chronic |
|---|---|---|---|
| Dose selection | ≥3 | Not specified | 2 (MTD and LOAEL from a 90-day dose screen) |
| Period of exposure | ≤14 days | 15–364 days | ≥365 days |
| Period of observation | 14 days | Every 12–24 hours | Every 24 hours |
| Body weight measured | Weekly | Weekly | Weekly to week 13 then every 2 weeks |

IV. Endpoint effects

1. Were appropriate methods used to measure endpoint effects?
2. Were these methods state-of-the-art?
3. Was a dose-response relationship established?
4. Did the study sufficiently demonstrate a NOAEL or LOAEL?
5. Were appropriate statistical analyses performed?
6. Were the results statistically significant (at least $p<0.05$)?
7. Optimal criteria at specific intervals:

| | Acute | Intermediate | Chronic |
|---|---|---|---|
| Organ weights recorded | Not specified | Liver, kidney, brain, gonads, heart, etc. | Same as intermediate |
| Histopathological gross exam | Gross necropsy | Necropsy and histopathology for target organs | All tissue in at least control and highest dose group |

## ATTACHMENT B. EVALUATING THE QUALITY OF AN EPIDEMIOLOGICAL STUDY

I. Overall criteria

1. The study has been published or peer reviewed.
2. The paper should provide:
   a. Background (i.e., supporting rationale, definition, and explanation of the problem).
   b. Study objectives and study design, including assumptions, limitations, and statement of purpose or hypothesis.
   c. Study population and control group (i.e., method of selection, rationale and criteria for inclusion/exclusion, appropriateness and limitations of control group).
   d. Data collection method, including direction and possible magnitude of any bias introduced into the study (i.e., may be single-, double-, or triple-blind to prevent bias). Quality assurance (QA), quality control (QC), or calibration data are presented for the data collection instrument (method).
   e. Type and length of follow-up.
   f. Account for (via matching, stratification, multivariate analysis, etc.) and clearly define major confounding factors.
   g. Procedures and statistical methods used for data analysis. Significance levels need to display a strong association ($p<0.05$) to rule out the probability of the results occurring by chance variation.
   h. Results that are related to the objectives of the study. Do the numbers in the tables add up?
   i. Discussion of limitations and biases that may have affected the results. The examination of causality (conclusion) should be supported by the results.
   j. A logical, temporal sequence of exposure-response that is toxicologically plausible.
   k. A demonstrated dose-response relationship using valid estimates of exposure and dose.

II. Types of epidemiological studies

1. Observational studies
   a. General points
      1) These studies are rarely designed to provide quantitative risk information.
      2) Groups are already divided on the basis of some experience or exposure (not created experimentally).
      3) Sample size (N) should consider the size of the difference being detected (i.e., rare or common).

4) Small N does not mean study should not be done, rather it might indicate that nothing could be found in the population. The study may need to state that numbers were too few to detect an excess risk.

b. Main types

1) Retrospective (case-control)

a) These studies are helpful for monitoring substance/drug exposure.

b) A positive association is demonstrated between the exposure and the disease/effect if the diseased group is more likely to be exposed than the group not diagnosed with the disease/effect. Researcher looks historically to determine exposure after the disease/effect has been determined.

c) Cases:

(1) The study group must be delineated precisely, not generalized (e.g., premenopausal women and lobular breast cancer).

(2) Optimally, the study should use newly diagnosed cases with specified characteristics during a specified period in a defined population. Deceased cases as well as those alive when study is undertaken should be included.

d) Controls:

(1) Controls should be representative of the general population in terms of probability and opportunity for exposure, and should represent the population from which cases arose.

(2) Individual matching is optimal.

e) Advantages

(1) The number of subjects can be small because the study is initiated by the identification of cases.

(2) More than one risk factor in the same set of data can be identified.

(3) Studies can take into consideration changes in exposure.

f) Disadvantages

(1) Information on past events may be inaccurately recorded or not available.

(2) Information supplied by an informant may be consciously or unconsciously biased.

(3) The study yields only an odds ratio that is an estimate of relative risk (i.e., a comparison of incidence for exposed versus unexposed populations). It is advisable to select more than one control group.

2) Prospective (cohort or longitudinal)
    a. Cohort is free of disease/effect but varies in exposure to the supposed factor. The exposed group is then followed to see if the disease/effect develops. The assumption is that exposed individuals are representative of all exposed persons regarding the risk of disease/effect development.
    b. A positive association is demonstrated between the exposure and the disease/effect if the exposed group develops the disease/effect at a greater rate than those not exposed.
    c. Cohort needs to be as similar as possible to the group it is intended to represent.
    d. Advantages:
        1) Permits calculation of incidence rates among exposed and not exposed. Incidence = number of new cases/total population at risk.
        2) Permits observation of many outcomes.
    e. Disadvantages:
        1) Long-term follow-up may be difficult.
        2) Large cohort (study group) is expensive.
3) Historical prospective
    a. Combines advantages of retrospective and prospective
    b. Follows historically identified healthy exposed and unexposed cohorts for the development of disease/effect.
    c. Can calculate actual incidence and relative risk.
4) Cross sectional (prevalence): Both risk factors and disease are determined at the same time (e.g., prevalence of coronary heart disease and serum cholesterol level).

2. Experimental studies: General points
    a. The impact of varying some controlled factor is studied.
    b. These studies are not common, for obvious reasons.
    c. Subjects should be divided into treatment groups by random allocation.

3. Occupational studies
    a. Ecological
        1) Generate hypotheses.
        2) A group rather than individual is the unit of comparison.
    b. Cross sectional (prevalence)

1) Observations of a group are made at one point in time, yielding prevalence rates. Prevalence = number of old and new cases/total population at risk.
2) These studies represent one of the most frequently used ways of identifying a disease/effect in a community (survey, screening).
3) Cases of short duration are less likely to be found than cases of long duration.
4) These studies are especially suited for subtle, subclinical health effects for which records are unlikely to exist.
5) The relationship between effects and time cannot readily be explored.

c. Case control

1) These studies are used when the disease/effect of interest is relatively rare and would require a large cohort for follow-up.
2) Environmental concentrations and biological levels are often measured.
3) Several occupations or substances may be associated with the disease/effect of interest.
4) The influence of various modifiers can be studied (synergism).
5) Previous jobs are often of greater relevance than current, therefore entire work history needs examination.

d. Cohort

1) Occupational cohort studies are usually mortality studies.
2) Cohort should be defined as broadly as possible, prevalence or incidence.
3) Eliminating workers from the cohort who are not active can lead to serious biases in assessing mortality because this can distort the age distribution of the cohort and omit workers who left because of ill health.
4) Dose-response relationships or high-risk jobs are searched for by dividing cohort into exposure level groups.

## ATTACHMENT C. CHECKLIST FOR PDF-READY COPIES

Toxicological Profile for _____

| Contractor | ATSDR | |
|---|---|---|
| | [] | Contractor Checklist Verified |
| **Title Page** | | |
| [] | [] | Month and Year of Release are Correct |
| [] | [] | Draft for Public Comment – No Data in Running Footer on any Page |
| [] | [] | Final – No Footer on any Page |
| [] | [] | Final – "Draft" Removed From Title |
| **Pagination** | | |
| [] | [] | Disclaimer is on Page ii |
| | | The following parts start on odd-numbered pages: |
| [] | [] | - Foreword |
| [] | [] | - Version History |
| [] | [] | - Contributors |
| [] | [] | - Contents |
| [] | [] | - List of Figures |
| [] | [] | - List of Tables |
| [] | [] | - Each Chapter |
| [] | [] | - Appendices |
| **Other** | | |
| [] | [] | Contents, List of Figures, List of Tables – Words and page numbers match the words and page numbers in the text |
| [] | [] | MRLs are expressed to one significant figure |
| [] | [] | Names and titles of peer reviewers have been verified |

_____ _____

Contractor/Author Date

_____ _____

ATSDR Chemical Manager Date

## ATTACHMENT D. GUIDANCE ON PREPARING THE SUPPLEMENTAL DOCUMENT

The supplemental document is not a public document. Produce it prior to the development of the profiled substance. The document consists of the summary tables that contain summary information from all studies reviewed for potential inclusion in the health effects chapter of the profile.

The supplemental document is produced using the ATSDR EZ-TOX database. Access to the database is administered by ATSDR. Basic guidance for the content development and appearance follows below.

### *Formatting*

- Tables are landscaped, center justified, and 9.5 inches wide
- Single line spacing
- Header from top and footer from bottom both set at 0.3”

The header font is in Arial 8-point, all caps. The header will contain the chemical name flush with the left margin and the page number flush with the right margin. The header should appear on all pages except the title pages, foreword, and legends.

The footer is in Arial 8-point font, all caps and is centered on the page. ***DRAFT – DO NOT CITE OR QUOTE – [Month day, year]*** (define with the date code so that MS Word automatically populates with the date of the draft). For the final pre-public-comment and post-public-comment versions, the date should be removed from the footer.

The table title must be 12-point bold Arial font, within the 1st row of the table. Table titles are first letter capitalized and centered, and there is a hard return after the title. Table titles are first letter capitalized and centered, and there is a hard return after the title. The title row has light blue shading (Red 222, Green 234, Blue 246). The header row has light gray shading (Red 242, Green 242, Blue 242) and text that is first letter capitalized and left justified in 10-point Arial font. Both title and header rows are defined so that they repeat over multiple pages. Use a darker gray shade (Red 217, Green 217, Blue 217), for rows that divide sections (e.g., acute, intermediate, chronic). The font for data entries in the table shall be Arial 10-point. There are horizontal lines separating study rows in tables; there is no horizontal line above the table title row. No vertical lines are used in tables.

## *Front Matter*

### Title Page

This should follow the format shown Figure D-1 within this attachment. The word "DRAFT" should remain in the title of all submissions of the supplemental document.

### Foreword

This should follow the format shown in Figure D-2.

### Legend

There are standard legends for the summary table (see Figure D-3). The legend defines the abbreviations and codes used within the table. Abbreviations for the tables and legends, which are also the result of the proper use of the worksheets, are shown in Appendix G of this guidance.

It may be necessary to abbreviate terms within descriptions of effects associated with LOAELs. Add an explanation of these abbreviations to the table legend. If these abbreviations are not substance-specific, then add them to the guidance.

The legends contain abbreviations for common prenatal and postnatal time measurements.

List the source of conversion factors at the bottom of each legend, as follows in the legend figures. ***The conversion factors used in the supplemental document are from EPA 1988 (see reference in this attachment).***

## *Summary Table for Toxicity Studies*

Figure D-4 is an example of the summary tables for toxicity studies. EZ-TOX, a computer database, generates these tables. The tables are generated from records which summarize the available toxicity data base for the profiled substance. Route-specific summary tables are prepared for inhalation, oral, and dermal exposure; discuss with the chemical manager whether a table for other routes of exposure should be prepared. Create separate records for studies testing multiple exposure routes, species, and/or durations. Genotoxicity and *in vitro* studies do not need summarization in the supplemental document.

## Study Design

### *Species, Strain, Number, and Sex*

- Include the species, animal strain in parenthesis, and the number of animals/sex/group.
- Use M and F to indicate males and females, use B (both) when the study does not specify how many animals per sex, and NS when the study does not report the number of animals per group or the sex.

### *Exposure Duration, Frequency, and Route*

- Include information on the duration of exposure and the frequency of exposure
- Use the term “once” instead of “1 time/day” if the substance was only administered once. For inhalation studies and dermal studies conducted for <1 day, use the actual time, e.g., 4 hours, 10 minutes.
- For studies involving gestational and/or lactational exposure, indicate the exposure days.

  Example: GDs 6–18, LDs 1–4, where GD = gestation day(s), LD = lactation day(s).
- When an exposure occurs at a time other than adulthood, specify the age of the animal in the “Exposure duration/frequency” category.
- If the study involved pulse or complicated dosing regimens, briefly explain the regimen as fully and succinctly as possible under the "Exposure Duration/Frequency" column. You may need to resort to an inexact, simplified portrayal of the regimen that more fully conveys the effective dose, as opposed to simply listing as many details as space permits and having the rest deleted. Then, explain the regimen fully in the Description section.
- Be consistent in the order in which entering information in the "Exposure Duration/Frequency" category, e.g., total number of days and frequency of exposure.

  Example: 13 wk<br>6 hr/d<br>5 d/wk
- For the oral and other routes of exposure tables, indicate the dosing method using appropriate abbreviations in parentheses

| | |
|---|---|
| F | Dietary exposure |
| W | Drinking water |
| G | Gavage, neat or not specified vehicle |
| GW | Gavage with aqueous vehicle |

| | |
|---|---|
| GO | Gavage with oil vehicle |
| C | Capsule |
| IM | Intramuscular |
| IP | Intraperitoneal |
| IT | Intratracheal |
| IV | Intravenous |
| SC | Subcutaneous |

***Doses***

- All exposure levels shall be expressed as concentrations (air), administered dose (oral and other routes of exposure), or applied dose (dermal). Do not attempt to estimate absorbed dose.
    - If the investigator estimated absorbed dose or dose to specific organs, it may be appropriate to include this in the text of the profile.
- Use ppm or mg/$m^3$ for inhalation (ppm for gases, mg/$m^3$ for particulates) and mg/kg/day for oral exposure. If necessary use µg, ng, or another unit, instead of mg to keep dose information in the whole-number range; the same unit should be used throughout an LSE table.
- Convert all salts to the parent substance, express in the table as units of parent compound (e.g., mg Cr/$m^3$ and mg Cr/kg/day, for chromium).
- Consistently use the same units (as found in the "dose" column headings) for oral and inhalation data.
- For dermal exposure records, remove the units from the "dose" column heading and enter the units for each level in the table beside the level. The data for dermal exposure are often in different units and conversions are often not possible or useful (i.e., reference or standard conversions are not helpful).
- Do not adjusted concentrations or doses for intermittent exposure

***Parameters Monitored***

- List the parameters monitored in the study, using the following codes:

| | |
|---|---|
| BC | serum (blood) chemistry |
| BI | biochemical changes |
| BW | body weight |
| CS | clinical signs |
| DX | developmental toxicity |
| FI | food intake |
| GN | gross necropsy |
| HE | hematological |
| HP | histopathology |
| IX | immune function |
| LE | lethality |
| NX | neurological function |

OF organ function
OP ophthalmology
OW organ weight
RX reproductive function
UR urinalysis
WI water intake

- Use BC for all non-hematological parameters measured in serum/blood including enzymes, electrolytes, serum proteins, albumin, urea, cholesterol, etc.
- Use BI for biochemical indices measured in non-blood tissues, such as enzyme levels, liver cholesterol, bone collagen, etc.

## Study Results

### *System*

- The systems are the same as the health effect categories used in Chapter 2 (i.e., death, body weight, respiratory, cardiovascular, etc.).
- List to the developing organism or offspring as "developmental" in the table and not under specific effect categories, e.g., neurological. List maternal effects under the specific effect.
- Do not include *in vivo* genotoxicity studies in the supplemental document.

### *NOAEL and LOAEL*

- See the discussion in the Chapter 2 section on categorizing NOAEL and LOAEL effects and health effect-specific less serious and serious LOAELs in Sections 2.3–2.18 (Tables 2-A–2-T).
- Identify the highest NOAEL and lowest LOAEL for each endpoint; if applicable include both less serious and serious LOAEL values
- If the study shows both less serious and serious effects at the same dose level for the endpoints, only list the effects under the serious category.
- Do not list NOAEL values for death or cancer
- Indicate sex differences under the following four conditions. In all cases, the sex of the animal should be entered one space to the right of the dose, using "M" to indicate male and "F" to indicate female.
    1. Where effect and/or effect levels are different between males and females (see Example A).

**Example A**.

| | NOAEL | LOAEL | LOAEL (serious) |
|---|---|---|---|
| Neurological | 180 M | 320 M | 30 F |

2. Where the study used different doses (accounting for differences greater than 10%) for males and females. For example, for male rats dosed at 50, 180, and 320 mg/kg and for female rats dosed at 30, 125, and 280 mg/kg, report results as shown in Example B.

**Example B**.

| | NOAEL | LOAEL |
|---|---|---|
| Respiratory | 50 M<br>125 F | 180 M<br>280 F |

3. When the study examined both sexes but only one sex for a particular endpoint enter the sex after each level in the table.
4. Where both sexes exhibited only NOAELs for a certain endpoint, list the NOAELs for each sex (see Example C).

**Example C**.

| | NOAEL | LOAEL |
|---|---|---|
| Renal | 320 M<br>250 F | |

***Effect***

- Briefly describe the effect.
- When presenting a health effect that occurs with varying severity, clearly describe the degree or magnitude of the effect (e.g., hepatic, less serious LOAEL of "small, infrequent foci of necrosis with no biochemical or functional alterations;" hepatic, serious LOAEL of "frequent focal and coalescing areas of necrosis with markedly elevated AST and ALT"). Include all adverse effects noted under the endpoint.

  Example:

| | NOAEL | LOAEL | LOAEL (serious) | Effect |
|---|---|---|---|---|
| Hepatic | 25 | 75 | 250 | Elevated ALT and AST at ≥75 ppm, severe necrosis at ≥250 mg/kg/day |

- Clearly define the endpoint effect(s), and do not use "generic descriptions." If alternate descriptions are available then avoid nonspecific terms such as degeneration, severe signs of neurotoxicity, necrosis, neurological effects, central nervous system effects, fatty changes, and the like. For example, rather than "increased enzyme activity," specify "increased levels of ALT, AST."

- For developmental studies, including those conducted during lactation, indicate if the effects were to the dam or fetus if it is not intuitively obvious (e.g., delayed vaginal opening in pups, increased heme synthesis in dams).
- If available, include percentages or ratios for effects that may occur with varying incidence or magnitude (e.g., percent resorption, percent decrease in body weight/body weight gain and number of deaths/number of test animals).
- For cancer effects, use the term CEL rather than LOAEL. Always list the CEL dose/concentration should be listed in the serious LOAEL column. *Note: In some cases, it may be appropriate to use levels that are NOT statistically significant, because the resolving power of the study may be insufficient to capture cases where tumors are historically rare. Discuss this fact with the chemical manager.* When providing CELs in the table, include the type of cancer in the effect(s) column, e.g., 25 CEL: liver tumors, 10 CEL: acute myelogenous leukemia. If there are observable difference in cancer effects (e.g., at levels higher than the lowest CEL), include both levels and effects in the Effect column; listing the lower CEL first. For example, CEL: lymphoma at ≥10 ppm; CEL: hepatocellular carcinoma at ≥100 ppm

**Comment Sections**

***Compound***

- When studies were conducted using various forms of the compound (e.g., salts, isomers, isotopes), indicate the form used in the line above the reference.
- If multiple compounds are included in one table, indicate the compound in the line above the reference.

***Calculation***

- Describe any dose calculations in this section. Omit this section if there are no dose calculations.
- Make the purpose of a conversion readily apparent. Show units and the conversion factor name parenthetically unless the function of the conversion is obvious (such as 5–5,000 mg). *Do not* restate conversion descriptions for subsequent, identical conversions (in the same record).
- If conversion from concentration in food or drinking water to estimated daily dose is required, and the necessary information (e.g., body weight, food or water consumption) is not in the reference, refer to the following document for standard assumptions:

EPA. 1988. Recommendations for and documentation of biological values for use in risk assessment. Cincinnati, OH: U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, for the Office of Solid Waste. PB88-179874.

- Include calculations to convert doses from administered salts to parent compound.
- Example of calculation text:

  Doses were estimated using reported TWA dietary concentrations (0.008 and 0.03% for males and 0.004 and 0.01% for females) and reference body weights (0.380 and 0.229 kg for males and females) and food intakes (0.030 and 0.021 kg/day). M: 0.008% = 80 mg/kg food; 80 mg/kg food x 0.030 kg diet/day x 1/0.380 kg body weight = 6 mg/kg/day; 0.03% =20 mg/kg/day. F: 0.004% = 40 mg/kg food; 40 mg/kg food x 0.021 kg/day x 1/0.229 kg =4 mg/kg/day; 0.01% =9 mg/kg/day.
- Significant figures
  - Do *not* round numbers until after *all* conversions have taken place. At that point, round value to the same number of significant figures as the datum point. Using the numbers from the example above, the lowest number of significant figures is one for dietary concentrations (0.008 and 0.03% for males and 0.004 and 0.01% for females). Therefore, round the daily doses to one significant figure; resulting in doses of 6 and 20 mg/kg/day for males and 4 and 9 mg/kg/day for females.
  - If more than one experimentally determined datum point enters into the conversion, use the number of significant figures that the datum with the least number of significant figures. In some cases, authors will want to use more significant figures than are technically appropriate. A common instance where this might be done is when experimenters report a dietary concentration as 5 and 7.5%. It is safe to assume that the experimenters actually measured the substance with more precision than the single significant figure stated (i.e., 5.0%). In this example, report the calculated dose using two significant figures. Disregard the number of significant figures that are in the conversion factors themselves unless your scientific judgment dictates that the precision of a certain conversion factor is pertinent.
  - Standard scientific practice is to resort to scientific notation in order to present large numbers with unambiguous precision. Nevertheless, scientific notation would be confusing to some lay readers and burdensome for table preparation. Therefore, do not use scientific notation only to retain precision. For example, round 2,557 to 2,600, *not* as $2.6x10^3$.

- Double-check all calculations and conversions during the preparation and quality control of the supplemental document.

***Description***

- Describe the protocol and procedures of the study in detail. Including the goal of the study may be a good way to structure the Results section by determining what effects are most important. Include the number and sex of animals, the strain and species, the exposure regimen including dose levels, duration, number of exposures and route of exposure, techniques used, and tissues or activities monitored when reported.

  Example: Groups of five male and five female Sprague-Dawley rats were administered 10 or 100 mg/kg/day chloroform in oil once daily via gavage for 5 days. Five rats of each sex served as controls. Measures included liver and kidney weights and ALT and AST activities. Liver, kidney, and lung tissues underwent histopathological examination.

***Results***

- Summarize the results of the study and the author's conclusions. Note the most essential results of the study. Summarize all effects vital to the profile in the Results section. Discuss the following relevant or useful information.
  - Lowest levels at which nonadverse effects were seen (if different from lowest adverse levels)
  - Gradation of effects
  - Incidence of effect (for cancer) or magnitude of effect
  - Sex and/or strain difference.

***Comments***

- Include any supporting information in this section; omit this section if there is no additional information.
- The type of information which can be included in this section:
  - Citations for other reports discussing the same study, e.g., the results of this study are also reported in Smith et al. (1987)
  - Supporting information for calculations
  - Author’s conclusions
  - Study limitations

- When considering study limitations, include the following: Were appropriate statistical analyses performed? Are results biologically significant? Were the data presented accurately (do the numbers in tables and figures add up and agree with the text)? Is the overall quality of the study adequate? Negative answers to any of the above questions, should be noted in this section. Additionally, consider the following when describing study adequacy and/or the appropriateness: test material purity, vehicle, procedural problems, mortality, methods to evaluate endpoints, endpoints measured, observation frequency, number of animals, number of controls, control appropriateness, number of treatment groups, exposure route, treatment regimen.

### *Quality Assurance/Quality Control*

Quality control procedures should be in place to ensure minimal discrepancies in the information extracted from papers and calculations. Authors shall use as much care as is necessary to produce a quality supplemental document because it is the underpinning for making statements. Complete the supplemental document with the first draft of the toxicological profile.

**Figure D-1**

# DRAFT

# SUPPLEMENTAL DOCUMENT FOR [Substance x]

Prepared by:

[Contractor Name and Address]

Prepared for:

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

[Month, year]

**Figure D-2**

# FOREWORD

This document presents summary tables for studies reviewed for the Toxicological Profile for [Substance x].

# Figure D-3

## Legend for Summary Tables for Toxicity Studies For [Substance X]

**Header**

| | |
|---|---|
| kg | kilogram |
| LOAEL | Lowest-observed-adverse-effect-level |
| $m^3$ | Cubic meters |
| mg | Milligram |
| mg/kg/day | Milligram per kilogram per day |
| NOAEL | No-observed-adverse-effect-level |
| ppm | Parts per million |

**Species/Strain/No. & Sex**

| | | | |
|---|---|---|---|
| F | female | NS | Not specified |
| M | Male | B | both |

**Exposure Duration/Frequency**

| | | | |
|---|---|---|---|
| x | Time | Wk | Week(s) |
| D | Day(s) | Mo | Month(s) |
| Hr | Hour(s) | Yr | Year(s) |
| Min | Minute(s) | Sec | Second(s) |
| Ad lib | Ad libitum | NS | Not specified |
| GD | Gestation day | LD | Lactation day |
| PND | Post-natal day | gen | generation |
| PPD | Post-parturition day | | |

**Subroute**

| | | | |
|---|---|---|---|
| (G) | Gavage – not specified | (F) | Feed |
| (GO) | Gavage-oil | (W) | Water |
| (GW) | Gavage-water | (C) | Capsule |
| (IP) | Intraperitoneal | (IT) | Intratracheal |
| (IV) | Intravenous | (IM) | Intramuscular |
| (SC) | Subcutaneous | (inV) | In vitro |
| (environ) | Environmental | (Occup) | Occupational |
| (IN) | Ingestion | | |

**Parameters Monitored**

| | |
|---|---|
| BC | Serum (blood) chemistry |
| BI | Biochemical indices |
| BW | Body weight |
| CS | Clinical signs |
| DX | Developmental toxicity |
| GN | Gross necropsy |
| HE | Hematological |
| HP | Histopathology |
| IX | Immune function |
| LE | Lethality |
| NX | Neurological function |
| OF | Organ function |
| OW | Organ weight |
| RX | Reproductive toxicity |
| UR | Urinalysis |
| WI | Water intake |

**System**

| | |
|---|---|
| Bd wt | Body weight |
| Cardio | Cardiovascular |
| Derm | Dermal |
| Endocr | Endocrine |
| Gastro | Gastrointestinal |
| Hemato | Hematological |
| Immuno | Immunological |
| Musc/skel | Musculo/skeletal |
| Resp | Respiratory |

## Legend for Summary Tables for Toxicity Studies For [Substance X] (continued)

**Effect**

| | |
|---|---|
| AChE | acetylcholinesterase |
| aden | adenoma |
| ALT | alanine aminotransferase |
| AST | aspartate aminotransferase |
| behav | behavior |
| biochem | biochemical |
| BUN | blood urea nitrogen |
| bw | body weight |
| $^{14}C$ | carbon 14 |
| CBD | common bile duct |
| CEL | cancer effect level |
| CK | creatine kinase activities |
| CNS | central nervous system |
| decr | decrease |
| degen | degeneration |
| DMBA | 7,12-dimethylbenz[a]anthracene |
| MSO | dimethyl sulfoxide |
| ECG | electrocardiogram; electrocardiography |
| EDTA | ethylenediamine tetraacetic acid |
| EEG | electroencephalograph |
| EKG | electrocardiograph |
| EMG | electromyograph |
| ESWL | extracorporeal shock wave lithotripsy |
| FOB | functional observational battery |
| GGT | γ-glutamyl transferase |
| GSH | glutathione |
| Hb | hemoglobin |
| Hct | hematocrit |
| incr | increase |
| $LC_{50}$ | concentration producing 50% death |
| $LD_{50}$ | dose producing 50% death |
| LDH | lactate dehydrogenase |
| $LT_{50}$ | exposure time producing 50% death |
| MAI | motor activity index |
| MCHC | mean corpuscular hemoglobin concentration |
| MCV | mean corpuscular volume |
| MFO | mixed function oxidase |
| mL | milliliters |
| MNNG | N-methyl-N'-nitro-N-nitrosoguanidine |
| NADPH | nicotinamide adenine dinucleotide phosphate (reduced form) |
| NDMA | nitrosodimethylamine |
| ppb | parts per billion |
| QRS | region on the electrocardiogram produced by ventricular activation |
| RBC | red blood cell(s) |
| SDH | sorbital dehydrogenase |
| sec | second |
| SGOT | serum glutamic oxaloacetic transaminase |
| SGPT | serum glutamic pyruvic transaminase |
| TPA | 12-o-tetradecanoylphorbol-13-acetate |
| TWA | time-weighted average |
| UDPGT | uridine diphosphoglycuronyl transferase |
| WBC | white blood cell(s) |
| wt | weight |

## Figure D-4

**Summary Table for Toxicity Studies for Exposure to [Substance X]– Oral**

| Species/ Strain/No. & Sex | Exposure Duration/ Frequency (Route) | Doses | Parameters Monitored | System | NOAEL (mg/kg/day) | LOAEL | | Effect |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Less Serious (mg/kg/day) | Serious (mg/kg/day) | |
| **INTERMEDIATE EXPOSURE** | | | | | | | | |
| Rat Wistar 6F | 10-18 wk (F) | 0, 15, 150 | CS, BW, OW, HP, DX | Bd wt | | 15 | | Body weight decreased by 15% |
| | | | | Resp | 150 | | | |
| | | | | Cardio | 150 | | | |
| | | | | Hepatic | 15 | 150 | | Slight proliferation of bile duct epithelium |
| | | | | Renal | | 15 | 150 | Tubular degeneration and necrosis of cells at 15 mg/kg/day; extensive tubular degeneration at 150 mg/kg/day |
| | | | | Endocr | 150 | | | |
| | | | | Immuno | 150 | | | No histological alterations |
| | | | | Neuro | 15 | | 150 | Ataxia, demyelination and fragmentation of femoral nerve fibers |
| | | | | Repro | 15 | | 150 | Infertility |
| | | | | Develop | | 15 | | 16-19% reduction in pup weight |

**Harleman and Seinen 1979**

**Calculation:** Doses were calculated using a reference body weight of 0.156 kg and food consumption of 0.016 kg/day. 150 mg/kg diet x 0.016 kg diet x 1/0.156 kg = 15 mg/kg/day; 1,500 ppm = 150 mg/kg/day

***Description:*** Groups of 6 female Wistar rats were exposed to 0, 150, or 1,500 ppm hexachlorobutadiene in the diet (equivalent to a dose of 15 and 150 mg/kg/day). After 4 weeks on the diet, the rats were mated with untreated males during a 3-week mating period. It is unclear whether the animals were exposed after 4 weeks. At parturition, pups were weighed and the number of pups were reduced to 8 pups per litter. At study week 18, the rats were sacrificed and maternal heart, liver, kidney, spleen, brain, adrenal, thymus, and thyroid weights were measured. Histopathological examination of the major tissues and organs were examined histologically.

**Summary Table for Toxicity Studies for Exposure to [Substance X]– Oral**

| Species/ Strain/No. & Sex | Exposure Duration/ Frequency (Route) | Doses | Parameters Monitored | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Effect |
|---|---|---|---|---|---|---|---|---|
| **Results:** At 150 mg/kg/day, the rats progressively lost weight and displayed hindlimb weakness and unsteady gait, progressing to ataxia without paralysis by week 6; animals in this group were sacrificed during week 10. Decreases in body weight were observed in the 15 mg/kg/day; at termination, the rats weighed 15% less than controls. Increases in relative kidney weight were also observed at 15 mg/kg/day; no alterations were observed in other organ weights. Histological alterations were observed in the kidneys, consisting of hypocellularity of epithelial lining cells and hydropic degeneration and necrosis of individual cells in the straight limb of the proximal tubules at 15 mg/kg/day and extensive tubular degeneration at 150 mg/kg/day. Other histological alterations observed in the 150 mg/kg/day group only included slight proliferation of the bile duct epithelial cells in the liver, fragmentation and demyelination of single fibers in the femoral nerve. Infertility was observed at 150 mg/kg/day (no pregnancies were apparent). No alterations in fertility, the number of pups per litter, or pup survival were observed at 15 mg/kg/day. Significantly lower pup body weights were observed on PND 0, 10, and 20 (16, 16, and 19%, respectively). The investigators noted that gross malformations were not observed. | | | | | | | | |
| Rat Wistar 10M, 10F | 13 wk (GO) | 0, 0.4, 1, 2.5, 6.3, 15.6 | BW, FI, HE, BC, UR, OW, HP | Bd wt | 2.5 | | 6.3 | Body weight decreased by 29% in females and by 13% in males |
| | | | | Resp | 15.6 | | | |
| | | | | Cardio | 15.6 | | | |
| | | | | Gastro | 15.6 | | | |
| | | | | Hemato | 15.6 | | | |
| | | | | Hepatic | 6.3M | 15.6M | | Increased cytoplasmic basophilia |
| | | | | Renal | 2.5M<br>1F | 6.3M<br>2.5F | | Enlarged hyperchromatic nuclei in the proximal tubules in females at 2.5 mg/kg/day and male at 6.3 mg/kg/day; decreased urine osmolarity in females at 2.5 mg/kg/day |
| | | | | Endocr | 15.6 | | | |
| | | | | Immuno | 15.6 | | | |
| | | | | Neuro | 15.6 | | | |

**Harleman and Seinen 1979**

## Summary Table for Toxicity Studies for Exposure to [Substance X]– Oral

| Species/ Strain/No. & Sex | Exposure Duration/ Frequency (Route) | Doses | Parameters Monitored | System | NOAEL (mg/kg/day) | LOAEL | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Less Serious (mg/kg/day) | Serious (mg/kg/day) | Effect |
| ***Description:*** Groups of 10 male and 10 female Wistar rats were administered 0, 0.4, 1, 2.5, 6.3, or 15.6 mg/kg/day hexachlorobutadiene in arachid oil via gavage for 13 weeks. The following parameters were used to assess toxicity: body weights and food consumption measured twice weekly, hematological (hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts) and serum clinical chemistry (total protein, albumin, globulin, BUN, aspartate aminotransferase, γ-glutamyl transpeptidase, and alkaline phosphatase) indices measured at week 8 and termination, urinalysis indices (glucose, protein, hemoglobin, ketones, pH, total urine production, and osmolarity) assessed at week 10, organ weights (heart, liver, kidneys, spleen, brain, adrenals, thyumus, thyroids, gonads), and histopathological examination of major tissues and organs from the 15.6 mg/kg/day group (selected organs were also examined in other groups).<br><br>***Results:*** Significant decreases in body weight gain were observed at 6.3 and 15.6 mg/kg/day; a dose-related decrease in food consumption was also observed at 6.3 mg/kg/day in females and 15.6 mg/kg/day in males and females. No alterations in hematological or serum chemistry indices were noted. Urine osmolarity was significantly decreased in females at ≥2.5 mg/kg/day and in males at 15.6 mg/kg/day; a significant increase in total urine production was also observed in females at ≥6.3 mg/kg/day. Significant increases in relative liver (≥6.3 mg/kg/day in males and 15.6 mg/kg/day in females), kidney (≥0.4 mg/kg/day in males and ≥6.3 mg/kg/day in females), brain (15.6 mg/kg/day in males and ≥6.3 mg/kg/day in females), spleen (15.6 mg/kg/day in males and ≥6.3 mg/kg/day in females), and gonads (≥6.3 mg/kg/day in males); organ weight changes in the absence of histological evidence of damage were not considered adverse. Histological alterations were observed in the liver and kidneys. Liver effects were only observed in males and were characterized as increased basophilic, flocky granulation most prominent in zone I of the liver acinus at 15.6 mg/kg/day and in 2/10 rats at 6.3 mg/kg/day. Kidney effects included enlarged hyperchromatic nuclei in the proximal tubules in females at 2.5 mg/kg/day and males at 6.3 mg/kg/day, hypercellularity of the epithelial lining of straight segment of the proximal tubules, large hyperchromatic nuclei, focal necrotic cells and nuclear detritus in the lumen in females at 6.3 and 15.6 mg/kg/day and males at 15.6 mg/kg/day. | | | | | | | | |

# ATTACHMENT E. LSE TABLES AND FIGURES

## *Overview*

The purpose of the LSE tables and figures are to show the following:

- The effects noted as exposure levels increase
- The effects seen as exposure duration increases
- Differences in response by species, strain, and sex
- Levels of exposure to humans below which the risk of adverse effects is presumed to be a minimal risk (MRL) for effects other than cancer
- CELs, where data permit
- Effects that occur at concentrations less than those that result in 100% mortality

Exhibit 10, Chapter 2 Figures and Tables show examples of LSE tables and figures (Tables 2-1, 2-2, and 2-3 and Figures 2-3 and 2-4). Include a User's Guide for these tables and figures as Appendix D of the profile.

LSE tables and figures are computer-generated based on the supplemental document (see Attachment D). Generate tables and figures for inhalation, oral, or dermal (table only) data even if limited data points exist. Present these tables and figures in landscape format, with the table typeface matching other tables in the profile.

For profiles that include elements and compounds (e.g., a metal and its salts), express exposure levels in terms of the element, rather than the test compound. Title the LSE tables and figures "Levels of Significant Exposure to [Substance x]," and not "Levels of Significant Exposure to [Substance x] and Compounds," because the exposure levels in the table are for the metal.

## *What to Include in the LSE Tables and Figures*

As a general rule, enter all NOAELs and LOAELs for a given study in the tables. One exception is when effect levels are different between males and females; in this case, NOAEL and LOAEL values for each sex are included in the LSE table and only the more sensitive sex is presented in the LSE figure.

- Studies that lack quantitative estimates of NOAELs or LOAELs, or that are not reliable, should not be included in LSE tables. ***Important: Refer to General Guidance (Quality Criteria for Animal and Human Studies) and Attachment A and Attachment B of this guidance when writing this section.***
- For data-poor substances, include all reliable NOAELs, LOAELs, and CELs that are available for the substance in the LSE tables and figures.
- For data-rich substances, identify NOAELs and LOAELs for each specific effect, species, and duration (and, where applicable, each compound) from the available studies that satisfy the criteria in Attachment A (Evaluating the Quality of a Toxicological Study).
- Not all endpoints in a given study need to appear in the LSE tables and figures. In such cases, the authors must consult with their chemical manager.

Similarly, the principal author, and chemical manager may choose not to include in the LSE tables certain effects (or entire studies) that appear in the supplemental tables.

### *MRL Footnotes for LSE Tables*

Each data point used to derive an MRL is marked with a footnote in the LSE table. Shade the study entry in the LSE table light green (Red 226, Green 239, Blue 217). The footnote should use language similar to the following examples.

- Example 1: Oral LOAEL in animals where dose has been adjusted for intermittent exposure; if the MRL is based on a minimal LOAEL, note this in the footnote text:

  **Used to derive an intermediate oral minimal risk level (MRL) of [XXX] mg/kg/day; dose adjusted for intermittent exposure (5 days/week) and divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).**

- Example 2: Inhalation NOAEL in humans where dose has been adjusted for intermittent exposure:

  **Used to derive an acute inhalation minimal risk level (MRL) of [XXX] ppm; concentration adjusted for intermittent exposure and divided by an uncertainty factor of 10 (for human variability).**

- Example 3: Inhalation NOAEL in animals:

  **Used to derive a chronic inhalation minimal risk level (MRL) of [XXX] ppm; concentration adjusted for intermittent exposure ($NOAEL_{adj}$) and converted to a human equivalent concentration ($NOAEL_{HEC}$) by multiplying the $NOAEL_{adj}$ by the RGDR for extrathoracic respiratory effects. The $NOAEL_{HEC}$ of [YYY] ppm was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability).**

- Example 4: Oral MRL based on a BMDL in animals:

  **Used to derive an intermediate oral minimal risk level (MRL) of [XXX] mg/kg/day. The $BMDL_{10}$ of [YYY] mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).**

If more than one footnote for MRLs exists in a table, only spell out *minimal risk level* the first time the phrase is used; thereafter, use the abbreviation.

### *LSE Figures*

Plot the data in the inhalation and oral LSE tables need in the corresponding LSE figures. The dermal LSE table does not have an accompanying LSE figure.

When the LSE table lists separate NOAELs and LOAELs for males and females, choose the most sensitive sex (i.e., the one with the lowest LOAEL) and enter this LOAEL and the corresponding NOAEL for the same sex in the LSE figure. Where sex differences are indicated in an LSE table but not in the corresponding LSE figure, use the following boilerplate (or a modification thereof) as footnote "b" (after footnote "a"). .

**Differences in levels of health effects and cancer effects between males and females are not indicated in Figure 2-X. Where such differences exist, only the levels of effect for the most sensitive sex are presented.**

Each data point used to derive an MRL is marked with a dashed line and an anchor symbol in the LSE figure and with a footnote in the LSE table.

Next to each point plotted on the LSE figure, indicate the following information.

- The figure key number as shown in the corresponding LSE table.
- Species, using the following abbreviations. List abbreviations in this order in the key.

| | | |
|---|---|---|
| k = monkey | c = cat | e = gerbil |
| r = rat | h = rabbit ("hare") | b = cow |
| m = mouse | p = pig | x = chicken |
| g = guinea pig | f = ferret | j = pigeon |
| s = hamster | n = mink | o = other |
| d = dog | a = sheep | |

- Use the following symbols in the key to show effects.

  ■ Filled-in squares for $LD_{50}$ or $LC_{50}$ values

  ○ ● Circles for data from animals

  ▲ Δ Triangles for data from humans

  Half-filled symbols for LOAELs for "less serious" effects

  Filled symbols for LOAELs for "serious" effects

  Empty (not filled with color) symbols for NOAELs

  ♦ Filled-in diamonds for CELs in animals

  ▼ Filled-in inverted triangles for CELs in humans

  ▬ MRL symbol, usually with a vertical dotted line connecting to the study NOAEL or LOAEL

- Vertically align dots in LSE figures to clarify existing dose-response relationships for a particular species and to group effects appropriately (e.g., a LOAEL is above the corresponding NOAEL).
- When presenting CELs in the LSE figure, place an asterisk on the "Cancer" heading and add the following footnote to the key of the figure.

  ***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.**

See the sample LSE tables and figures in Chapter 2 Exhibits (Tables 2-1, 2-2, and 2-3 and Figures 2-3 and 2-4) for illustrations of the content and the appearance of these items.

## ATTACHMENT F. INTERPRETING RENAL PATHOLOGY IN THE MALE RAT

Risk assessment approaches generally assume that chemicals producing toxicity and neoplasia in laboratory animals pose similar hazards to humans. For most chemicals, this extrapolation remains appropriate. However, a growing body of evidence indicates that certain chemicals cause nephropathy and renal neoplasia through a mechanism that occurs in male rats but not in humans (or female rats, mice, or other species).

### Alpha2u-globulin induced renal pathology in male rats

Numerous investigations have demonstrated a consistent association between the accumulation of hyaline droplets containing alpha2-microglobulin (α2u-g) and certain lesions in the male rat kidney (Borghoff et al. 1991; EPA 1991; Hard et al. 1993; Swenberg et al. 1989). These renal lesions have not been identified in female rats, in mice, or in other laboratory species tested. A number of chemicals (e.g., unleaded gasoline) are capable of inducing accumulation of α2u-g, a low molecular weight protein, in the male rat kidney. The accumulation of this protein (which is synthesized in the liver) initiates a sequence of events that results in protein droplet nephropathy and eventually renal tumors. Exposure of male rats to chemicals inducing α2u-g accumulation (CIGA) results in the following histopathological sequence of renal lesions (EPA 1991).

- An excessive accumulation of hyaline droplets containing α2u-g in renal proximal tubules.
- Subsequent cytotoxicity and single-cell necrosis of the tubule epithelium.
- Sustained regenerative tubule cell proliferation, if exposure continues.
- Development of intraluminal granular casts from sloughed cell debris, along with tubule dilation and papillary mineralization.
- Foci of tubule hyperplasia in the convoluted proximal tubules.
- Renal tubule tumors.

Biochemical studies show that CIGA or their metabolites bind specifically, but reversibly, to male rat α2u-g. The resulting α2u-g-CIGA complex appears to be more resistant to hydrolytic degradation by lysosomal enzymes than native, unbound α2u-g. Inhibition of the catabolism of α2u-g, a protein only slowly hydrolyzed by renal lysosomal enzymes under normal physiological conditions, provides a possible basis for the initial stage of protein overload in the nephropathy sequence (EPA 1991; Hard et al. 1993). It has been hypothesized that the sustained protein overload results in single-cell necrosis in the

tubule epithelium and increased cell regeneration (a reparative process). The increased proliferative response caused by chemically induced cytotoxicity may be a plausible reason for the development of renal tumors in male rats.

EPA has established three criteria for determining that renal lesions in male rats are caused by α2u-g accumulation; a positive response in each criterion is required. These criteria are:

1. The number and size of hyalin droplets in renal proximal tubule cells of treated male rats have increased.

The abnormal accumulation of hyaline droplets in the P2 segment of the renal tubule is necessary to attribute the renal tumors to the α2u-g sequence of events. This finding helps differentiate α2u-g inducers from chemicals that produce renal tubule tumors through other mechanisms.

2. The accumulated protein in the hyaline droplets must be α2u-g.

Hyaline droplet accumulation is a nonspecific response to protein overload in the renal tubule and may not be due to α2u-g. Therefore, it is necessary to demonstrate, normally by immunohistochemistry, that α2u-g accounts for the hyaline droplet accumulation found in the male rat.

3. Additional aspects of the pathological sequence of lesions associated with α2u-g nephropathy must be demonstrated.

Typical lesions include single-cell necrosis, sloughing of epithelial cells into the proximal tubular lumen, formation of granular casts, linear mineralization of the papilla, and tubule hyperplasia and regeneration. If the response is mild, all of these lesions may not be observed; however, some elements consistent with the pathological sequence must be present.

It should not be expected that a compound that induces α2u-g accumulation will always be found to induce renal tubule tumor formation in the male rat. The ability to detect renal tumors depends on many features that may not be present in any individual experiment (e.g., sufficient dose to induce effect without early deaths of the animals, insufficient length of exposure or follow-up and incomplete histopathology).

Nephropathy and renal tumors associated with CIGA appear to be unique responses of the male rat. Therefore:

- Nephropathy in the male rat that is associated with α2u-g accumulation should not be used as an endpoint for quantitative noncarcinogenic risk assessment (MRL derivation).
- Renal tubule tumors in the male rat that are associated with α2u-g accumulation should not be used to support qualitative weight of evidence that a chemical poses a cancer risk in humans; these endpoints also should not be used for dose-response extrapolations that estimate human cancer risk.

Kidney effects data related to α2u-g accumulation in the male rat should be discussed in the profile and included in the LSE tables, even though it may not be used for MRL derivation. However, in these cases, the association of renal lesions to α2u-g accumulation and the relevance of these endpoints to risk assessment (human extrapolation) should be clearly discussed.

### Chronic progressive nephropathy (CPN)

Another factor to consider in using rat kidney effects for risk assessment is the species-related condition CPN. CPN is an age-related spontaneous disorder of rats that is more severe in males than in females, and that affects certain strains more than others. CPN is more common in Sprague-Dawley and F344 rats than in the Wistar strain (Gray 1986), and it is also common in the Osborne-Mendel rat (Goodman et al. 1980). The etiology of CPN is not known, but the severity of this condition may be influenced by a number of factors, particularly dietary manipulation affecting protein content or caloric intake (Masoro and Yu 1989). If their lifespan is long enough, most rats will have this renal lesion to some degree at the time of death.

Chronic administration of CIGA to male rats may result in exacerbation of CPN, characterized by increased severity and earlier onset of the disease. However, chemicals that do not induce α2u-g accumulation may cause damage by direct nephrotoxicity or may cause damage indirectly by accelerating the onset and increasing the severity of CPN. Histopathologic characteristics of CPN (EPA 1991; UAREP 1983) include some lesions that are also found in α2u-g nephropathy, as well as lesions that are distinctive. Single-cell necrosis, regenerating tubules, and focal hyperplasia of proximal tubule epithelium are common to CPN and to α2u-g nephropathy. CPN lesions that are *not* components of α2u-g nephropathy include prominent thickening of tubules and glomerular basement membranes, hyaline casts

consisting of homogenous, proteinaceous material (distinct from granular casts containing cellular debris), interstitial mononuclear cell infiltration, fibrosis, tubule atrophy, and sclerotic glomeruli. In advanced cases of CPN, sporadic tubules may contain excessive numbers of hyaline droplets similar in appearance to those induced by CIGA. However, these droplets do not show immunochemical evidence of α2u-g (Hard et al. 1993).

CPN in the aging male rat can complicate the interpretation of other renal lesions. However, nephropathy in the male rat that is not attributable to either CPN or α2u-g accumulation may provide endpoints that are suitable for consideration in the risk assessment process, particularly if similar effects are seen in female rats, in mice, or in other species. Generally, lesions of CPN in exposed rats should be excluded as endpoints used in quantitative risk assessment (MRL derivation). However, there may be an exception to this guideline in a few cases. Lesions of CPN in exposed rats may be considered potential endpoints for estimating noncarcinogenic risk if exposed male and female, or only female[1], rats have lesions of CPN that exhibit a clearly defined dose response. More specifically, with increasing exposure doses there should be a progressive significance of CPN lesions as characterized by (a) an earlier age of onset, (b) increasing severity, (c) an increased frequency of animals affected (one or any combination of these three items may be present). Observation of renal effects in other similarly exposed species contributes to the weight of evidence in these cases.

In cases where the above criteria are met, NOAEL values for lesions of CPN can be considered for quantitative risk assessment. A NOAEL in this situation is defined as a test dose that produces no statistically significant enhancement of CPN lesions compared with the controls. The effect description for NOAEL values should read "no enhancement of CPN in females" (and males, if appropriate). At those doses where enhancement of CPN lesions is observed, effects should be classified as less serious LOAELs or serious LOAELs, depending upon their magnitude. Less serious LOAEL values can be considered for MRL derivation if NOAELs have not been identified. The effect description for LOAEL endpoints should read "dose-related enhancement of CPN in females" (and males, if appropriate).

[1]In untreated rats, CPN is typically much more severe in males. If exposed females exhibit a dose-response, such a pattern may be obscured in the exposed male rat due to the severity of the lesion.

## ATTACHMENT G. ASSESSING CHOLINESTERASE ACTIVITY INHIBITION

Organophosphorus (OP) and carbamate compounds share a common pathophysiology; they combine with and thereby inhibit cholinesterase enzymes, of which acetylcholinesterase in nerve tissue is the most important. Inactivation of acetylcholinesterase results in accumulation of acetylcholine at synapses and neuromuscular junctions. Exposure to OP (e.g., disulfoton, malathion) or carbamate (e.g., baygon, carbaryl) compounds produces a broad spectrum of clinical effects indicative of massive overstimulation of the cholinergic system, including muscarinic effects (parasympathetic), nicotinic effects (sympathetic and motor), and central nervous system effects (ATSDR 1993; Gallo and Lawryk 1991; Kaloyanova and El Batawi 1991). These effects present clinically as symptoms of headaches, weakness, dizziness, blurred vision, psychosis, respiratory difficulty, paralysis, convulsions, and coma. Other typical findings include increased salivation, lacrimation, urination, and defecation. In the following discussion, OP compounds will be used as the prototype-cholinesterase inhibiting toxin.

In principle, cholinesterase activity correlates with the amount of OP compound absorbed in the organism. Therefore, cholinesterase activity is a specific test for exposure to OP compounds (Morgan 1989). There are two principal human cholinesterases: acetylcholinesterase and pseudocholinesterase. Acetylcholinesterase, also referred to as true cholinesterase or erythrocyte acetyl cholinesterase, is found mainly in nervous tissue and erythrocytes, as well as in lymphocytes (Goldfrank et al. 1990). Pseudocholinesterase is often referred to as plasma cholinesterase or serum cholinesterase. Pseudocholinesterase and lymphocyte acetylcholinesterase activities are depressed before erythrocyte cholinesterase activity, suggesting that these cholinesterases are more sensitive indicators of exposure to OP compounds (Fitzgerald and Costa 1993; Iyaniwura 1991; Sundlof et al. 1984). However, erythrocyte cholinesterase recovers more slowly (90–120 days) than pseudocholinesterase or lymphocyte acetylcholinesterase (days to weeks), and is therefore a better indicator after exposure ceases.

Depression of pseudocholinesterase activity only indicates possible exposure to OP compounds, whereas depression of erythrocyte and lymphocyte acetylcholinesterases not only indicates exposure, but also a neurologic effect, as they reflect inhibition of brain acetylcholinesterase activity. The toxicologic effects of OP compounds are almost entirely due to the inhibition of acetylcholinesterase in the nervous system. Thus, the toxicity of OP compounds is most appropriately assessed in the laboratory by measurement of the erythrocyte (true) cholinesterase rather than the plasma (pseudo-) cholinesterase. Inhibition of plasma cholinesterase has not been associated with toxicity.

For the purpose of health effect assessment associated with OP compound exposure, the laboratory parameter to be used in profiles is measurement of acetylcholinesterase activity (in erythrocytes and/or the brain). If the rate of acetylcholinesterase inhibition is rapid, the correlation between enzyme inhibition and the severity of clinical symptoms tends to be good. When the rate of acetylcholinesterase inhibition is slow, the correlation may be low or nonexistent. This may happen during long-term occupational OP compound exposure, because the body adapts to the high levels of acetylcholine accumulated in the synapses and neuromuscular junctions (Kaloyanova and El Batawi 1991). Chronic moderate OP compound exposure results in cumulative inhibition of acetylcholinesterase activity. The appearance of symptoms depends more on the rate of fall in acetylcholinesterase activity than on the absolute level of activity reached. Some workers may exhibit 70–80% inhibition of acetylcholinesterase activity after several weeks of moderate exposure without manifesting cholinergic symptoms. Other individuals may develop symptoms at first exposure, even though the inhibition of acetylcholinesterase activity is <30%.

In classifying the neurological health effect endpoint of "inhibition of acetylcholinesterase activity" (in erythrocytes and/or the brain), the following guidelines should be followed. A 20–59% inhibition of enzyme activity is classified as a less serious LOAEL; enzyme activity inhibition of ≥60% is classified as a serious LOAEL. However, in addition to the aforementioned guidelines, consideration should be given to associated clinical symptoms. If clinical effects observed at a particular exposure level are most consistent with those symptoms described in the table under moderate or severe poisoning, this exposure level should be classified as a serious LOAEL, even if the degree of inhibition of acetylcholinesterase activity is <60%. In those cases where inhibition of enzyme activity of <60% is classified as a "serious" LOAEL, the specific clinical effects that lead to this classification (as well as the percentage of enzyme inhibition) should be clearly stated in the text of Chapter 2 and in the LSE tables. Inhibition of acetylcholinesterase activity of ≥60% should always be classified as a serious effect. Clinical signs, if present, should be discussed in Chapter 2 and listed in the LSE table entry. In cases where erythrocyte or brain acetyl-cholinesterase is inhibited by <20% (NOAEL) and statistically significant decreases in plasma cholinesterase are observed, this fact should be stated in Chapter 2. This information is useful in quantitative risk assessment since it proves that significant absorption occurred.

## ATTACHMENT H. AGE AT WEANING AND SEXUAL MATURITY FOR COMMON LABORATORY SPECIES AND HUMANS

| Species | Age at weaning[a] | Age at sexual maturity[b] (puberty) |
|---|---|---|
| Mouse | 21 days | 50 days |
| Rat | 21 days | 56 days |
| Dog (Beagle) | 42 days | 240 days |
| Cat | 49 days | 240 days |
| Guinea pig | 14 days | 70 days |
| Hamster | 21 days | 60 days |
| Rabbit (New Zealand) | 56 days | 195 days |
| Gerbil | 21 days | 70 days |
| Monkey (Rhesus) | 130 days | 1,825 days |
| Pig | 14–35 days[c] | 150 days |
| Mink | 56 days | 300 days |
| Human | 6 months–2 years | 13 years (female)<br>15 years (male) |

[a]Age of weaning is the age that breastfeeding or formula is stopped by mother or child. Some toddlers are breastfed longer than 2 years, and some infants are on solid foods before 6 months; the latter is not recommended.
[b]EPA. 1988. Recommendations for and documentation of biological values for use in risk assessment. U.S. Environmental Protection Agency. Environmental Criteria and Assessment Office, Office of Research and Development, Cincinnati, OH. EPA/600/6-87-008.
[c]Dr. G. Gomez. North Carolina State University, Veterinary School. Personal communication, April 8, 1998. Commercial operations often begin the weaning process at 2 weeks. At 5 weeks, the sow begins a drastic reduction in milk production.

## ATTACHMENT I. HISTORICAL BACKGROUND RATES FOR VARIOUS DEVELOPMENTAL OUTCOMES

*Used in interpreting National Toxicology Program (NTP) developmental studies on rabbits, rats, and mice. Appendices from Research Triangle Institute (RTI) contracted studies, used with permission of NTP. [Tables]*

## ATTACHMENT J. METABOLIC ENZYMES WHOSE EXPRESSION OR ACTIVITY VARIES DEVELOPMENTALLY

Taken from Leeder, JS and Kearns, GL. 1997. Pharmacogenetics in Pediatrics: Implications for Practice. Pediatric Clinics of North America 44:55-77; Table 2.

### Developmental Patterns for the Ontogeny of Important Drug-Metabolizing Enzymes

***Phase I Enzymes***

CYP2D6 - Low to absent in fetal liver but uniformly present at 1 week of postnatal age. Poor activity (approximately 20% of adult) at 1 month of postnatal age. Adult competence attained by approximately 3–5 years of age.

CYP2C19 - Not apparent in fetal liver. Inferential data using phenytoin disposition as a nonspecific pharmacologic

CYP2C9 - probe suggest low activity in first week of life, with adult activity reached by 6 months of age. Peak activity (as reflected by average values for Vmax, which are 1.5–1.8-fold adult values) may be reached at 3–4 years of age, which declines to adult values at the conclusion of puberty.

CYP1A2 - Not present to an appreciable extent in human fetal liver. Adult levels reached by 4 months and may be exceeded in children 1–2 years of age. Activity slowly declines to adult levels which are attained at the conclusion of puberty. Gender differences in activity are possible during puberty.

CYP3A7 - Functional activity in fetus is approximately 30–75% of adult levels of CYP3A4.

CYP3A4 - Low activity in the first month of life, with approach toward adult levels by 6-12 months of postnatal age. Pharmacokinetic data for CYP3A4 substrates suggest that adult activity may be exceeded between 1 and 4 years of age. Activity then progressively declines, reaching adult levels at the conclusion of puberty.

***Phase II Enzymes***

NAT2 (N-acetyltransferase-2) - Some fetal activity present by 16 weeks. Virtually 100% of infants between birth and 2 months of age exhibit the slow metabolizer phenotype. Adult phenotype distribution reached by 4–6 months of postnatal age, with adult activity present by approximately 1–3 years of age.

TPMT (thiopurine methyltransferase) - Levels in fetal liver are approximately 30% of those in adult liver. In newborn infants, activity is approximately 50% higher than in adults, with a phenotype distribution that parallels that in adults. In Korean children, adult activity appears at approximately 7–9 years of age.

UGT (glucuronosyltransferase) - Ontogeny is isoform specific as reflected by pharmacokinetic data for certain substrates (e.g., acetaminophen or chloramphenicol). In general, adult activity as reflected from pharmacokinetic data seems to be achieved by 6–18 months of age.

ST (sulfotransferase) - Ontogeny (based on pharmacokinetic studies) seems to be more rapid than that for UGT; however, it is substrate specific. Activity for some isoforms (e.g., that responsible for acetaminophen metabolism) may exceed adult levels during infancy and early childhood.

## ATTACHMENT K. ALTERNATE NAMES FOR ENZYMES

| **Phase I enzymes** | | |
|---|---|---|
| CYP1A2 | 7-ethoxy resorufin o-deethylase | $MC_4$ (hamster) |
| | EC1 | Mkah2 (monkey) |
| | P450d (human, rat)) | P-450-D3-P-450-D2, Dah2 (dog) |
| | P-448 (rat) | DP-4501A-61 (chicken) |
| | $LM_4$ (rabbit) | $P_3$, $P_2$ (mouse |
| CYP2C9 | (methyl) hydroxylase (e.g., tolbutamide, phenytoin, tieneilic acid | EC1.14.99 |
| | MP-1 | IIC1 |
| | MP-2, | Human-2 |
| | mp-4 | pHLS.5 |
| | HM2 (human) | hPA6 |
| CYP2C19 | S-mephenytoin hydroxylase | EC1.14.13 |
| | 11a (human) | |
| CYP2D6 | Nifedipine oxidase | EC1.14.99 |
| | db1 (human) | |
| CYP3A4 | Nf-25 | hpCN1 |
| | Nf-10 (human) | |
| CYP3A7 | HFLa | HFL33 |
| | Hlp2 (human) | |

CYP = cytochrome P450 (Nelson et al 1993; Parkinson 1996)

| **Phase II enzymes** | | |
|---|---|---|
| NAT2<br>N-acetyl-transferase-2 | Acetyltransferase | Acetyl CoA-arylamine N-acetyltransferase |
| | Arylamine | Acetyltransferase, 2-naphthylamine |
| | Beta.-Naphthylamine N-acetyltransferase | N-Acetyltransferase, 4-aminobiphenyl |
| | 4-Aminobiphenyl- N-acetyltransferase | Acetyltransferase, p-aminosalicylate N- |
| | 5-HT N-acetyltransferase | Acetyltransferase, procainamide N- |
| | Aromatic amine N-acetyltransferase | Arylamine acetylase |
| | Arylamine acetyltransferase | Arylamine N-acetyltransferase |
| | Dapsone N-acetylase | Dapsone N-acetyltransferase |
| | E.C. 2.3.1.5 | Indoleamine N-acetyltransferase |
| | p-Aminosalicylate N-acetyltransferase | |
| PMT<br>thiopurine s-methyltransferase | Methyltransferase | mercaptopurine (9CI) |
| | Mercaptopurine methyltransferase | Thiopurine methyltransferase |

| | | |
|---|---|---|
| UGT Glucurono-syltransferase | uridine diphospho-(9CI) | 1-Naphthol-UDP-glucuronosyltransferase |
| | 4-Hydroxybiphenyl UDP-glucuronosyltransferase | 4-Methylumbelliferone UDP-glucuronosyltransferase |
| | 4-Nitrophenol UDP-glucuronyltransferase | Ciramadol UDP-glucuronyltransferase |
| | E.C. 2.4.1.17 | Uridine diphosphoglucuronate-4-hydroxybiphenyl |
| | Nitrophenol UDP-glucuronosyltransferase | Uridine diphospho Glucuronyltransferase |
| | p-Hydroxybiphenyl UDP glucuronyltransferase | p-Nitrophenol UDP-glucuronyltransferase |
| | p-Nitrophenylglucuronosyltransferase | phenol-UDP-glucuronosyltransferase |
| | p-Phenylphenol glucuronyltransferase | UDP glucuronosyltransferase |
| | UDP glucuronyltransferase | UDP-glucuronate-4-hydroxybiphenyl glucuronosyltransferase |
| | UDPGA transferase | UDPGA-glucuronyltransferase |
| | Uridine 5'-diphosphoglucuronic acid transferase | Uridine 5'-diphosphoglucuronyltransferase |
| | Uridine diphosphate glucuronyltransferase | Uridine diphosphoglucuronosyltransferase |
| | Uridine diphosphoglucuronyltransferase | |
| ST sulfo-transferase | 3'-Phosphoadenosine 5'-phosphosulfate sulfotransferase | 6-Hydroxymethylbenzo[a]pyrene sulfotransferase |
| | PAPS-Sulfotransferase/PAPS-Sulphotransferase | Phosphoadenosine phosphosulfate transferase |
| | S-Sulfotransferase | |

(Chemical Abstracts as searched February 1998)

# LIST OF EXHIBITS

**Exhibit #, Description**

EXHIBITS FOR GUIDANCE

*In the exhibits, you will often find italicized text. The text is guidance for the figure, table, or contents. The text is not to be included in the toxicological profile.*

*The author and chemical manager team may not have the data needed to build all the required tables. Alternatively, they may want optional tables in each of the chapters, especially Chapters 3 and 5. As always, begin each chapter with figure/table 1 and number consecutively for each table and figure, e.g., Figure 5-1, Table 5-1, Figure 5-2, Figure 5-3, Table 5-2, etc.*

## EXHIBIT 1. OUTLINE FOR TOXICOLOGICAL PROFILES

Title Page (A or B)

Foreword (A - Pre-public or B - Post-public)

Version History (A or B - *Targeted Toxicological Profiles Only*)

Contributors & Reviewers (A or B - *Targeted Toxicological Profiles Only*)

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List of Tables

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- 1.2 Summary of Health Effects
- 1.3 Minimal Risk Levels (MRLs)

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- 2.2 Death
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- 2.5 Cardiovascular
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- 2.19 Cancer
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2.21 Mechanisms of Action *(as needed)*

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3.1.2 Distribution

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3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

3.1.6 Animal to Human Extrapolation

3.2 Children and Other Populations That Are Unusually Susceptible

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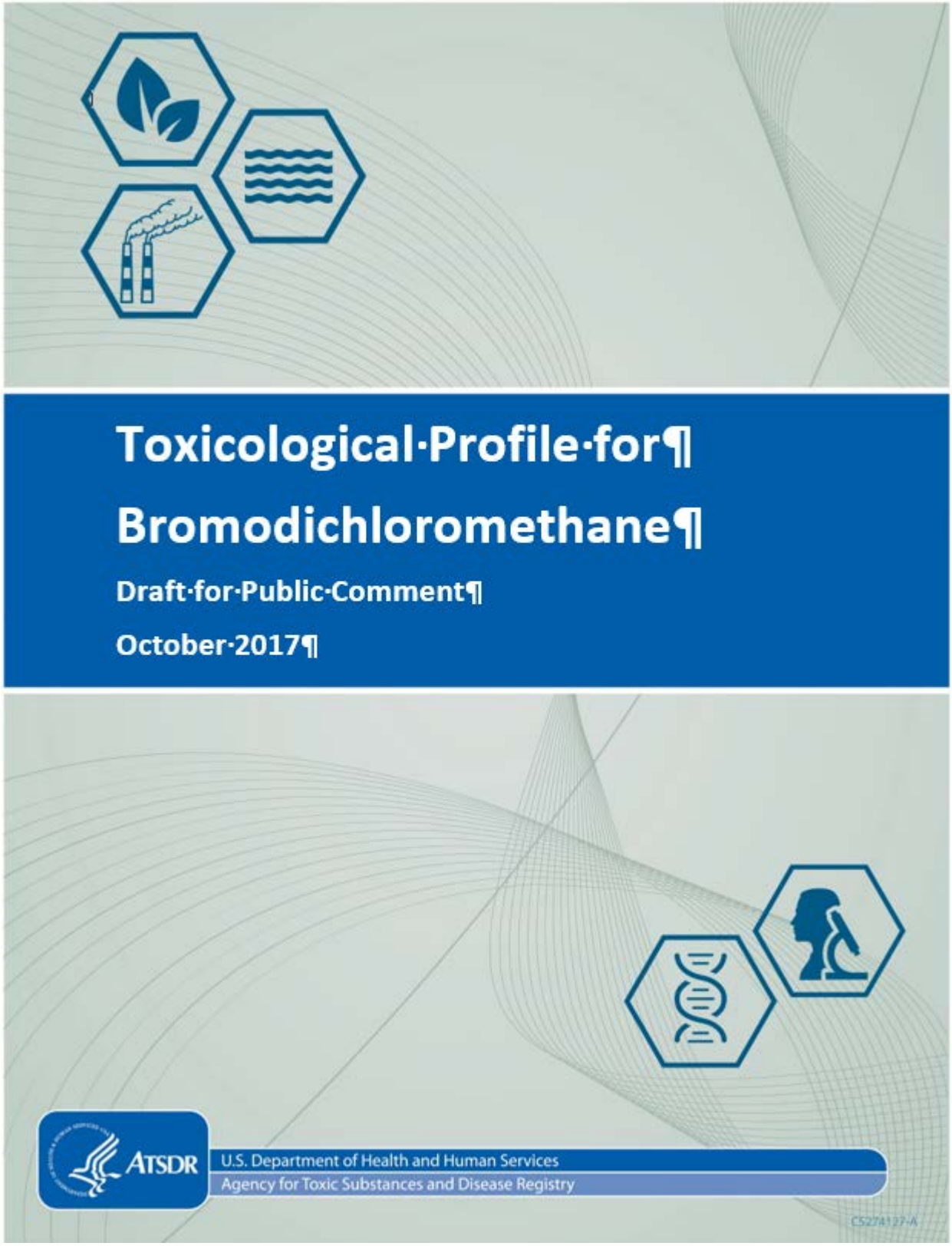
5.5.2 Water

** Appendix letters are dependent on whether or not a systematic review is included as Appendix C.*

## EXHIBIT 2. EXAMPLE TITLE PAGE



## EXHIBIT 3A. FOREWORD [PRE-PUBLIC DRAFTS]

*<All writing is single spaced, 11 pt Times New Roman font>*

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 these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a 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 relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. 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 the 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 toxic substance to ascertain the levels of significant human exposure for the substance and the associated acute, intermediate, 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 the levels of exposure that present a significant risk to human health due to acute, intermediate, and chronic duration exposures; 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. ATSDR plans to revise these documents in response to public comments and as additional data become available. Therefore, we encourage comments that will make the toxicological profile series of the greatest use.

Electronic comments may be submitted via: www.regulations.gov. Follow the on-line instructions for submitting comments.

Written comments may also be sent to: Agency for Toxic Substances and Disease Registry
Division of Toxicology and Human Health Sciences
Environmental Toxicology Branch

Regular Mailing Address:
1600 Clifton Road, N.E.
Mail Stop F-57
Atlanta, Georgia 30329-4027

Physical Mailing Address:
4770 Buford Highway
Building 102, 1st floor, MS F-57
Chamblee, Georgia 30341

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL) and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the NPL, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staffs 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.

Patrick N. Breysse, Ph.D., CIH
Director, National Center for Environmental Health and
Agency for Toxic Substances and Disease Registry
Centers for Disease Control and Prevention

## EXHIBIT 3B. FOREWORD [POST-PUBLIC DRAFTS]

*<All writing is single spaced, 11 pt Times New Roman font>*

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 these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a 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 relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. 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 the protection of public health are identified by ATSDR.

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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.

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Patrick N. Breysse, Ph.D., CIH
Director, National Center for Environmental Health and
Agency for Toxic Substances and Disease Registry
Centers for Disease Control and Prevention

*Legislative Background

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL) and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the NPL, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

## EXHIBIT 4A. VERSION HISTORY

# VERSION HISTORY

| Date | Description |
|---|---|
| [Month Year] | Draft for public comment released |
| [Month Year] | Addenda released (when applicable) |
| [Month Year] | Final toxicological profile released |

Guidance on version history:

1. Page name is centered on page, capitalize each word in bold, 13 pt Arial font.
2. Date and description columns are Arial 11 pt font and left justified in columns.
3. Dates and description rows (entries) are Arial 10 pt font.
4. Date to be month followed by four-digit year (e.g., March 1987).
5. The final publication date is always at the top.
6. Include a line for the release for public comment draft.
7. Have older changes at the bottom of the table and new changes at the top.
8. The lines must state the changes to the profile if it is less than the complete profile, e.g., Chapter 4 and 7 updated.

## EXHIBIT 4B. VERSION HISTORY (*TARGETED PROFILES ONLY*)

# VERSION HISTORY

| Date | Description |
|---|---|
| [Month Year] | Final toxicological profile released |
| [Month Year] | Addendum to the toxicological profile released (*when applicable*) |
| [Month Year] | Update of data in Chapters 2, 3, and 7 |

Guidance on version history:

1. Page name is centered on page, capitalize each word in bold, 13 pt Arial font.
2. Date and description columns are Arial 11 pt font and left justified in columns.
3. Dates and description rows (entries) are Arial 10 pt font.
4. Date to be the month followed by four-digit year (e.g., March 1987).
5. The final publication date is always at the top.
6. Include a line for the release for public comment.
7. Have older changes at the bottom of the table and new changes at the top.
8. The lines must state the changes to the profile if it is less than the complete profile, e.g., Chapter 4 and 7 updated.

**EXHIBIT 5A. CONTRIBUTORS & REVIEWERS**

# CONTRIBUTORS & REVIEWERS

## CHEMICAL MANAGER TEAM

[Chemical Manager 1] (Lead)
[Chemical Manager 2]

ATSDR, Division of Toxicology and Human Health Sciences, Atlanta, GA

[Contractor Author 1]
[Contractor Author 2]

[Contractor Name, City, State]

## REVIEWERS

**Interagency Minimal Risk Level Workgroup:**

Includes ATSDR; National Center for Environmental Health (NCEH); National Institute of Occupational Health and Safety (NIOSH); U.S. Environmental Protection Agency (EPA); National Toxicology Program (NTP).

**Additional reviews for science and/or policy:**

ATSDR, Division of Community Health Investigations; NCEH, Division of Laboratory Science; U.S. Department of Defense.

## PEER REVIEWERS

1.
2.
3.

These experts collectively have knowledge of toxicology, chemistry, and/or health effects. All reviewers were selected in conformity with Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The listing of peer reviewers should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with ATSDR.

**EXHIBIT 5B. CONTRIBUTORS & REVIEWERS (*TARGETED TOXICOLOGICAL PROFILES ONLY*)**

## CONTRIBUTORS & REVIEWERS

## CHEMICAL MANAGER TEAM

| | |
|---|---|
| [Chemical Manager 1] | [Contractor Author 1] |
| ATSDR, Division of Toxicology and Human Health Sciences, Atlanta, GA | [Contractor Name, City, State] |

*Guidance: Include only the chemical manager and lead author.*

## EXHIBIT 6. CONTENTS

# CONTENTS

## EXHIBIT 7. LIST OF FIGURES FOR PROFILE

Below is a list of figures that are included in most toxicological profiles. Additional figures may be included in the profile.

### Chapter 1

Health Effects Found in Humans and Animals Following Inhalation Exposure to [Substance X]
Health Effects Found in Humans and Animals Following Oral Exposure to [Substance X]
Summary of Sensitive Targets of [Substance X] – Inhalation
Summary of Sensitive Targets of [Substance X] – Oral

### Chapter 2

Overview of the Number of Studies Examining [Substance X] Health Effects
Levels of Significant Exposure to [Substance X] – Inhalation
Levels of Significant Exposure to [Substance X] – Oral

### Chapter 3

Metabolic Scheme for [Substance X]

### Chapter 5

Number of NPL Sties with [Substance X]

### Chapter 6

Summary of Health Effects Sudies on [Substance X] By Route and Endpoint

*As always with figure and table numbering, begin each chapter with figure/table 1 and number consecutively for each table and figure, e.g., Figure 5-1, Table 5-1, Figure 5-2, Figure 5-3, Table 5-2, etc.*

## EXHIBIT 8. LIST OF TABLES FOR PROFILE

Below is a list of tables that are included in most toxicological profiles. Additional tables may be included in the profile.

### Chapter 1

Minimal Risk Levels (MRLs) for [Substance x]

### Chapter 2

Levels of Levels of Significant Exposure to [Substance x] – Inhalation

Levels of Significant Exposure to [Substance x] – Oral

Levels of Significant Exposure to [Substance x] – Dermal

Genotoxicity of [Substance x] *In Vitro*

Genotoxicity of [Substance x] *In Vivo*

### Chapter 4

Chemical Identity of [Substance X]

Physical and Chemical Properties of [Substance x]

### Chapter 5

Facilities that Produce, Process, or Use [Substance x]

Releases to the Environment from Facilities that Produce, Process, or Use [Substance x]

Lowest Limit of Detection Based on Standards

[Substance x] Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Blood [Substance x] Levels (ng/mL) in the NHANES U.S. Population

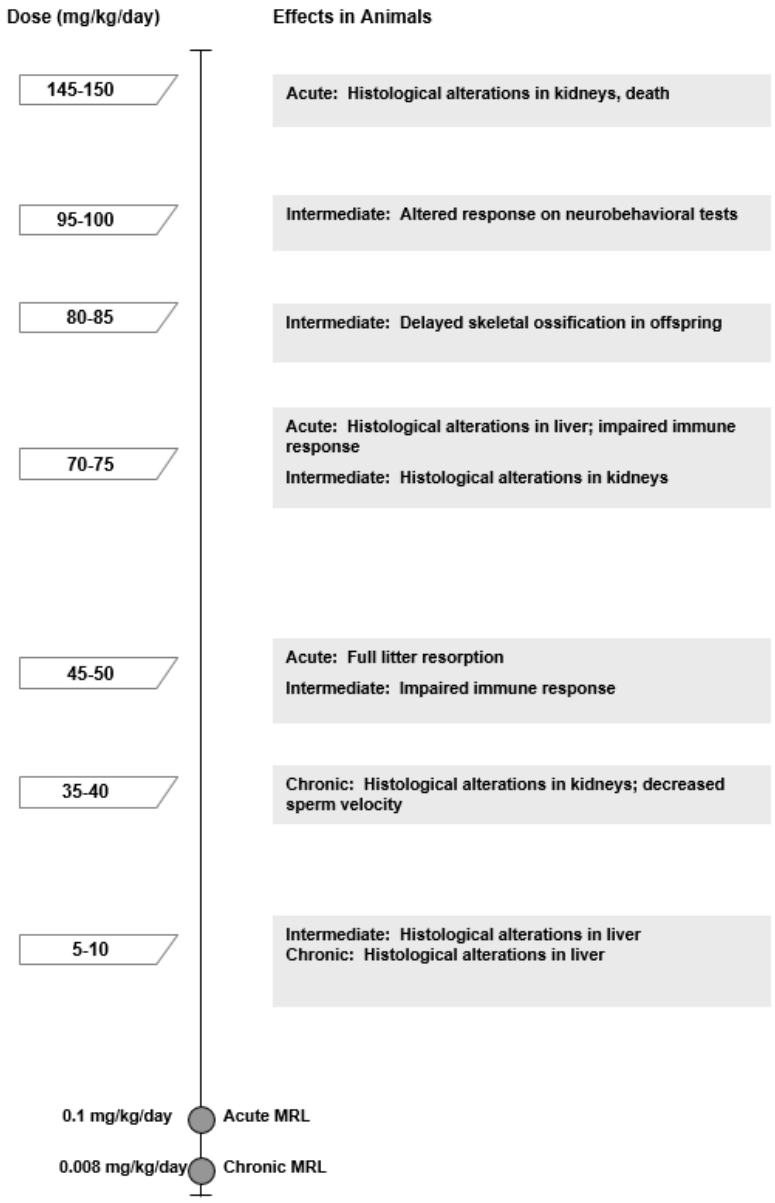
### Chapter 7

Regulations and Guidelines Applicable to [Substance x]

*As always with figure and table numbering, begin each chapter with figure/table 1 and number consecutively for each table and figure; e.g., Figure 5-1, Table 5-1, Figure 5-2, Figure 5-3, Table 5-2, etc.*

## EXHIBIT 9. CHAPTER 1 FIGURES AND TABLES

### Figure 1-1. Health Effects* Found in [Animals/Humans] Following [Inhalation/Oral] Exposure to [Substance x]




*Guidance for figure:*

- *Chemical manager decides whether there is a single thermometer for animals and humans versus multiple thermometers*
- *Do not include NOAELs*

- *In general, do not adjust exposures to be continuous or daily. For profiles with mix of continuous and intermittent exposure protocols for inhalation studies, discuss with the chemical manager whether the concentrations should be duration-adjusted.*
- *Show the most sensitive sex on the figure*
- *Specify duration (acute, intermediate, or chronic) associated with effect*
- *Don't list every effect occurring within a range; instead include only dose + effect points where effects are just beginning*
- *Select lowest LOAEL for x exposure type*
- *Put some white space between the MRLs when there is an order of magnitude between them*
- *Use up to 12 endpoints*
- *Make the graphic fill the page in width*
- *Develop a thermometer even when there are no MRLs*
- *Doses and text boxes will not be spaced equidistant on the thermometer unless the data are truly this way; let doses dictate the bunching/grouping of the health effect*

## Figures 1-2 and 1-3. Summary of Sensitive Targets of [X] – Inhalation & Oral

**Figure 1-2. Summary of Sensitive Targets of [Substance x]—Inhalation**

**The [endpoint(s)] [is/are] the most sensitive target(s) of [Substance x] inhalation exposure.**
Numbers in triangles and circles are the lowest LOAELs among health effects in humans and animals, respectively.

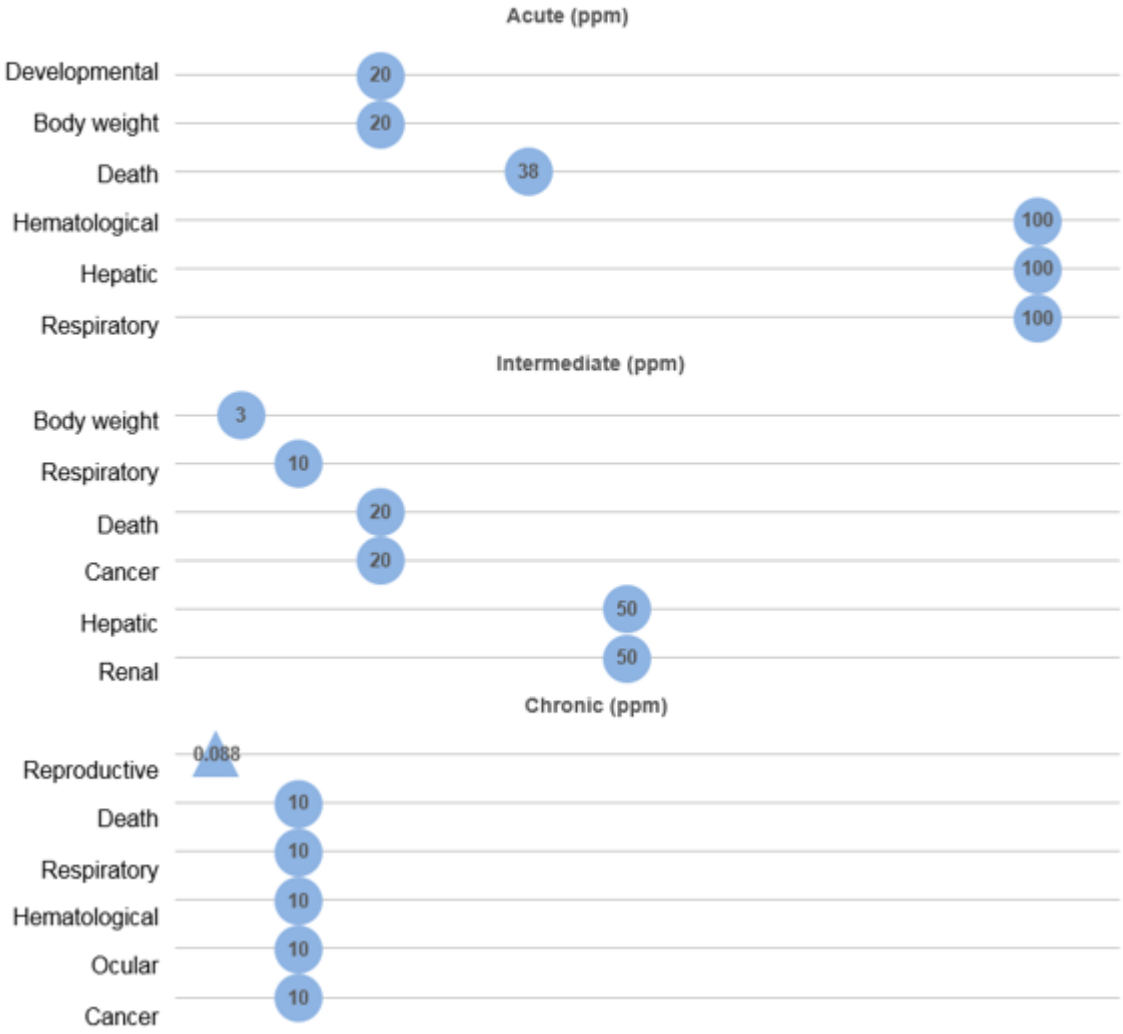
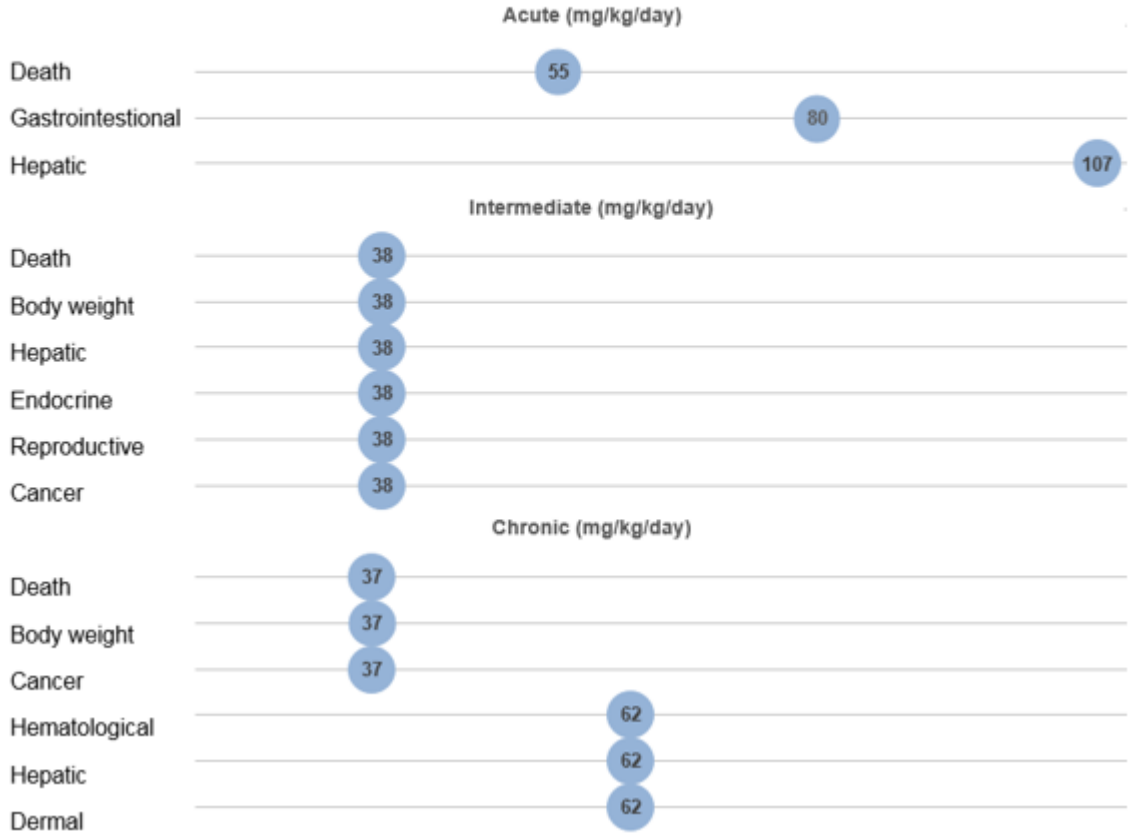


**Figure 1-3. Summary of Sensitive Targets of [Substance x]—Oral**

**The [endpoint(s)] [is/are] the most sensitive target(s) of [Substance x] oral exposure.**
Numbers in circles are the lowest LOAELs for all health effects in animals.
No reliable dose response data were available for humans.




*Guidance for Figures 1-2 and 1-3: Organize the figure by duration then in ascending order for each duration. This means to graph the lowest LOAEL first, followed by the second lowest and so on. When possible, make portrait and have two per page. The lowest LOAELs shall be the first entries for these figures. Use a maximum of four health endpoints per duration. If necessary, mention in text/table footer that there are more. Do not adjust exposures to be continuous or daily.*

**Table 1-1. Minimal Risk Levels (MRLs) for [Substance x]**

**Table 1-1. Minimal Risk Levels (MRLs) for [Substance x][a]**

| Exposure duration | MRL | Critical effect | Point of departure | Uncertainty factor | Reference |
|---|---|---|---|---|---|
| **Inhalation exposure (ppm)** | | | | | |
| Acute | Insufficient data for MRL derivation | | | | |
| Intermediate | Insufficient data for MRL derivation | | | | |
| Chronic | Insufficient data for MRL derivation | | | | |
| **Oral exposure (mg/kg/day)** | | | | | |
| Acute | 0.1 | Full-litter resorption in rats | 10 ($BMDL_{05}$) | 100 | Narotsky et al. 1997 |
| Intermediate | Insufficient data for MRL derivation; chronic MRL considered protective for intermediate duration exposure | | | | |
| Chronic | 0.008 | Hepatocellular fatty degeneration in rats | 0.78 ($BMDL_{10}$) | 100 | Aida et al. 1992 |

[a]See Appendix A for additional information.

## EXHIBIT 10. CHAPTER 2 FIGURES AND TABLES

### Figure 2-1. Profile Studies

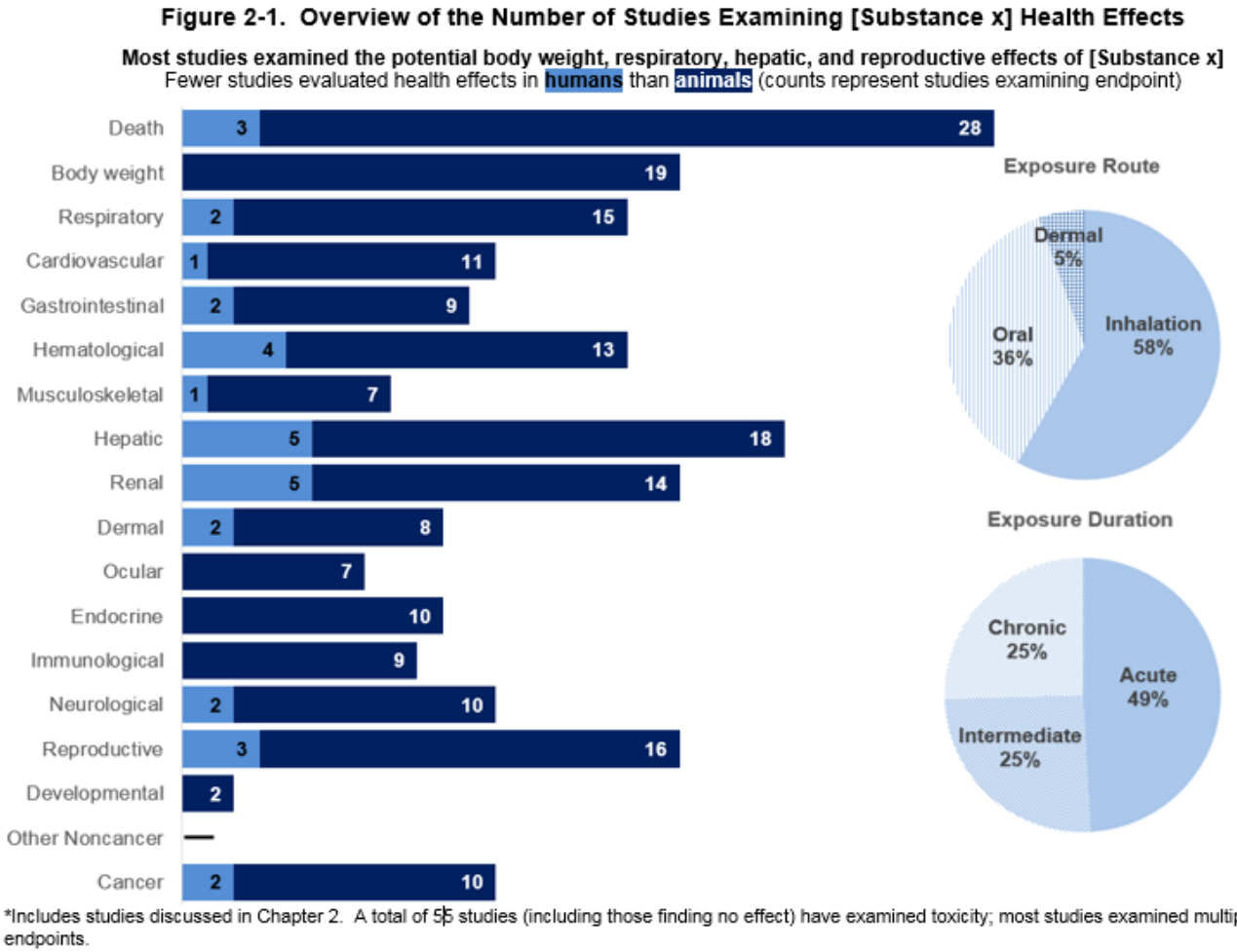
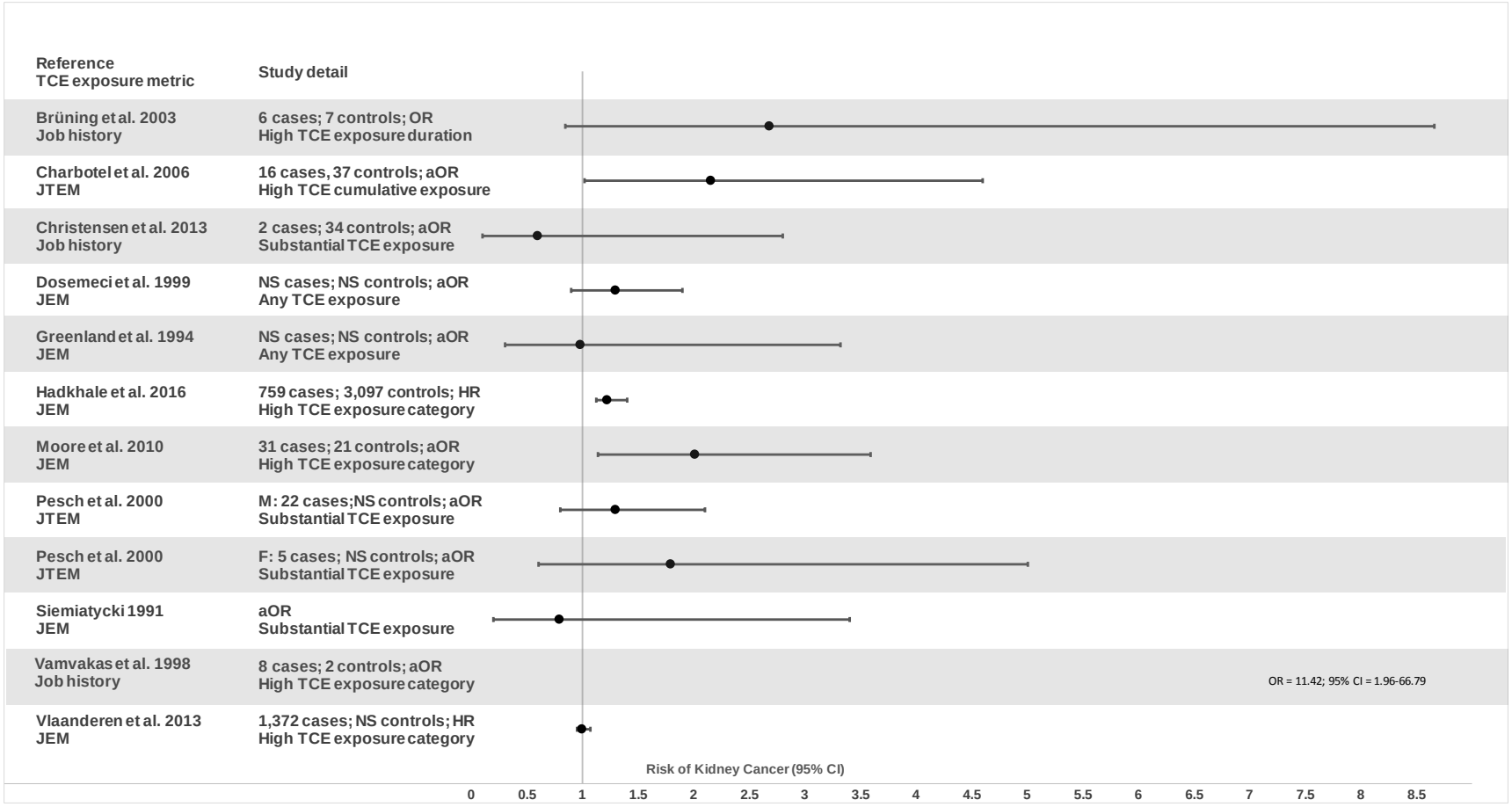



*Figure guidance: Can shrink the size of figure to reduce white space. Do not use decimal points in pie charts. This figure needs to match the discussion in the Health Effects Chapter and the Chapter 6 figure. Human studies, including case studies, discussed in Chapter 2 are to be counted in this figure and in the Chapter 6 figure.*

## Figure 2-2. Forest Plot

### Figure 2-2. Selected Case-Control Studies of [Substance X] Exposure and Risk of Kidney Cancer




C = number of kidney cancer morbidities/mortalities; CI = confidence interval; HR = hazard ratio; HRR = hazard rate ratio; JEM = job exposure matrix; N = number TCE-exposed subjects; NS = not specified; RR = rate ratio; SIR = standardized incidence ratio; SMR = standardized mortality ratio; TCA = trichloroacetic acid (trichloroethylene urinary metabolite); TCE = trichloroethylene

**Table 2-1. Inhalation LSE**

**Table 2-1. Levels of Significant Exposure to [Substance x] – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effect |
|---|---|---|---|---|---|---|---|---|---|
| **ACUTE** | | | | | | | | | |
| 1 | Mouse (C57BL/6) 6M | 6 hours/day 7 days/week 1 week | 1, 10, 30, 100, 150 | LE, BW, OW, HP | Death | | | 30 | 2/6, 1/6, 3/6 deaths in wild type strain at 30, 100, and 150 ppm, respectively |
| | | | | | Bd wt | 10 | 30 | | Decreased body weight gain |
| | | | | | Hepatic | 10 | 30 | | Centrilobular hepatocellular degeneration at ≥30 ppm and hepatocellular necrosis at ≥100 ppm |
| | | | | | Renal | 1 | 10 | | Tubular degeneration and nephrosis |
| **Torti et al. 2001** | | | | | | | | | |
| **INTERMEDIATE** | | | | | | | | | |
| 2 | Mouse (C57BL/6) 6NS | 6 hours/day 7 days/week 3 weeks | 0, 0.3, 1, 3, 10, 30 | LE, BW, OW, HP | Bd wt | 30 | | | |
| | | | | | Hepatic | 30 | | | Centrilobular hepatocellular degeneration was observed at ≥10 ppm in heterozygous strains |
| | | | | | Other noncancer (urinary bladder) | 30 | | | |
| **Torti et al. 2001** | | | | | | | | | |

[a]The number corresponds to entries in Figure 2-[x] *(link to corresponding LSE figure).*
[b]Used to derive an intermediate-duration inhalation MRL of 0.001 mg [Substance x]/$m^3$ calculated using benchmark dose analysis. The BMCL10/NOAEL/LOAEL of 0.94 mg /m3 was adjusted for intermittent exposure (6 hours/day, 5 days/week), multiplied by the RDDR of 0.206, and divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability). See Appendix A for details.
[c](*When necessary, add this footnote*) Intermediate-duration inhalation MRL also adopted for acute-duration MRL.

BW or Bd wt = body weight; F = female(s); Gastro = gastrointestinal; HP = histopathology; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight

*Guidance: Please note that if there are chronic inhalation studies this would also be added to this table.*

**Table 2-2. Oral LSE**

**Table 2-2. Levels of Significant Exposure to [Substance X] – Oral**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (mg/kg/day) | Parameters monitored | Endpoint | NOAEL (mg/kg/day) | Less serious LOAEL (mg/kg/day) | Serious LOAEL (mg/kg/day) | Effect |
|---|---|---|---|---|---|---|---|---|---|
| **ACUTE EXPOSURE** | | | | | | | | | |
| 1 | Rat (Wistar) 4 M, 4 F | Once (GO) | 0, 200 | HP | Hepatic | | 200 | | Cytoplasmic lipid droplets and apoptotic cells in liver |
| | | | | | Renal | | | 200 | Extensive epithelial necrosis and degeneration of epithelia in proximal tubules |
| **Birner et al. 1995** | | | | | | | | | |
| 2 | Rat (Wistar) 6 M, 6 F | 14 d (F) | M: 0, 5.9, 19, 59 F: 0, 6.2, 20, 62 | BW, OW, HP | Bd wt | 5.9M | 19 M 6.2 F | | Body weight was reduced by 9.5% in females |
| | | | | | Hepatic | 59M 62F | | | |
| | | | | | Renal | | 5.9 M[b] 6.2 F | | Proximal convoluted tubule degeneration |
| **Harleman and Seinen 1979** | | | | | | | | | |
| 3 | Rat (Wistar) 5 M | Once (GO) | 0, 10, 100, 200 | FI, BW, BC, UR, OW, HP | Renal | 10 | 100 | 200 | Increased relative kidney weight, proximal tubular necrosis, increases in serum creatinine; at 200 mg/kg, increases in BUN, and urinary protein, and glucose, increased urine volume and decreased urine density |
| **Jonker et al. 1993a** | | | | | | | | | |

**Table 2-2. Levels of Significant Exposure to [Substance X] – Oral**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (mg/kg/day) | Parameters monitored | Endpoint | NOAEL (mg/kg/day) | Less serious LOAEL (mg/kg/day) | Serious LOAEL (mg/kg/day) | Effect |
|---|---|---|---|---|---|---|---|---|---|
| **INTERMEDIATE EXPOSURE** | | | | | | | | | |
| 4 | Rat (Wistar) 10 M, 10 F | 13 weeks (GO) | 0, 0.4, 1, 2.5, 6.3, 15.6 | BW, FI, HE, BC, UR, OW, HP | Bd wt | 2.5 | | 6.3 | Body weight decreased by 29% in females and by 13% in males |
| | | | | | Resp | 15.6 | | | |
| | | | | | Cardio | 15.6 | | | |
| | | | | | Gastro | 15.6 | | | |
| | | | | | Hemato | 15.6 | | | |
| | | | | | Hepatic | 6.3 M | 15.6 M | | Increased cytoplasmic basophilia |
| | | | | | Renal | 2.5 M<br>1 F | 6.3 M<br>2.5 F | | Enlarged hyperchromatic nuclei in the proximal tubules in females at 2.5 mg/kg/day and male at 6.3 mg/kg/day; decreased urine osmolarity in females at 2.5 mg/kg/day |
| | | | | | Endocr | 15.6 | | | |
| | | | | | Immuno | 15.6 | | | |
| | | | | | Neuro | 15.6 | | | |
| **Harleman and Seinen 1979** | | | | | | | | | |
| 5 | Rat (Wistar) 6 F | 10–18 weeks (F) | 0, 15, 150 | CS, BW, OW, HP, RX, DX | Bd wt | | 15 | | Body weight decreased by 15% |
| | | | | | Resp | 150 | | | |
| | | | | | Cardio | 150 | | | |
| | | | | | Hepatic | 15 | 150 | | Slight proliferation of bile duct epithelium |

**Table 2-2. Levels of Significant Exposure to [Substance X] – Oral**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (mg/kg/day) | Parameters monitored | Endpoint | NOAEL (mg/kg/day) | Less serious LOAEL (mg/kg/day) | Serious LOAEL (mg/kg/day) | Effect |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Renal | | 15 | 150 | Tubular degeneration and necrosis at 15 mg/kg/day; extensive tubular degeneration at 150 mg/kg/day |
| | | | | | Endocr | 150 | | | |
| | | | | | Immuno | 150 | | | |
| | | | | | Neuro | 15 | | 150 | Ataxia, demyelination and fragmentation of femoral nerve fibers |
| | | | | | Repro | 15 | | 150 | Infertility |
| | | | | | Develop | | 15 | | 16–19% reduction in pup body weight |
| **Harleman and Seinen 1979** | | | | | | | | | |
| 6 | Rat (Wistar) 5 M, 5 F | 4 weeks (F) | 0, 2.5, 9.9, 40 | BW, HP, OW, UR | Bd wt | 2.5 | 9.9 | | Reduced body weight in males (9.7%) and females (15%) |
| | | | | | Hepatic | 40 | | | |
| | | | | | Renal | | 2.5 F | | Decreased BUN at ≥2.5 mg/kg/day in females; diffuse tubular cytomegaly in the inner cortex in females at ≥9.9 mg/kg/day and males at 40 mg/kg/day |
| **Jonker et al. 1993b** | | | | | | | | | |
| 7 | Rat (Wistar) 5 F | 32 days (GO) | 0, 1, 4 | BW, HP, OW, UR | Bd wt | 4 | | | |
| | | | | | Renal | 1 | 4 | | Increased GGT in urine (79%), increased relative kidney weight (12.6%), and focal tubular vacuolization |
| **Jonker et al. 1996** | | | | | | | | | |

**Table 2-2. Levels of Significant Exposure to [Substance X] – Oral**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (mg/kg/day) | Parameters monitored | Endpoint | NOAEL (mg/kg/day) | Less serious LOAEL (mg/kg/day) | Serious LOAEL (mg/kg/day) | Effect |
|---|---|---|---|---|---|---|---|---|---|
| 8 | Rat (Sprague-Dawley) 4 F | 30 days (F) | 0, 1, 3, 10, 65, 100 | CS, BC, HE, FI, BW, OW, GN, HP | Bd wt | 10 | 30 | | Decreased body weight gain; 10.5% at 30 mg/kg/day |
| | | | | | Resp | 100 | | | |
| | | | | | Cardio | 100 | | | |
| | | | | | Gastro | 100 | | | |
| | | | | | Hemato | 3 | 10 | | Increased hemoglobin concentration |
| | | | | | Hepatic | 65 | 100 | | Centrilobular hepatocellular swelling |
| | | | | | Renal | 10 | 30 | | Tubular degeneration, necrosis, regeneration |
| | | | | | Endocr | 100 | | | |
| | | | | | Immuno | 100 | | | |
| | | | | | Neuro | 100 | | | |
| **Kociba et al. 1971** | | | | | | | | | |
| 9 | Rat (Wistar) 3 M | 3 weeks (F) | 0, 7.1, 37, 190 | BW, HP | Bd wt | 7.1 | 37 | | 15% reduction in body weight |
| | | | | | Renal | 37 | 190 | | Proximal tubules lined with basophilic epithelium |
| **Nakagawa et al. 1998** | | | | | | | | | |
| 10 | Rat (Wistar) 21 M | 30 weeks (F) | 0, 94 | BW, OW, HP | Bd wt | | 94 | | 23% decrease in mean final body weight |
| | | | | | Renal | 94 | | | No change in BUN or creatinine levels, no histological alterations |
| **Nakagawa et al. 1998** | | | | | | | | | |

**Table 2-2. Levels of Significant Exposure to [Substance X] – Oral**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (mg/kg/day) | Parameters monitored | Endpoint | NOAEL (mg/kg/day) | Less serious LOAEL (mg/kg/day) | Serious LOAEL (mg/kg/day) | Effect |
|---|---|---|---|---|---|---|---|---|---|
| 11 | Rat (Sprague-Dawley) 10–12 M, 20–24 F | 90 days premating, 15-day mating, GDs 1–21, LDs 1–21 (F) | 0, 0.2, 2, 20 | CS, BW, FI, BC, UR, HE, OW, HP, RX, DX | Bd wt | 2 | 20 | | 7–17% decrease body weight gain in females; decreases in food intake also observed. |
| | | | | | Resp | 20 | | | |
| | | | | | Cardio | 20 | | | |
| | | | | | Gastro | 20 | | | |
| | | | | | Hemato | 20 | | | |
| | | | | | Musc/Skel | 20 | | | |
| | | | | | Hepatic | 20 | | | |
| | | | | | Renal | 0.2 F | 2 F | | Tubular dilatation and hypertrophy with foci of epithelial degeneration and regeneration in females at ≥2 mg/kg/day and males at 20 mg/kg/day |
| | | | | | Neuro | 20 | | | |
| | | | | | Repro | 20 | | | |
| | | | | | Develop | 2 | 20 | | 13% decrease in neonatal weight |
| **Schwetz et al. 1977** | | | | | | | | | |
| 12 | Mouse (B6C3F1) 5 M, 5 F | 15 days (F) | M: 0, 3, 12, 40, 19, 24; F: 0, 5, 16, 49, 30, 36 | CS, BW, OW, GN, HP | Death | | | | 100% mortality at the two highest doses |
| | | | | | Bd wt | 3 M<br>5 F | | 12 M<br>16 F | Weight loss |
| | | | | | Neuro | 12 M<br>16 F | | 40 M<br>49 F | Lethargy, hunched position, incoordination |
| **NTP 1991** | | | | | | | | | |

**Table 2-2. Levels of Significant Exposure to [Substance X] – Oral**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (mg/kg/day) | Parameters monitored | Endpoint | NOAEL (mg/kg/day) | Less serious LOAEL (mg/kg/day) | Serious LOAEL (mg/kg/day) | Effect |
|---|---|---|---|---|---|---|---|---|---|
| 13 | Mouse (B6C3F1) 10 M, 10 F | 13 weeks (F) | M: 0, 0.1, 0.4, 1.5, 4.9, 16.8 F: 0, 0.2, 0.5, 1.8, 4.5, 19.2 | CS, BW, FI, OW, GN, HP | Bd wt | 1.5 M<br>4.5 F | 4.9 M<br>19.2 F | | Body weight gain reduced by 9.9% in males |
| | | | | | Resp | 19.2 M<br>16.8 F | | | |
| | | | | | Cardio | 19.2 M<br>16.8 F | | | |
| | | | | | Gastro | 19.2 M<br>16.8 F | | | |
| | | | | | Musc/skel | 19.2 M<br>16.8 F | | | |
| | | | | | Hepatic | 19.2 M<br>16.8 F | | | |
| | | | | | Renal | 1.5 M<br>0.2 F[c] | 4.9 M<br>0.5 F | | Tubular epithelial regeneration in females at ≥0.5 mg/kg/day and in males at ≥4.9 mg/kg/day |
| | | | | | Endocr | 19.2 M<br>16.8 F | | | |
| | | | | | Dermal | 19.2 M<br>16.8 F | | | |
| | | | | | Immuno | 19.2 M<br>16.8 F | | | No histological alterations |
| | | | | | Neuro | 19.2 M<br>16.8 F | | | No histological alterations |

**Table 2-2. Levels of Significant Exposure to [Substance X] – Oral**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (mg/kg/day) | Parameters monitored | Endpoint | NOAEL (mg/kg/day) | Less serious LOAEL (mg/kg/day) | Serious LOAEL (mg/kg/day) | Effect |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Repro | 19.2 M<br>16.8 F | | | No dose-related decreases in sperm motility at 1.5 mg/kg/day; no alterations in sperm count, incidence of abnormal sperm, estrual cyclicity, average estrous cycle length |
| **NTP 1991; Yang et al. 1989** | | | | | | | | | |
| **CHRONIC EXPOSURE** | | | | | | | | | |
| 14 | Rat (Sprague-Dawley) 40 M, 40 F | 2 years (F) | 0, 0.2, 2, 20 | BW, HE, BC, UR, OW, GN, HP | Death | | | 20M | Increased mortality in males |
| | | | | | Bd wt | 2 | 20 | | Mean body weight reduced by 8–20% in males and 5–12% in females |
| | | | | | Resp | 20 | | | |
| | | | | | Cardio | 20 | | | |
| | | | | | Gastro | 20 | | | |
| | | | | | Hemato | 20 | | | |
| | | | | | Musc/skel | 20 | | | |
| | | | | | Hepatic | 20 | | | |
| | | | | | Renal | 0.2 | 2 | | Tubular epithelial hyperplasia |
| | | | | | Endocr | 20 | | | |

**Table 2-2. Levels of Significant Exposure to [Substance X] – Oral**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (mg/kg/day) | Parameters monitored | Endpoint | NOAEL (mg/kg/day) | Less serious LOAEL (mg/kg/day) | Serious LOAEL (mg/kg/day) | Effect |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Neuro | 20 | | | |
| | | | | | Repro | 20 | | | |
| | | | | | Cancer | | | 20 | CEL: kidney tumors |
| **Kociba et al. 1977a** | | | | | | | | | |

[a]The number corresponds to entries in Figure 2-x *(link to corresponding LSE figure)*; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-x *(link to corresponding LSE figure).* Where such differences exist, only the levels of effect for the most sensitive gender are presented.
[b]Used to derive an acute oral Minimal Risk Level (MRL) of 0.006 mg/kg/day; the LOAEL dose was divided by an uncertainty factor of 1,000 (10 for the use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability)
[c]Used to derive an intermediate oral MRL of 0.002 mg/kg/day; dose divided by an uncertainty factor of 1,000 (10 for extrapolation from animals to humans, 10 for use of a LOAEL and 10 for human variability).

BC = blood chemistry; Bd wt or BW = body weight; BUN = blood urea nitrogen; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; G = gavage; Gastro = gastrointestinal; GGT = gamma-glutamyl transferase; GN = gross necropsy; (GO) = gavage in oil vehicle; HE = hematology; Hemato = hematological; HP = histopathological; Immuno = immunological; LOAEL = lowest-observed-adverse-effect level; $LD_{50}$ = lethal dose, 50% kill; M = male(s); Musc/skel = muscular/skeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; UR = urinalysis

**Table 2-3. Dermal LSE**

**Table 2-3. Levels of Significant Exposure to [Substance x] – Dermal**

| Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effect |
|---|---|---|---|---|---|---|---|---|
| **ACUTE EXPOSURE** | | | | | | | | |
| Rabbit (NS) 4 NS | Once | NS | CS | Dermal | | 0.5 mL | | Slight erythema |
| **Torkelson et al. 1961** | | | | | | | | |
| **INTERMEDIATE EXPOSURE** | | | | | | | | |
| Rabbit (NS) 1 NS | 20 days 1 time/day | NS | CS | Dermal | | 0.5 mL | | Crustiness of skin |
| **Torkelson et al. 1961** | | | | | | | | |
| **CHRONIC EXPOSURE** | | | | | | | | |
| Mouse (Ha:ICR Swiss) 30 F | 63–85 weeks 3 days/week 1 time/day | 0, 11.7 35.0 | GN, HP | Cancer | | | 11.7[a] | CEL: stomach carcinoma |
| **Van Duuren et al. 1979** | | | | | | | | |

[a]Cumulative dose based on exposure to 390 mg/kg, 3 days/week up to 85 weeks.

CEL = cancer effect level; CS = clinical signs; F = female(s); GN = gross necropsy; HP = histopathology; $LD_{50}$ = lethal dose, 50 % kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-effect-level; NS = not specified

**Figures 2-3 and 2-4. LSE**

**Figure 2-3. Levels of Significant Exposure to [Substance X] – Inhalation**

Acute (≤14 days)

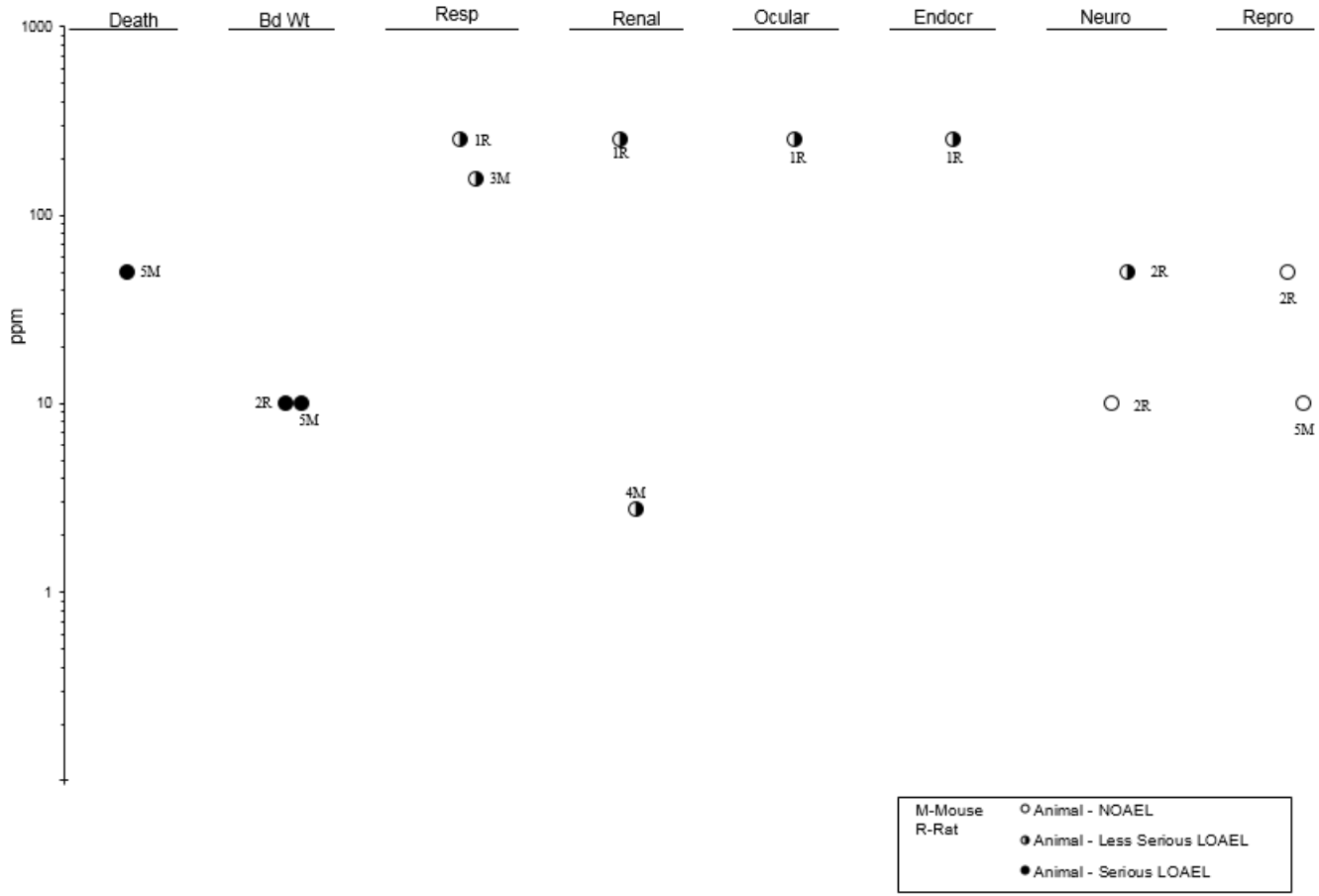
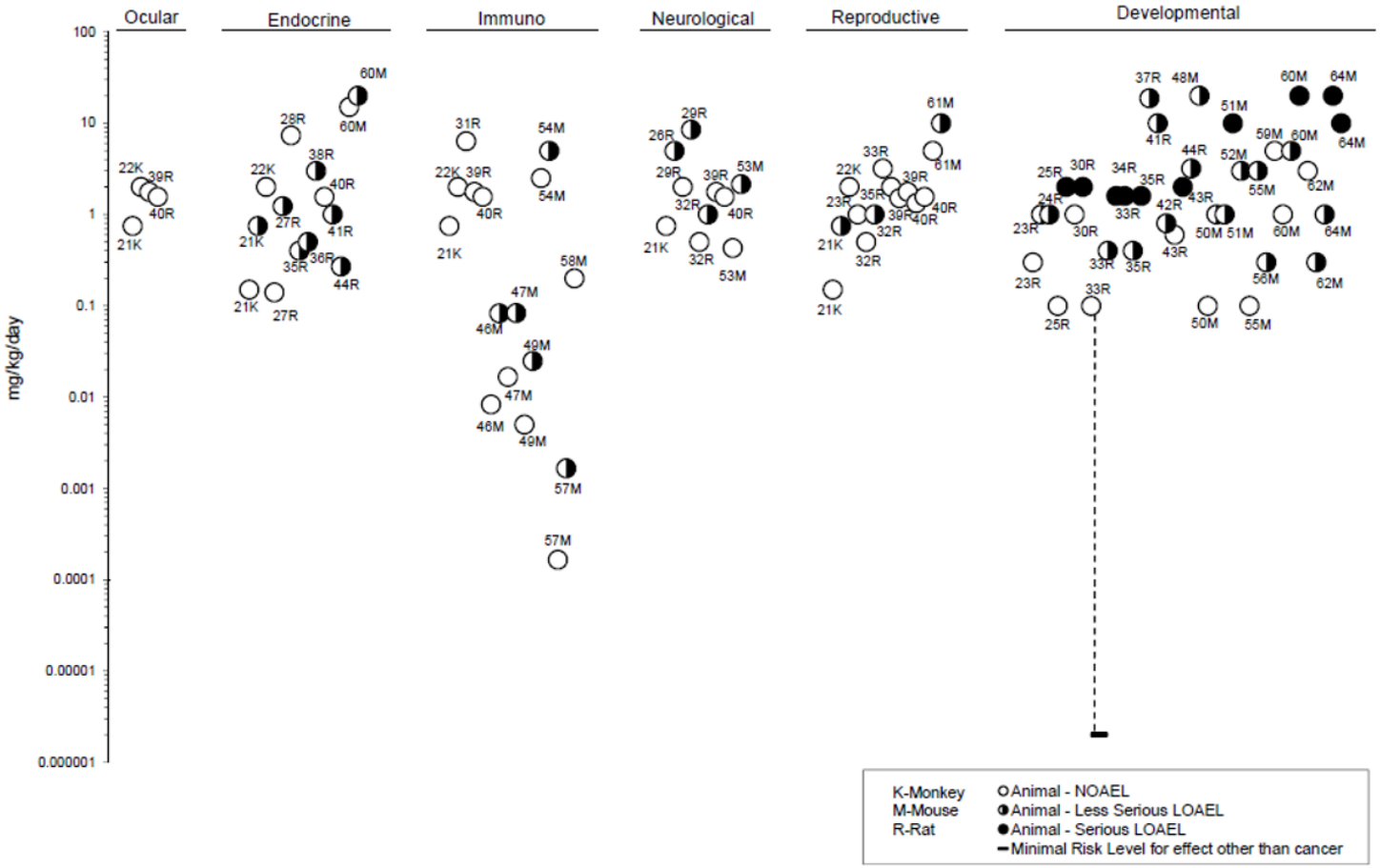


**Figure 2-4. Levels of Significant Exposure [Substance x] – Oral**
Intermediate (15-364 days)




*Guidance: For inhalation and oral exposure routes, landscape figure(s) for each duration (acute, intermediate, chronic) on separate pages. Figure 2-2 displays inhalation exposures. Figure 2-3 displays oral exposures. Due to the number of potential endpoints (19), a single duration may use more than one page. This is dependent on whether studies exist. If an endpoint has not been studied, it is not included in the LSE table. For instance, hematological endpoints have not been studied following acute inhalation exposure (see above) and the effect category is not listed. If there are no studies for an exposure duration then do not include the figure. The study chosen for the MRL has a dashed line to the MRL. The numbers on the figure correspond to the figure key numbers in the LSE table. There are no dermal LSE figures.*

**Table 2-4 and 2-5. Genotoxicity of [Substance x] *In Vitro* and *In Vivo***

**Table 2-4. Genotoxicity of [Substance x] *In Vitro***

| Species (test system) | Endpoint | Results: Activation: With | Results: Activation: Without | Reference |
|---|---|---|---|---|
| Prokaryotic organisms | | | | |
| *Salmonella typhimurium* | Gene mutation | + | + | Novotná and Duverger-van Bogaert 1994 |
| *S. typhimurium* | Gene mutation (reverse mutation) | ND | + | Ames and Yanofsky 1971 |
| *S. typhimurium* | Gene mutation (Ara test) | + | + | Roldán-Arjona et al. 1991 |
| *S. typhimurium* | DNA damage (SOS uma test) | + | + | Oda et al. 1996 |
| *Escherichia coli* | Gene mutation | ND | + | Watanabe et al. 1998 |
| *Bacillus subtilis* | Gene mutation (forward mutation; spot test) | + | – | Shiau et al. 1980 |
| *B. subtilis* | DNA damage (spot test) | ND | – | Shiau et al. 1980 |
| *Aspergillus nidulans* | Gene mutation (forward mutation) | ND | + | Principe et al. 1981 |
| *Streptomyces coelicolor* | Gene mutation (forward mutation) | ND | – | Principe et al. 1981 |
| *Neurospora crassa* | Gene mutation (recessive lethal) | ND | + | Malling 1969 |
| Mammalian cells | | | | |
| Human (epithelial cells) | Gene mutation (forward mutation) | ND | + | Ferreri et al. 1983 |
| Human (lymphoblasts; Tk6) | Gene mutation (forward mutation) | ND | + | Crespi et al. 1985 |
| Human (lymphoblasts; AAH-1) | Gene mutation (forward mutation) | ND | + | Crespi et al. 1985 |
| Human (testicular germ cells) | DNA damage (single strand breaks) | ND | + | Bjørge et al. 1996 |
| Human (hepatocytes) | DNA adducts (DNA binding) | ND | + | Cmarik et al. 1990 |
| Rat (testicular germ cells) | DNA damage (single strand breaks) | ND | + | Bjørge et al. 1996 |
| Rat (hepatocytes) | DNA damage (double strand breaks) | ND | – | Storer et al. 1996 |
| Rat (hepatocytes) | DNA adducts (DNA binding) | ND | + | Cmarik et al. 1990 |
| Chinese hamster ovary (CHO) cells | Gene mutation | ND | + | Ballering et al. 1998 |
| CHO cells | Gene mutation | ND | + | Graves et al. 1996 |
| CHO cells | Gene mutation (forward mutation) | + | + | Tan and Hsie 1981; Brimer et al. 1982 |

– = negative result; + = positive result; $Ara^r$ = L-arabinose resistance; DNA = deoxyribonucleic acid; ND = not determined

**Table 2-5. Genotoxicity of [Substance x] *In Vivo***

| Species (exposure route) | Endpoint | Results | Reference |
|---|---|---|---|
| *Drosophila melanogaster* (vapor exposure of adult males) | Sex-linked recessive lethal mutations in sperm cells | – | Kale and Baum 1982a |
| *D. melanogaster* (feed of adult males) | Sex-linked recessive lethal mutations | + | Zimmering 1983 |
| *D. melanogaster* (feed of adult males) | Sex-linked recessive lethal mutations | + | Inoue et al. 1982 |
| *D. melanogaster* (vapor exposure of adult males) | Genetic crossing over in sperm cells | + | Kale and Baum 1982a |
| *D. melanogaster* (feed of adult males) | Heritable translocations | + | Yoon et al. 1985 |
| *D. melanogaster* (feed of adult males) | Chromosome loss | + | Zimmering 1983 |
| Muta-mouse (intraperitoneal) | Gene mutation in testicular cells | (+) | Hachiya and Motohashi 2000 |
| Mouse (intraperitoneal) | Specific-locus gene mutations | – | Russell et al. 1986 |
| Mouse (intraperitoneal) | Somatic cell mutagenicity (spot test) | + | Sasaki et al. 1986 |
| Mouse (intraperitoneal) | Chromosomal aberrations in bone marrow | – | Shelby and Witt 1995 |
| Rat (intraperitoneal) | DNA damage in cells from multiple organs | + | Brunborg et al. 1988, 1996 |
| Rat (intraperitoneal) | DNA damage in kidney and testicular cells | + | Lag et al. 1991 |
| Rat, mouse, guinea pig, hamster (intraperitoneal) | DNA damage in kidney cells | + | Soderlund et al. 1990 |
| Mouse (oral) | DNA damage | + | Sasaki et al. 1998 |
| Rat (oral) | Micronuclei in bone marrow | + | Albanese et al. 1988; George et al. 1990 |
| Mouse (oral) | Micronuclei in bone marrow, stomach, liver, kidney, lung | + | Sasaki et al. 1998 |
| Mouse (intraperitoneal) | Micronuclei in bone marrow | – | Shelby and Witt 1995; Shelby et al. 1993 |
| Rat (oral) | Dominant lethality | + | Teramoto et al. 1980 |
| Rat (inhalation) | Dominant lethality | + | Rao et al. 1983 |
| Mouse (oral) | Dominant lethality | – | Teramoto et al. 1980 |
| Mouse, prepubertal males (intraperitoneal) | Unscheduled DNA synthesis in premeiotic germ cells | + | Lee and Suzuki 1979 |
| Mouse, adult males (intraperitoneal) | Unscheduled DNA synthesis in spermatozoa | – | Lee and Suzuki 1979 |

– = negative result; + = positive result; (+) = weakly positive result; DNA = deoxyribonucleic acid

## EXHIBIT 11. CHAPTER 3 FIGURES AND TABLES

### Table 3-1. Tissue Distribution and Excretion

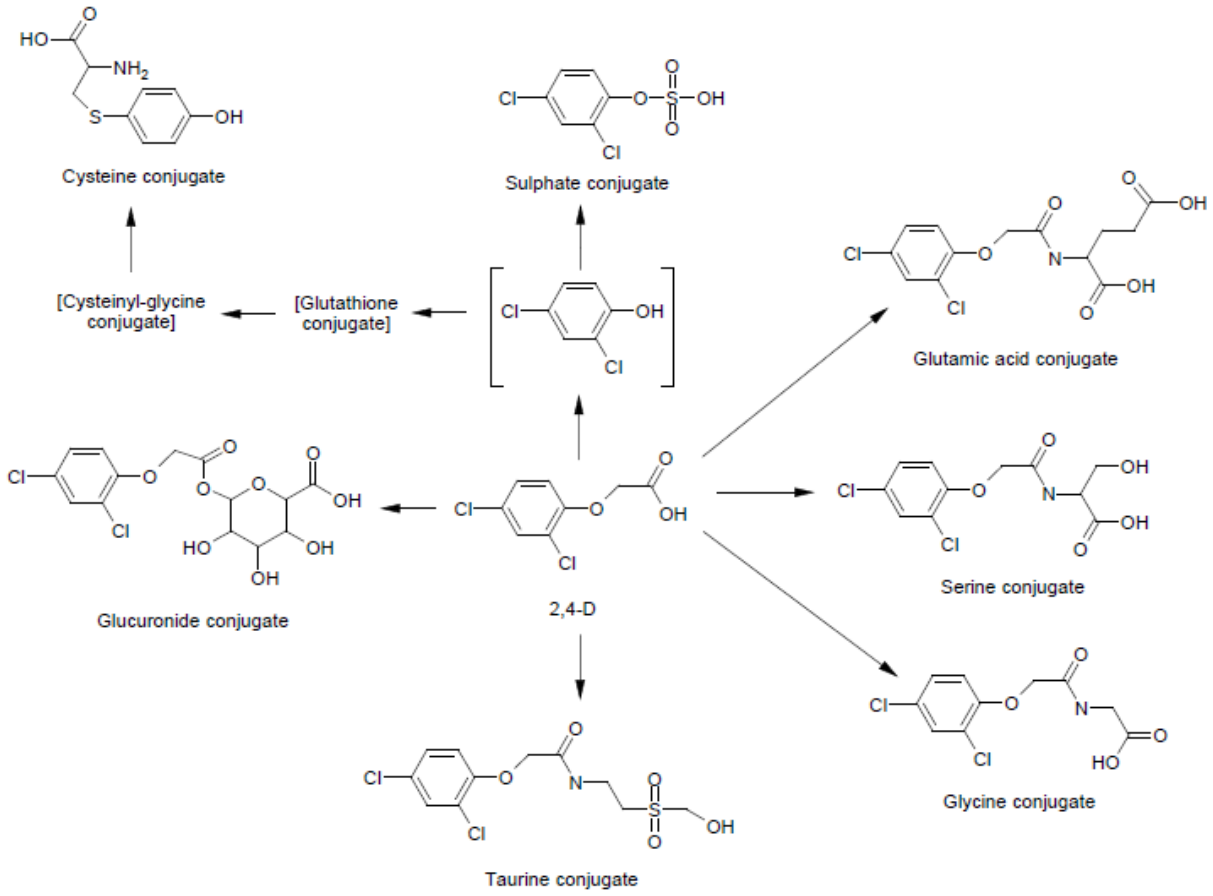
**Table 3-1. Tissue Distribution and Excretion of 14C-Radioactivity From Animals Dosed with 14C-Labeled [Substance x][a]**

| | µg Equivalent per g (mL) wet weight[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Rat | | Mouse | | Hamster | | Rabbit | |
| Sample | Male | Female | Male | Female | Male | Female | Male | Female |
| Blood | 23.5 | <0.1 | 13.8 | 10.1 | 0.1 | 8.8 | <0.1 | 0.1 |
| Liver | 40.0 | <0.1 | 43.2 | 45.3 | 0.3 | 7.3 | 0.1 | 1.5 |
| Kidneys | 24.0 | <0.1 | 2.9[c] | 2.2[c] | 0.2 | 7.1 | 0.1 | 0.4 |
| Lungs | 8.7 | <0.1 | 1.4[c] | 1.3[c] | <0.1 | 3.8 | <0.1 | 0.1 |
| Heart | 6.4 | <0.1 | 1.2[c] | 0.6[c] | <0.1 | 2.9 | <0.1 | <0.1 |
| Skin | 4.8 | <0.01 | 3.5 | 0.2 | <0.1 | 3.4 | <0.1 | <0.1 |
| Muscle | 1.9 | <0.1 | 1.1 | 0.5 | <0.1 | 0.9 | <0.1 | <0.1 |
| Fat | 1.7 | <0.1 | 1.6 | 1.3 | <0.1 | 1.5 | <0.1 | <0.1 |
| Brain | 0.6 | <0.1 | 0.2[c] | 0.8[c] | <0.1 | 0.3 | <0.1 | <0.1 |
| | Percent of dose | | | | | | | |
| Tissues | 59.6 | 0.6 | 73.6 | 50.0 | 0.7 | 26.5 | <0.1 | 0.3 |
| Urine | 25.6 | 73.9 | 3.4 | 6.7 | 90.3 | 45.3 | 76.8 | 87.9 |
| Feces | 9.2 | 27.8 | 8.3 | 5.4 | 8.2 | 9.3 | 4.2 | 4.6 |
| Expiration | 3.6 | 1.5 | 5.2 | 4.4 | 1.3 | 2.9 | No data | No data |
| Cage wash | 0.6 | 0.8 | 4.9 | 4.9 | 0.6 | 2.1 | 0.5 | 4.8 |
| Percent recovered | 98.5 | 104.6 | 95.4 | 71.4 | 101.1 | 86.1 | 81.6 | 97.6 |

[a]The rabbits were sacrificed 168 hours after dosing; all other animals were sacrificed 120 hours after dosing.
[b]The µg equivalent calculations were based on the specific activity of $^{14}$C-labeled X, which was 1.1x10$^{6}$ DPM/mg. The µg equivalent per g wet weight could not accurately be determined below 0.1 µg/g.
[c]Represents the µg equivalents for the entire organ.

Source: Clark 2018

**Figure 3-1. Proposed Metabolic Pathway for [Substance x] in Y**




2,4-D = 2,4-dichlorophenoxyacetic acid

Source: Van Ravenzwaay et al. 2003

**Table 3-2. Table of PBPK Model Parameters**

## Table 3-2. Parameter Values for Sweeney et al. (2010) PBPK Model of [Substance X] in Rats and Humans

| Description | Value | | Source |
|---|---|---|---|
| | Rat | Human | |
| Body weight (*BW*, kg) | 0.3 | 70 | Observed |
| Cardiac output (*KQC*, L/hour/kg$^{0.74}$) | 15 | 14 | Brown et al. 1997, as cited in Sweeney et al. 2010; Krishnan et al. 2009; Timchalk et al. 2002 |
| Blood flow (K*Q*) fraction of cardiac output | | | |
| Liver (K*QL*) | 0.25 | 0.175 | Brown et al. 1997, as cited in Sweeney et al. 2010; Krishnan et al. 2009; Timchalk et al. 2002 |
| Brain (*KQB*) | 0.03 | 0.114 | |
| Fat (*KQF)* | 0.09 | 0.085 | |
| Slowly perfused tissues (*KQS*) | 0.20 | 0.2449 | |
| Rapidly perfused tissues (*KQR*) | 0.43 | 0.3811 | 1-(*KQL+KQB+KQF+KQS*) |
| Compartment volumes ($V_i$) fraction of body weight | | | |
| Liver (*KVL)* | 0.04 | 0.026 | Brown et al. 1997, as cited in Sweeney et al. 2010; Krishnan et al. 2009; Timchalk et al. 2002 |
| Brain (*KVB*) | 0.012 | 0.02 | |
| Fat (*KVF)* | 0.07 | 0.21 | |
| Rapidly perfused tissues (*KVR*) | 0.04 | 0.052 | |
| Blood (*KVV*) | 0.06 | 0.079 | |
| Slowly perfused tissues (*KVS*) | 0.688 | 0.523 | 0.91 – (*KVL+KVB+KVF+KVR+KVV*) |
| Tissue:blood partition coefficients | | | |
| Liver *(PL*) | 1.2 | 1.3 | Krishnan et al. 2009 (predicted from n-octanol:water partition coefficient) |
| Brain (*PB*) | 1.4 | 1.6 | |
| Rapidly perfused tissues (*PS*) | 1.4 | 1.6 | |
| Fat (*PF*) | 5.57 | 5.57 | Optimized—intravenous rat data[a] |
| Slowly perfused tissues (*PR*) | 0.15 | 0.15 | |
| Liver metabolism | | | |
| Metabolism (*KfC*, kg$^{0.33}$/hour) | 2.6 | 11.2 | Optimized—intravenous rat data[a]<br>Optimized—oral human data[b] |
| Gastrointestinal absorption | | | |
| Absorption from stomach (*KAS*, hour$^{-1}$) | | 0.033 | Optimized—oral human data[b] |
| gavage (rat) | 0.83 | NA | Optimized—oral rat data[c] |
| capsule (rat) | 0.12 | NA | |
| coarse (rat) | 0.005 | NA | |
| Transfer to duodenum (*KT*, hour$^{-1}$) | | 0 | Optimized—oral human data[b] |
| gavage (rat) | 1.37 | NA | Optimized—oral rat data[c] |
| capsule (rat) | 0 | NA | |
| coarse (rat) | 0 | NA | |
| Absorption from duodenum (*KAD*, hour$^{-1}$) | | NA | Optimized—oral human data[b] |
| gavage (rat) | 0.0258 | NA | Optimized—oral rat data[a] |
| capsule (rat) | NA | NA | |
| coarse (rat) | NA | NA | |

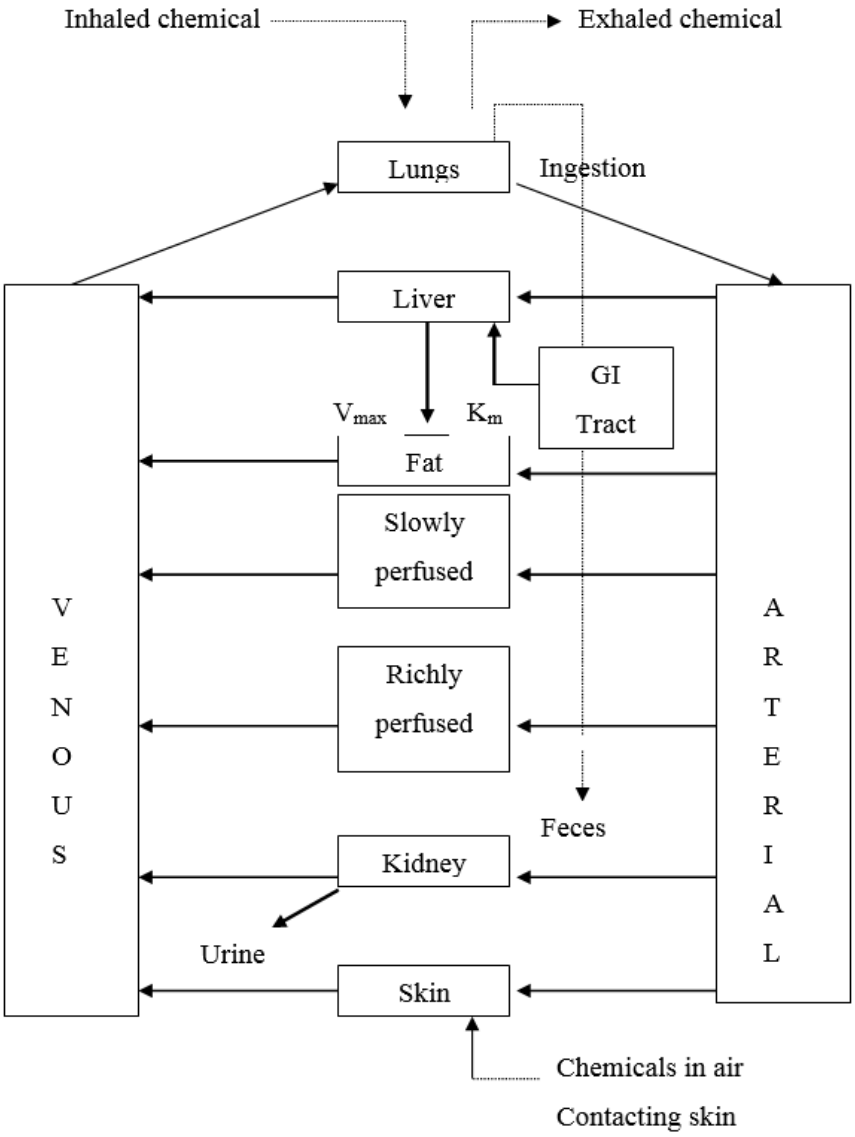
[a]Krishnan et al. 2009.
[b]Özhan et al. 2003; Woody et al. 1986.
[c]Bannon et al. 2009a; Crouse et al. 2008; Krishnan et al. 2009; Schneider et al. 1977.

Source: Sweeney et al. 2010

**Figure 3-2. PBPK Model**

*(use the author's model when MRL derived from it)*
*Note: This is a generic representation just for understanding.*

## Figure 3-2. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



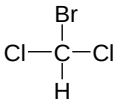

Source: Krishnan et al. 1994

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.

## EXHIBIT 12. CHAPTER 4 TABLES

### Table 4-1. Chemical Identity

**Table 4-1. Chemical Identity of [Substance x]**

| Characteristic | Information | Reference |
|---|---|---|
| Chemical name | [Substance x] | HSDB 2012 |
| Synonym(s) and Registered trade name(s) | AKA-1; AKA-2; AKA-3; AKA-4, AKA-5; AKA-6 | HSDB 2012; NIOSH 2015 |
| Chemical formula | $CHBrCl_2$ | HSDB 2012 |
| Chemical structure |  | Haynes 2014 |
| CAS registry number | 75-27-4 | HSDB 2012 |

CAS = Chemical Abstracts Service

**Table 4-2. Physical and Chemical Properties**

**Table 4-2. Physical and Chemical Properties of [Substance x]**

| Property | Information | Reference |
|---|---|---|
| Molecular weight | 163.829 | Haynes 2014 |
| Color | Colorless | O'Neil 2013 |
| Physical state | Liquid | O'Neil 2013 |
| Melting point | -56.0°C | Haynes 2014 |
| Boiling point | 90°C | Haynes 2014 |
| Density: | | Haynes 2014 |
| at 20°C/4°C | 1.980 | |
| Odor | No data | |
| Odor threshold: | | |
| Water | No data | |
| Air | No data | |
| Taste threshold | No data | |
| Solubility: | | |
| Water | 3,030 mg/L at 30 °C | Yalkowsky et al. 2010 |
| Organic solvent(s) | Very soluble in ethanol, acetone, and benzene; slightly soluble in carbon tetrachloride | Haynes 2014 |
| Partition coefficients: | | |
| Log $K_{ow}$ | 2.00 | HSDB 2012 |
| Log $K_{oc}$ | 1.8 | Mabey et al. 1982 |
| Vapor pressure | | |
| at 20°C | 50 mm Hg | HSDB 2012 |
| Henry's law constant | $2.12 \times 10^{-3}$ at 25 °C | EPA 1987 |
| Autoignition temperature | No data | |
| Flashpoint | No data | |
| Flammability limits | No data | |
| Conversion factors | 1 ppm=6.70 mg/m$^3$<br>1 mg/m$^3$=0.15 ppm | Verschueren 1977 |
| Explosive limits | No data | |

## EXHIBIT 13. CHAPTER 5 FIGURES AND TABLES

*Chapter 5 requires several tables and one figure; these are listed below. In addition, Chapter 5 has a number of required tables if monitoring data are available. Optional tables may be included in Chapter 5 to present other relevant data. The Perfluoroalkyls Toxicological Profile has many examples. The contractor and chemical manager must agree to the content in Chapter 5.*

Table 5-X. Facilities that Produce, Process, or Use [Substance x]

Table 5-X. Releases to the Environment from Facilities that Produce, Process, or Use [Substance x]

Table 5-X. Lowest Limit of Detection Based on Standards

Table 5-X. [Substance x] Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Table 5-X. Blood [Substance x] Levels (ng/mL) in the NHANES U.S. Population

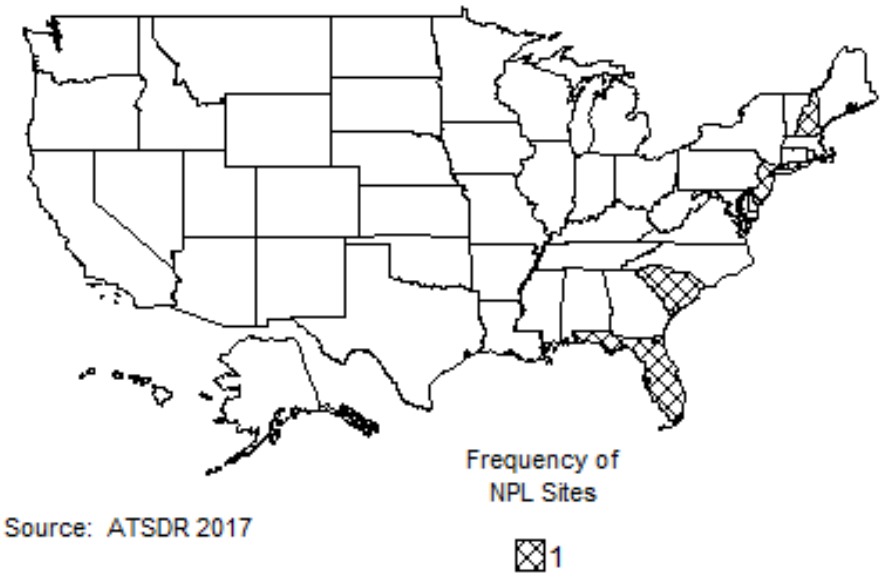
Figure 5-X. Number of NPL Sites with [Substance X] Contamination

*As always with figure and table numbering, begin each chapter with figure/table 1 and number consecutively for each table and figure, e.g., Figure 5-1, Table 5-1, Figure 5-2, Figure 5-3, Table 5-2, etc.*

**Figure 5-1. NPL Map**

*The figure is of an NPL map, as displayed below. It belongs in the overview (Section 5.1).*

**Figure 5-1. Number of NPL Sites with [Substance x] Contamination**



## Tables 5-1A and 5-1B. Production

*In Section 5.2.1, the contractor is to speak with the chemical manager to decide which of the two (or both) tables will be used in the toxicological profile.*

**Table 5-1A. Facilities that Produce, Process, or Use [Substance x]**

| State[a] | Number of facilities | Minimum amount on site in pounds[b] | Maximum amount on site in pounds[b] | Activities and uses[c] |
|---|---|---|---|---|
| CA | 2 | 0 | 49,999,999 | 7, 12 |
| LA | 2 | 0 | 999 | 1, 5, 7 |
| MN | 1 | 1,000 | 9,999 | 7, 9 |
| MS | 1 | 10,000 | 99,999 | 7 |
| MT | 1 | 10,000 | 99,999 | 2, 3, 4, 7, 9, 10 |
| NY | 1 | 100 | 999 | 12 |
| OH | 1 | 1,000 | 9,999 | 12 |
| TX | 3 | 10,000 | 99,999 | 2, 4, 7, 9 |

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/Uses:

1. Produce
2. Import
3. Used Processing
4. Sale/Distribution
5. Byproduct
6. Reactant
7. Formulation Component
8. Article Component
9. Repackaging
10. Chemical Processing Aid
11. Manufacture Aid
12. Ancillary
13. Manufacture Impurity
14. Process Impurity

Source: TRI16 2017; Data are from 2016

## Table 5-2B. Facilities that Produce, Process, or Use [Substance x]

| Facility | State[a] | Minimum amount on site in pounds[b] | Maximum amount on site in pounds[b] | Activities and uses[c] |
|---|---|---|---|---|
| Mesquite Mine | CA | 999,999 | 49,999,999 | 12 |
| Quemetco Inc. | CA | 0 | 99,999 | 7 |
| Noranda Alumina LLC. | LA | 0 | 999 | 1, 7 |
| Shell Norco Chemical Plant East Site | LA | 0 | 999 | 5 |
| Gopher Resource LLC. | MN | 1,000 | 9,999 | 7, 9 |
| Chemours Delisle Plant | MS | 10,000 | 99,999 | 7 |
| Colstrip Steam Electric Station | MT | 10,000 | 99,999 | 2, 3, 4, 7, 9, 10 |
| CWM Chemical Services LLC. | NY | 100 | 999 | 12 |
| Columbus Castings | OH | 1,000 | 9,999 | 12 |
| Bestolife Corp. | TX | 10,000 | 99,999 | 2 |
| Dow Chemical Co. Freeport Facility | TX | 10,000 | 99,999 | 2, 4 |
| Dupont Sabine River Works | TX | 10,000 | 99,999 | 2, 7, 9 |

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/Uses:

1. Produce
2. Import
3. Used Processing
4. Sale/Distribution
5. Byproduct
6. Reactant
7. Formulation Component
8. Article Component
9. Repackaging
10. Chemical Processing Aid
11. Manufacture Aid
12. Ancillary
13. Manufacture Impurity
14. Process Impurity

Source: TRI16 2017; Data are from 2016

**Table 5-2. Releases**

*Section 5.3 has one required table, below.*

**Table 5-2. Releases to the Environment from Facilities that Produce, Process, or Use [Substance x]**

| | | 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 |
| OH | 1 | 0 | 0 | 0 | 0 | No data | 0 | No data | 0 |
| Total | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[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 onsite 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: TRI16 2017; Data are from 2016

### Tables 5-3 and 5-4. Levels in the Environment

*Before the subsections in Section 5.5, levels in the environment, there are two required tables, as displayed below.*

**Table 5-3. Lowest Limit of Detection for [Substance x] Based on Standards[a]**

| Media | Detection limit | Reference |
|---|---|---|
| Air | 0.0003–1 ppm | NIOSH 1987 |
| Water | 0.01 µg/L | EPA 1987b |
| Soil | ≤0.018 µg/g | Sawhney et al. 1988 |
| Biological tissues | 0.5 µg/g | Letz et al. 1984 |

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.

**Table 5-4. [Substance x] Levels in Water, Soil, and Air of National Priorities List (NPL) Sites**

| Medium | Median[a] | Geometric mean[a] | Geometric standard deviation[a] | Number of quantitative measurements | NPL sites |
|---|---|---|---|---|---|
| Water (ppb) | 2.4 | 2.53 | 15,800 | 30 | 16 |
| Soil (ppb) | 118,000 | 65,600 | 47,400 | 5 | 3 |
| Air (ppbv) | 0.01 | 0.029 | 4,503.15 | 5 | 4 |

[a]Concentrations found in ATSDR site documents from 1981 to 2017 for 1,832 NPL sites (ATSDR 2017). Maximum concentrations were abstracted for types of environmental media for which exposure is likely. Pathways do not necessarily involve exposure or levels of concern.

## Tables 5-5–5-7. Air

*In the air section (5.5.1), the following three tables are required if data are available.*

**Table 5-5. Percentile Distribution of Annual Mean [Substance x] Concentrations (ppbv) Measured in Ambient Air at Locations Across the United States**

| Year | Number of U.S. locations | 25th | 50th | 75th | 95th | Maximum |
|---|---|---|---|---|---|---|
| 2010 | 151 | 0.0089 | 0.010 | 0.033 | 0.10 | 0.47 |
| 2011 | 127 | 0.0079 | 0.012 | 0.029 | 0.099 | 0.47 |
| 2012 | 124 | 0.0072 | 0.010 | 0.050 | 0.075 | 0.23 |
| 2013 | 117 | 0.0095 | 0.0097 | 0.050 | 0.052 | 0.24 |
| 2014 | 116 | 0.0090 | 0.012 | 0.050 | 0.067 | 0.12 |
| 2015[a] | 52 | 0.0090 | 0.0090 | 0.050 | 0.11 | 0.23 |

[a]Data from January 1, 2015 to November 27, 2015.

Source: EPA 2015c

**Table 5-6. Outdoor Air Monitoring Data for [Substance x]**

| Location(s) | Geographic type | Date(s) | Range | Mean concentration | Notes | Reference |
|---|---|---|---|---|---|---|
| Texas, North Carolina, Arkansas | Suburban, urban, source dominated | Not specified (1983 or earlier) | 0.00076–0.180 ppbv | 0.0011 ppbv | Not detected in two of the rural, remote sites monitored in Arkansas | Brodzinsky and Singh 1983 |
| California | Urban, industrial | 1982/1983 | | 0.020–0.100 ppbv | Detected above 0.01 ppbv in 35% of the samples | Shikiya et al. 1984 |
| Atlantic Ocean | Open ocean | 1982/1984/1985 | 0.001–0.007 ppbv | | Air samples at several locations; attributed to releases from macroalgae | Class et al. 1986 |
| Texas, Louisiana, North Carolina, Arkansas | Suburban, urban, source dominated | Not specified (2005 or earlier) | | 0.74 µg/m$^3$ (0.11 ppbv) | Outdoor air | EPA 2005b |
| Italy, United States, and Germany | Surface air above swimming pools | 1986–1999 | <0.1 µg/m$^3$ (0.01 ppbv) (200 cm above water surface) | 0.1 µg/m$^3$ (0.01 ppbv) (150 cm above water surface) | Measured above the water surface of indoor and outdoor pools and hot tubs | WHO 2006 |
| Italy, United States, and Germany | Surface air above swimming pools | 1986–1999 | 100 µg/m$^3$ (14.9 ppbv) (20 cm above water surface) | 19.5 µg/m$^3$ (2.91 ppbv) (20 cm above water surface) | Measured above the water surface of indoor and outdoor pools and hot tubs | WHO 2006 |

**Table 5-7. Indoor Air Monitoring Data for [Substance x]**

| Location(s) | Geographic type | Date(s) | Mean concentration | Notes | Reference |
|---|---|---|---|---|---|
| New Jersey | Suburban | Not specified (1999 or earlier) | 0.38–0.75 µg/m$^3$ (0.056–0.11 ppbv) | Indoor air of 48 households | EPA 2005b |
| Southwestern United States | Urban living space air[a] | August 1997 | 0.01–0.49 ppbv | Outdoor air concentrations from 24-hour integrated samples 0.2–0.9 µg/m$^3$ (0.03–0.13 ppbv); air exchange rates in the home influenced concentrations | Kerger et al. 2005 |

[a]The average concentration of [Substance x] in the household water samples was reported as 42.0 µg/L.

## Tables 5-8–5-10. Water

*In Section 5.5.2, Water Levels in the Environment, tables for surface, ground, and drinking water are required if data are available.*

**Table 5-8. Surface Water Monitoring Data for [Substance x]**

| Location(s) | Geographic type | Date(s) | Range | Mean concentration | Notes | Reference |
|---|---|---|---|---|---|---|
| Atlantic Ocean | Open ocean; African coast, West Africa, Porto Santo, Sao Miguel, Bermuda Islands, Tenerife | 1982/1984/1985 | 0.1–1 ng/L (seawater); 0.4 ng/L (rain) | Not reported | Surface water concentrations attributed to releases from macroalgae | Class et al. 1986 |
| United States | Surface water at National Priority List (NPL) sites | | | Not reported | [Substance x] was detected at only 4 of 818 sites on the NPL and at 7% of a number of other sites being investigated under Superfund; quantitative data for surface water were not available | CLPSD 1988 |
| Salt River Phoenix, Arizona | River surface water | 1997–1998 | 0.3–1.1 µg/L | Not reported | | Rostad et al. 2000 |
| The Rhine, Meuse, northern delta area, and Westerscheld | Surface water | 1992–1997 | <0.1 mg/L | Not reported | | Miermans et al. 2000 |

**Table 5-9. Groundwater Monitoring Data for [Substance x]**

| Location(s) | Type | Date(s) | Range | Mean concentration | Notes | Reference |
|---|---|---|---|---|---|---|
| Salt Lake Valley, Utah | Well | 1999 | 0.02–0.51 µg/L | Not reported | Detected in 17 of 30 wells sampled; attributed to the recharge of chlorinated public supply waters used to irrigate lawns and gardens in residential areas | USGS 2003 |
| United States | Shallow groundwater | 1996 and 2002 | Trace: ≤0.2 µg/L | Not reported | Detected in 14% of samples; ≥0.2 in 1.7% of the samples | Squillace et al. 2004 |
| United States | Domestic wells | 1986–2001 | 0.2–7.0 µg/L | Not reported | Detected in 124 of 2,400 wells sampled | USGS 2006b |
| United States | Public wells | 1986–2001 | 0.2–21 µg/L | Not reported | Detected in 46 of 1,095 wells sampled | USGS 2006b |
| United States | Untreated groundwater and source water | 1985–2002 | 0.02–23 µg/L | Not reported | Detected in 1–3% of the aquifers samples; 0.1–1.7% shallow groundwaters; more frequently detected in groundwater samples collected from urban areas as compared to agricultural areas | USGS 2006b |
| United States | Untreated ground; public and domestic wells | 1997–2007 | 0.08–0.09 µg/L (median values) | Not reported | 10% (66 out of 631) of the public well samples; 1.7% (33 out of 1,861) of the domestic well samples; detected at a higher frequency in wells surrounded by urban areas compared with undeveloped, mixed, and agricultural surroundings | Carter et al. 2012 |
| United States | Public wells | 1993–2007 | | Not reported | Detected in 11% of the samples (932 wells) | USGS 2010b |

**Table 5-10. Drinking Water Monitoring Data for [Substance x]**

| Location(s) | Type | Date(s) | Range | Mean concentration | Notes | Reference |
|---|---|---|---|---|---|---|
| United States | Finished water | August 1973–February 1974 | 1.1–20.8 µg/L | Not reported | Sampling sites not reported | Bellar et al. 1974 |
| Tampa Bay, Florida | Finished water | August–September of 2004 | 0.053–7.48 µg/L | Not reported | Detected in 10 of 10 finished water samples | USGS 2007 |
| United States | Drinking water | 2000–2004 | | 1.0, 15.0, and 20.3 µg/L | Three locations were sampled weekly; it was found that all trihalomethanes were removed after heating the drinking water; faucet filters completely removed trihalomethanes and pitcher filters removed on average 40% of the trihalomethanes. | Savitz et al. 2006 |
| United States | Drinking/finished water | 1991–2003 | | 1.62 µg/L | Detected in 3 out of 34 tap water samples | FDA2006 |
| Italy | Italian tap water | Not specified (2005 or prior) | 0.249 µg/L | Not reported | Not detected in Italian mineral water, contaminated mineral water, Italian superficial snow, or Antarctic superficial snow | Zoccolillo et al. 2005 |

## Table 5-11. Sediment and Soil

*Section 5.5.3 has one required table, below, if data are available.*

**Table 5-11. Concentrations of [Substance x] in Soil and Sediment**

| | Percent detection and concentration (ng/g) | | | | | |
|---|---|---|---|---|---|---|
| Location | Substance x | Isomer1 | Isomer2 | Isomer3 | Isomer4 | Reference |
| Lansing, Michigan | | | | | | |
| Soil | | | | | | |
| Boring samples (n=50–108) | | | | | | 3M 2007b |
| Percent detected | 100% | — | 95% | 90% | 60% | |
| Maximum | 21,800 | — | 104,000 | 3,470 | 139 | |
| Alabama | | | | | | |
| Soil | | | | | | |
| Off-site soil (n=23) | | | | | | 3M 2008c |
| Percent detected | 100% | — | — | — | — | |
| Mean | 3.68–4.6 | | | | | |
| Range | 0.72–7.85 | — | — | — | — | |
| Off-site sediment (n=30) | <1–5 | | | | | |
| U.S. Highway 10 | | | | | | Xiao et al. 2015 |
| Percent detected | 100% | — | 100% | — | — | |
| Median | 8.0 | | 12.2 | | | |
| Range | 5.5–125.7 | | 0.2–28.2 | | | |

[a]Analyte was reported as total.

ND = not detected

## Table 5-12. Other Media

*Section 5.5.4, other media, there is one required table, if data are available, which reports food monitoring data. See example below.*

**Table 5-12. [Substance x] Detections in Food from the U.S. Food and Drug Administration (FDA) 1991–2003 Market Basket Survey**

| Food | Number of detections | Number of samples | Mean concentration (ppb) |
|---|---|---|---|
| Processed American cheese | 1 | 44 | 0.07 |
| Boiled beef/pork frankfurters | 4 | 44 | 0.39 |
| Beef/pork bolognas | 2 | 44 | 0.43 |
| Salami lunch meats | 1 | 44 | 0.09 |
| Raw/frozen strawberry samples | 1 | 43 | 0.07 |
| Regular carbonated colas | 4 | 44 | 0.43 |
| Fast food tacos with beef and cheese | 1 | 44 | 0.09 |
| Take out pizzas | 1 | 44 | 0.11 |
| Bottled cranberry juice cocktails | 1 | 4 | 1.75 |
| Orange juices | 1 | 4 | 0.75 |
| Prepared potato salads | 1 | 4 | 1.0 |
| Creamy peanut butter | 1 | 44 | 0.23 |
| Bottled apple juice | 1 | 44 | 0.75 |
| Fresh/frozen, boiled collards | 1 | 44 | 0.32 |
| Tomatoes | 1 | 44 | 0.25 |
| Green peppers | 1 | 44 | 0.32 |
| Fast food quarter-pound hamburgers on a bun | 1 | 44 | 0.84 |
| Creamy low calorie salad dressing | 1 | 4 | 2.5 |

Source: FDA 2006

## Tables 5-13 and 5-14. General Population Exposure

*When data are available, the required tables in Section 5.6, general population exposure, include NHANES monitoring data. See below.*

**Table 5-13. Geometric Mean and Selected Percentiles of Blood [Substance x] (in pg/mL) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) (CDC 2015)**

| | Survey years | Geometric mean (95% CI) | Selected percentiles (95% confidence interval) | | | | Sample size |
|---|---|---|---|---|---|---|---|
| | | | 50th | 75th | 90th | 95th | |
| Total | 2001–2002 | 2.21 (1.65–2.97) | 2.30 (1.56–3.21) | 4.63 (3.24–6.20) | 8.45 (5.86–12.0) | 12.0 (7.68–19.2) | 785 |
| | 2003–2004 | 1.50 (1.20–1.86) | 1.40 (1.10–1.90) | 3.40 (2.60–4.20) | 6.20 (5.30–7.00) | 9.50 (7.00–12.0) | 1,322 |
| | 2005–2006 | 1.41 (1.09–1.83) | 1.30 (0.880–1.80) | 3.00 (2.10–4.40) | 6.30 (4.30–9.70) | 10.0 (6.80–14.0) | 3,139 |
| Age group | | | | | | | |
| 12–19 years | 2005–2006 | 1.23 (0.954–1.58) | 1.00 (0.620–1.60) | 2.80 (1.70–4.10) | 5.50 (4.10–7.20) | 8.20 (6.20–12.0) | 932 |
| 20–59 years | 2001–2002 | 2.21 (1.65–2.97) | 2.30 (1.56–3.21) | 4.63 (3.24–6.20) | 8.45 (5.86–12.0) | 12.0 (7.68–19.2) | 785 |
| | 2003–2004 | 1.50 (1.20–1.86) | 1.40 (1.10–1.90) | 3.40 (2.60–4.20) | 6.20 (5.30–7.00) | 9.50 (7.00–12.0) | 1,322 |
| | 2005–2006 | 1.45 (1.11–1.89) | 1.30 (0.900–1.90) | 3.10 (2.10–4.60) | 6.40 (4.30–10.0) | 11.0 (6.90–14.0) | 1,537 |
| Sex | | | | | | | |
| Females | 2001–2002 | 2.24 (1.66–3.01) | 2.28 (1.49–3.24) | 4.63 (3.09–7.01) | 8.62 (5.26–12.9) | 11.1 (7.68–25.0) | 403 |
| | 2003–2004 | 1.51 (1.21–1.90) | 1.50 (1.10–1.90) | 3.30 (2.50–4.20) | 6.10 (4.69–7.30) | 7.80 (6.40–12.0) | 672 |
| | 2005–2006 | 1.44 (1.10–1.88) | 1.30 (0.900–1.90) | 3.10 (2.10–4.60) | 6.20 (4.20–9.40) | 9.40 (6.30–13.0) | 1,650 |
| Race/ethnicity | | | | | | | |
| Non-Hispanic blacks | 2001–2002 | 2.32 (1.82–2.94) | 2.50 (1.56–3.55) | 4.57 (3.60–5.56) | 8.69 (5.63–9.49) | 10.0 (5.89–13.5) | 130 |
| | 2003–2004 | 1.56 (1.15–2.13) | 1.70 (1.10–2.20) | 2.90 (2.15–3.80) | 5.10 (3.80–6.60) | 6.60 (4.90–13.0) | 290 |
| | 2005–2006 | 1.74 (1.27–2.37) | 1.70 (1.00–2.70) | 3.80 (2.70–4.80) | 6.40 (4.50–8.90) | 8.70 (6.60–11.0) | 817 |

**Table 5-14. Concentrations of [Substance x] and [Isomer1] in Human Serum Collected in the United States**

| | Detection and concentration (ng/mL [ppb])[a] | | |
|---|---|---|---|
| Location | Substance x | Isomer1 | Reference |
| U.S. residents—NHANES | | | |
| 2003–2004 (n=2,094) | | | Calafat et al. 2007b |
| Percent >LOD | 99.7% | 99.9% | |
| Geometric mean | 3.95 | 20.7 | |
| 95th percentile | 9.80 | 54.6 | |
| 2013–2014 (n=2,165) | | | CDC 2017 |
| Percent >LOD | NR | NR | |
| Geometric mean | 1.94 | 4.99 | |
| 95th percentile | 5.57 | 18.5 | |
| U.S. residents | | | Olsen et al. 2003c |
| (n=24) | | | |
| Percent >LLOQ | Not reported | 98% | |
| Geometric mean | 2.5 | 14.7 | |
| Minimum | <3.0 | <6.1 | |
| Maximum | 7.0 | 58.3 | |
| Midwestern United States | | | De Silva and Mabury 2006 |
| Atlanta, Georgia | | | Kuklenyik et al. 2004 |
| 2003 (n=20) | | | |
| Percent >LOD | 100% | 100% | |
| Mean | 4.9 | 55.8 | |
| Minimum | 0.2 | 3.6 | |
| Maximum | 10.4 | 164.0 | |
| Boston, Massachusetts; Charlotte, North Carolina; Hagerstown, Maryland; Los Angeles, California; Minneapolis-St. Paul, Minnesota; Portland, Oregon | | | Olsen et al. 2008 |
| 2006 (n=600) | 99% | 99% | |
| Percent >LLOQ | | | |
| Geometric mean | 3.4 | 14.5 | |
| 95th percentile CI geometric mean | 3.3–3.6 | 13.9–15.2 | |

[a]"Less than" values indicate that the concentration was reported as below the LOD or LLOQ. For cases where samples had concentrations below the limit of detection or lower limit of quantification, a value between zero and the LOD or LLOQ was assigned when calculating the mean concentration.
[b]Reported as bias-corrected estimates.
[c]One sample purchased separately with no origin information supplied.

CI = confidence interval; LLOQ = lower limit of quantification; LOD = limit of detection; NR = not reported; [Substance x] = ; [Isomer1] =

## Table 5-15 and Table 5-16. Populations with Potentially High Exposures

*In Section 5.7, the below tables are required when data are available.*

### Table 5-15. Concentrations of [Substance x], [Isomer1], and [Isomer2] in Human Serum for Occupationally Exposed Individuals

| | Concentration (µg/mL [ppm]) | | | |
|---|---|---|---|---|
| Location | Substance x | Isomer1 | Isomer2 | Reference |
| Decatur, Alabama | | | | |
| 2000 (n=263) | 1.78; 0.04–12.70 | 1.32; 0.06–10.06 | — | Olsen et al. 2003a |
| 1999–2004 (n=26)[a] | | | | Olsen et al. 2007a |
| Initial | 0.691 (0.072–5.1) | 0.799 (0.145–3.49) | 0.290 (0.016–1.30) | |
| Final | 0.262 (0.017–2.44) | 0.403 (0.037–1.74) | 1.85 (0.01–0.791) | |
| Cottage Grove, Minnesota | | | | |
| 1993 (n=111) | 0.00–80.00; 88% <8.92 | — | — | Olsen et al. 2000 |
| 2000 (n=122) | 4.63 (0.01–92.03) | 0.86 (0.03–4.79) | — | Olsen and Zobel 2007 |
| Washington Works, Little Hocking, Ohio | | | | |
| 2004–2005 | | | | Emmett et al. 2006a |
| No occupational exposure (n=312) | 0.423 (0.175–0.537)[b] | — | — | |
| Substantial occupational exposure (n=18) | 0.824 (0.422–0.999)[b] | — | — | |
| Washington Works | | | | |
| 2004 | | | | |
| Current occupational exposure (n=259) | 0.494 (0.0174–9.550) | — | — | Sakr et al. 2007b |
| Intermittent current occupational exposure (n=160) | 0.176 (0.0081–2.070) | — | — | Sakr et al. 2007b |
| Past occupational exposure (n=264) | 0.195 (0.0086–2.590) | — | — | Sakr et al. 2007b |

[a]Data include results from three retirees from the 3M plant in Cottage Grove, Minnesota.
[b]Reported as the interquartile range.
[c]Reported as the median value.

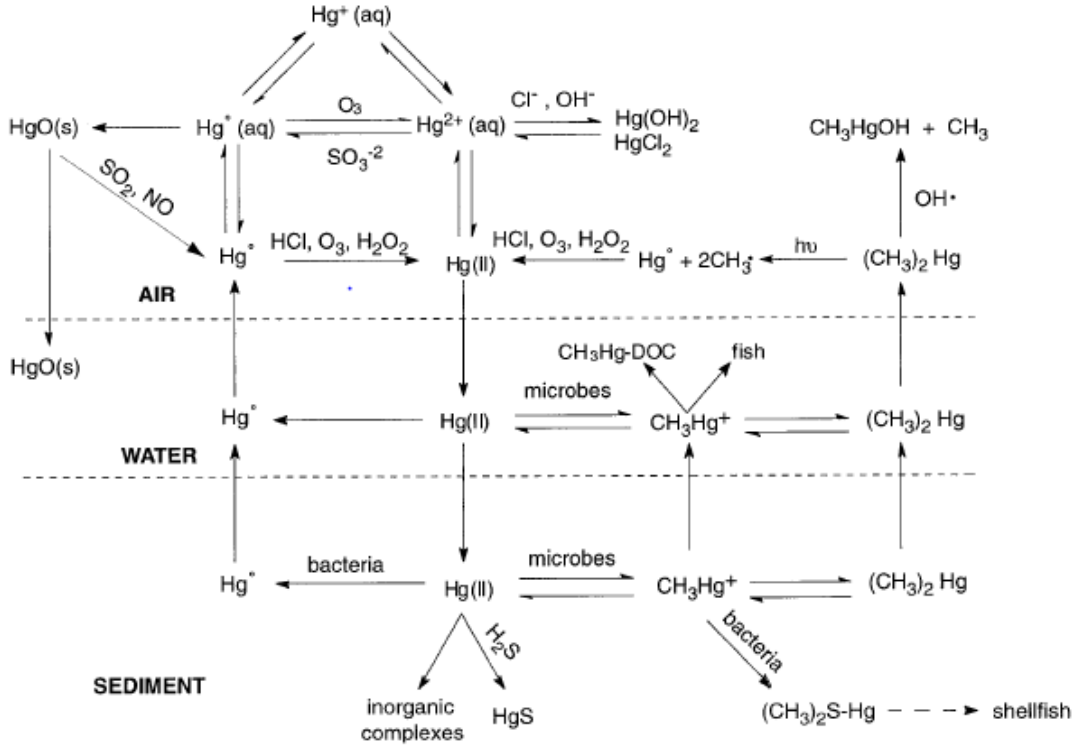
**Table 5-16. Blood Serum Levels for 69,030 Current and Former Residents of Six Water Districts in the Mid-Ohio Valley (2005–2006)**

| Age (years) | Number (percentage of total) | Median [Substance X] level (ng/mL) |
|---|---|---|
| 0–9 | 4,915 (7.1) | 32.8 |
| 10–19 | 9,658 (14.0) | 26.6 |
| 20–29 | 10,073 (14.6) | 21.0 |
| 30–39 | 10,547 (15.3) | 22.7 |
| 40–49 | 12,113 (17.6) | 28.0 |
| 50–59 | 10,515 (15.2) | 33.6 |
| 60–69 | 6,881 (10) | 42.9 |
| ≥70 | 4,328 (6.3) | 40.1 |

Source: Steenland et al. 2009a

**Figure 5-2. Transformation in Air, Water, and Sediment**

## Figure 5-2. Transformation of Mercury in Air, Water, and Sediment



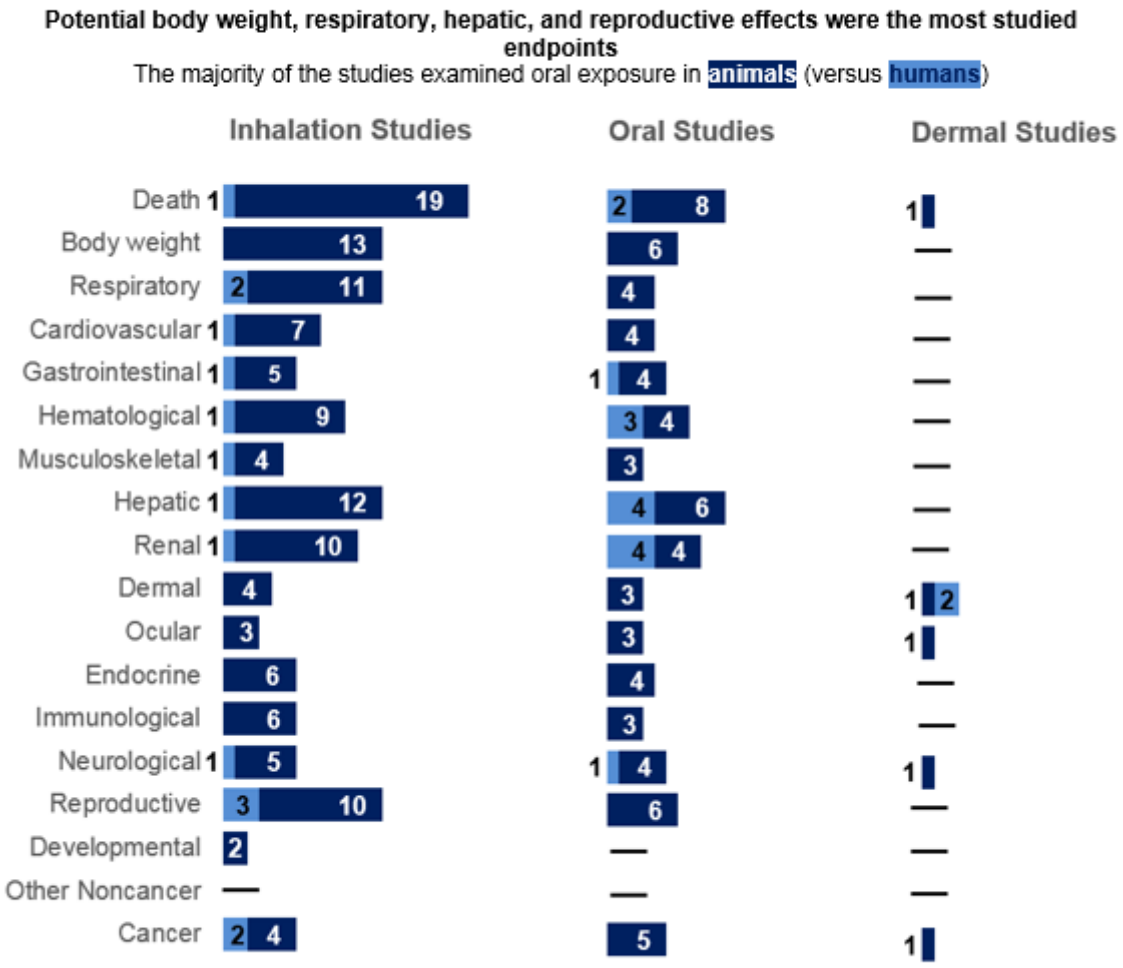

Dashed lines represent the boundary between environmental compartments.

aq = associated with aqueous; DOC = dissolved organic carbon; s = solid

Source: Stern et al. 1996

## EXHIBIT 14. CHAPTER 6 FIGURE (6-1)

**Figure 6-1. Summary of Existing Health Effects Studies on [Substance x] by Route and Endpoint***




*Includes studies discussed in Chapter 2; the number of studies include those finding no effect.

## EXHIBIT 15. CHAPTER 7 TABLE (7-1)

**Table 7-1. Regulations and Guidelines Applicable to [Substance x]**

| Agency | Description | Information | Reference |
|---|---|---|---|
| | **Air** | | |
| EPA | RfC | No data | IRIS 2002 |
| WHO | Air quality guidelines | No data | WHO 2011 |
| | **Water & Food** | | |
| EPA | Drinking water standards | | |
| | 1-day health advisory for a 10-kg child | 1 mg/L | |
| | 10-day health advisory for a 10-kg child | 0.6 mg/L | |
| | DWEL | 0.1 mg/L | |
| | MCL (total trihalomethanes) | 0.08 mg/L | EPA 2012 |
| | RfD | 0.02 mg/kg/day | IRIS 2002 |
| WHO | Disinfection by-products-drinking-water | 0.06 mg/L (60 µg/L)[b] | WHO 2011 |
| FDA | EAFUS | No data[c] | FDA 2013 |
| | **Cancer** | | |
| HHS | Carcinogenicity classification | Reasonably anticipated to be human carcinogen | NTP 2016 |
| EPA | Carcinogenicity classification | Group B2[a] | IRIS 2002 |
| IARC | Carcinogenicity classification | Group 2B[b] | IARC 2016 |
| | **Occupational** | | |
| OSHA | PEL (8-hour TWA) for general industry, shipyards and construction | No data | OSHA 2013<br>29 CFR 1910.1000, Table Z-1 |
| NIOSH | REL (up to 10-hour TWA) | No data | NIOSH 2016 |
| | **Emergency Criteria** | | |
| EPA | AEGLs-air | No data | AEGLs 2015 |
| AIHA | ERPGs | No data | AIHA 2015 |
| DOE | PAC-1[d] | 1.3 mg/m$^3$ | DOE 2016b |

[a]Classification B2: Probable human carcinogen.
[b]Group 2B: Possibly carcinogenic to humans.
[c]The EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS.
[d]Definitions of PAC terminology are available from U.S. Department of Energy (DOE 2016a).

AEGL = acute exposure guideline levels; AIHA = American Industrial Hygiene Association; CFR = Code of Federal Regulations; HHS = Department of Health and Human Services; DOE = Department of Energy; DWEL = drinking water equivalent level; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; ERPG = emergency response planning guidelines; FDA = Food and Drug Administration; GRAS = Generally Recognized As Safe; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; MCL = maximum contaminant level; MCLG = maximum contaminant level goal; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PAC = Protective Action Criteria; PEL = permissible exposure limit; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; TLV = threshold limit values; TWA = time-weighted average; WHO = World Health Organization

# APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

(Page 1 of 4)

*Appendix A consists of a two-page introductory statement and MRL worksheets describing the methodology used and all calculations involved in deriving the MRLs. Separate worksheets must be completed for each MRL that is derived; a worksheet is also completed when the data were considered inadequate for MRL derivation. When using GrabIt! Software for data collection, the worksheet must contain a table of the data used in analysis. When using benchmark dose (BMD) analysis to derive the BMDL (lowest point of departure), the worksheet must also contain the BMD output table and BMD graph of the final selected endpoint for each MRL. See the sample worksheet.*

*The appendix begins with the following boilerplate.*

**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 route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered. 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 NOAEL/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) 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 substance-induced endpoint 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 Human Health Sciences, 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 MRLs. For additional information regarding MRLs, please contact the Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-57, Atlanta, Georgia 30329-4027.**

*The MRL worksheets begin on the page after the boilerplate. See the next page for an example.*

APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

*Example when no MRL developed.*

***Chemical Name*:** [Substance x]
***CAS Numbers:*** [X]
***Date:*** [Month, (4 digit) Year]
[Month, (4 digit) Year]—Updated literature search *(only when a targeted profile and the MRL was not revised from the previous tox profile)*
***Profile Status:*** (Draft [#], Final)
***Route:*** (Inhalation, Oral)
***Duration:*** (Acute, Intermediate, Chronic)

***When necessary include the following note.***
Note: The (exposure duration, exposure route) MRL has been adopted for use as the (exposure duration, exposure route) MRL.

*Guidance: If there is no MRL for a specific exposure route or duration within the route, provide a concise and accurate justification for the lack of MRLs. The explanation must be as clear as possible; communication between the author, chemical manager, and MRL workgroup is essential. Line spacing is single.*

***MRL Summary:*** There are insufficient data for derivation of an acute-duration inhalation MRL due to several data gaps: lack of developmental toxicity studies, lack of examination of the respiratory tract, and lack of incidence data.

***Rationale for Not Deriving an MRL:*** There are limited data on the acute inhalation toxicity of [Substance x]. Torti et al. (2001) reported hepatic, renal, body weight, and ocular effects in two strains of mice exposed to X vapor 6 hours/day, 7 days/week for 1 week. The kidney was the most sensitive target, with tubular degeneration and nephrosis observed at ≥10 ppm; the NOAEL was 1 ppm. Hepatocellular centrilobular degeneration and decreases in body weight gain were observed at ≥30 ppm; increases in mortality were also observed at ≥30 ppm. The identification of the kidney as a sensitive target is supported by a 3-week study conducted by Torti et al. (2001) and by acute-duration oral studies.

*Guidance: When adopting another duration's MRL for a lesser duration, include a reasoning as to why it is considered protective.*

***Agency Contacts (Chemical Managers):*** [Chemical Manager Name]

# MINIMAL RISK LEVEL (MRL) WORKSHEET

*Example when MRL developed.*

| | |
|---|---|
| ***Chemical Name*:** | [Substance x] |
| ***CAS Numbers:*** | [X] |
| ***Date:*** | [Month, (4 digit) Year]<br>[Month, (4 digit) Year]—Updated literature search *(only when a targeted profile and the MRL was not revised from the previous tox profile)* |
| ***Profile Status:*** | ***(***Draft [#], Final) |
| ***Route:*** | (Inhalation, Oral) |
| ***Duration:*** | (Acute, Intermediate, Chronic) |
| ***MRL:*** | [##] units (e.g., ppm, mg/kg/day) |
| ***Critical Effect:*** | (effect) |
| ***Reference:*** | Author (4 digit) year |
| ***Point of Departure:*** | (NOAEL, LOAEL, BMDL) of [##] units |
| ***Uncertainty Factor:*** | (3, 10, 30, 100, 300, 1,000, 3,000) |
| ***LSE Graph Key:*** | [#, (##, ###; if necessary)] |
| ***Species:*** | (animal) |

*Guidance: Discuss the key study, effect level, and target organ of effect for the MRL and provide a brief description of studies that support the derivation or that provide evidence for the sensitivity of the endpoint selected. Line spacing is single.*

***MRL Summary:*** An acute-duration oral MRL of 0.1 mg/kg/day was derived for [Substance X] based on an increased incidence of full-litter resorptions in rats administered via gavage on GDs 6–15 (Narotsky et al. 1997). The MRL is based on a $BMCL_{05}$ of 10 mg/kg/day and a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

***Selection of the Critical Effect:*** A number of studies have evaluated the toxicity of [Substance X] following acute oral exposure; these studies examine a wide range of potential endpoints including liver and kidney effects (Condie et al. 1983; Keegan et al. 1998; Lilly et al. 1994, 1996; Munson et al. 1982; Ruddick et al. 1983; Thornton-Manning et al. 1994), immunotoxicity (French et al. 1999), reproductive toxicity (Bielmeier et al. 2001), and developmental toxicity (Bielmeier et al. 2001, 2004; Narotsky et al. 1997; Ruddick et al. 1983). The LOAELs for these studies range from 50 to 400 mg/kg/day; a summary of select LOAELs is presented in Table A-1 (studies identifying LOAELs for body weight effects were not included since this is not considered a primary effect of X).

The available data suggest that developmental toxicity, particularly full-litter resorption, is the most sensitive endpoint following acute-duration oral exposure. In multiple studies conducted by Bielmeier et al. (2001) and Narotsky et al. (1997), full-litter resorptions have been observed at 50 mg/kg/day (8–17% resorptions) and ≥75 mg/kg/day (17–100% resorptions). Similar LOAELs (≥74–75 mg/kg/day) were identified for liver and immunological effects. The liver effects consisted of centrilobular pallor, vacuolar degeneration and necrosis, and increases in liver enzymes (Condie et al. 1983; Keegan et al. 1998; Lilly et al. 1994, 1995; Munson et al. 1982; Thornton-Manning et al. 1994). Two studies demonstrated impaired immune responses in rats and mice administered ≥75 mg/kg/day (French et al. 1999; Munson et al. 1982). The kidney appears to be slightly less sensitive than other targets, with LOAEL values ranging from 148 to 400 mg/kg/day. The effects included tubular degeneration, hyperplasia, and necrosis, and increases in

BUN levels (Condie et al. 1983; Lilly et al. 1994, 1996; Munson et al. 1982; Thornton-Manning et al. 1994).

**Table A-1. Summary of Relevant NOAEL and LOAEL Values Considered for Derivation of an Acute Oral MRL for [Substance x]**

| Species | Duration/ route | NOAEL (mg/kg/day) | LOAEL (mg/kg/day) | Effect | Reference |
|---|---|---|---|---|---|
| Developmental effects | | | | | |
| F344 rat | GDs 6–15 (GW) | 25 | 50 | 17% full-litter resorption | Narotsky et al. 1997 |
| Kidney effects | | | | | |
| CD-1 mouse | 14 days (GO) | 74 | 148 | Intratubular mineralization, epithelial hyperplasia, and cytomegaly | Condie et al. 1983 |
| Liver effects | | | | | |
| Fischer 344 rat | Once (GW) | 200 | 400 | Centrilobular necrosis and vacuolar degeneration | Lilly et al. 1996 |
| Immunological effects | | | | | |
| F344 rat | 5 days (GW) | – | 75 | Impaired response to T-lymphocyte stimulants | French et al. 1999 |
| CD-1 mouse | 14 days (GW) | 125 | 250 | Altered response to sheep red blood cells | Munson et al. 1982 |

[a]Considered a serious LOAEL.

G = gavage; GD = gestation day; GO = gavage in oil vehicle; GW = gavage in water vehicle; LOAEL = lowest observed adverse effect level; NOAEL = no-observed-adverse-effect level

The lowest LOAEL for an acute-duration study was [….]

***Selection of the Principal Study:*** As summarized in Table A-1, Bielmeier et al. (2001) and Narotsky et al. (1997) conducted several studies evaluating full-litter resorptions in rats. Together, the studies demonstrate a dose-response relationship between [Substance X] exposure and full-litter resorption. The incidence of full-litter resorptions in selected studies conducted by these investigators are presented in Table A-2. Since the Narotsky et al. 1997 studies tested lower concentrations and identified a NOAEL, it was selected as the principal study for the MRL.

**Table A-2. Incidence of Full-Litter Resorptions in F344 Rats Administered [Substance x] via Gavage**

| | Dose (mg/kg/day) | | | | |
|---|---|---|---|---|---|
| | 0 | 25 | 50 | 75 | 100 |
| Narotsky et al. 1997 (GW) | 0/14 (0%) | 0/12 (0%) | 2/12 (17%) | 3/14 (21%) | |
| Narotsky et al. 1997 (GO) | 0/12 (0%) | 0/14 (0%) | 1/13 (8%) | 10/12 (83%) | |
| Bielmeier et al. 2001 (GDs 6–15) | 0% | | | 50% | |
| Bielmeier et al. 2001 (GDs 9) | 0% | | | 64% | 100% |

G = gavage; GD = gestation day; GO = gavage in oil vehicle; GW = gavage in water vehicle

***Summary of the Principal Study:***

Narotsky MG, Pegram RA, Kavlock RJ. 1997. Effect of dosing vehicle on the developmental toxicity of bromodichloromethane and carbon tetrachloride in rats. Fundam Appl Toxicol 40:30-36.

Groups of pregnant F344 rats (12–14/group) were administered 0, 25, 50, or 75 mg/kg/day [Substance X] by gavage in corn oil or an aqueous vehicle on GDs 6–15. Endpoints monitored included maternal weight and clinical signs. Pups were examined and weighed individually on PNDs 1 and 6. Dams were killed on PND 6, and the number of uterine implantations were recorded. The uteri of rats that did not deliver were stained to detect cases of full-litter resorptions.

Clinical signs seen only in the corn oil vehicle rats included hunched back (75 mg/kg/day) and chromodacryorrhea/lacrimation (≥50 mg/kg/day). Piloerection occurred at 75 mg/kg/day with both vehicles and at 50 mg/kg/day with the aqueous vehicle. Body weight gain on GDs 6–8 was reduced about 83% in rats dosed with 25 mg/kg/day in aqueous vehicle and about 61% with the oil vehicle (statistically significant only in aqueous vehicle group). Rats in the higher dose groups lost weight (both vehicles). Body weight gains were not reported at other time periods. Full-litter resorptions occurred in 50 and 75 mg/kg/day groups for both vehicles, but were not observed in controls or 25 mg/kg/day groups. The incidences of full-litter resorption are presented in Table A-2. In surviving litters, there was no significant effect on gestation length, postnatal viability, or pup weight on PND 1 or 6. In a toxicokinetic study also conducted, [Substance X] levels in the blood declined faster in aqueous vehicle groups than in corn oil vehicle groups; the blood half-times were 2.7 and 3.6 hours, respectively.

***Selection of the Point of Departure for the MRL:*** The $BMCL_{05}$ of 10 mg/kg/day for full-litter resorption was selected as the basis of the MRL.

Benchmark dose (BMD) modeling was conducted to identify a point of departure using the incidence data for full-litter resorptions in rats administered [Substance X] in a corn oil vehicle and in an aqueous

vehicle. The data were fit to all available dichotomous models in EPA's Benchmark Dose Software (BMDS, version 2.6.0) using the extra risk option. Adequate model fit was judged by three criteria: goodness-of-fit statistics (p-value >0.1), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined benchmark response (BMR). Among all of the models providing adequate fit to the data, the lowest BMCL (95% lower confidence limit on the benchmark concentration) was selected as the point of departure when the difference between the BMCLs estimated from these models was >3-fold; otherwise, the BMCL from the model with the lowest Akaike's Information Criterion (AIC) was chosen. Since the endpoint was developmental toxicity, a BMRs of 5% was used. The model predictions for the gavage in oil and gavage in aqueous solution are presented in Table A-3 and the fit of the selected models are presented in Figures A-1 and A-2.

$BMDL_{05}$ values of 10.43 and 33.49 mg/kg/day were calculated using the incidence data for rats administered [Substance X] via gavage in aqueous solution and gavage in oil vehicles, respectively. The $BMDL_{05}$ for the aqueous vehicle data set was selected as the point of departure for the MRL; the aqueous vehicle data were selected over the corn oil vehicle, but it is a more conservative value and it is most likely to mimic human exposure to [Substance X] in water. Although the $BMCL_{05}$ of 10 mg/kg/day was lower than the empirical NOAEL of 25 mg/kg/day identified in the study, it was selected as the point of departure because it provides a better indicator of the dose-response relationship than the NOAEL, which is a single data point.

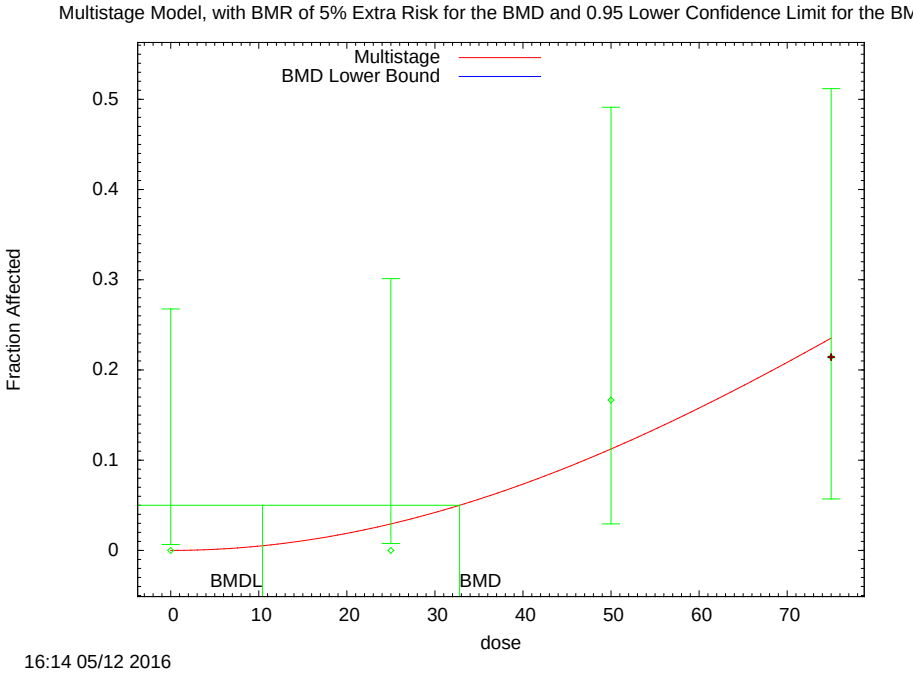
## Table A-3. Model Predictions for Full-Litter Resorptions in Rats Orally Administered [Substance x] in Aqueous or Oil Vehicles (Narotsky et al. 1997)

| Model | DF | $\chi^2$ | $\chi^2$ Goodness-of-fit p-value[a] | Scaled residuals[b] Dose below BMD | Scaled residuals[b] Dose above BMD | Scaled residuals[b] Overall largest | AIC | $BMD_{05}$ (mg/kg/day) | $BMDL_{05}$ (mg/kg/day) |
|---|---|---|---|---|---|---|---|---|---|
| **Aqueous Vehicle** | | | | | | | | | |
| Gamma[c] | 2 | 0.77 | 0.68 | -0.48 | 0.67 | 0.67 | 30.30 | 36.34 | 10.61 |
| Logistic | 2 | 1.49 | 0.47 | -0.57 | 0.98 | 0.98 | 31.10 | 41.00 | 25.03 |
| LogLogistic[d] | 2 | 0.77 | 0.68 | -0.52 | 0.66 | 0.66 | 30.34 | 35.59 | 9.60 |
| LogProbit[d] | 2 | 0.66 | 0.72 | -0.44 | 0.62 | 0.62 | 30.17 | 36.54 | 20.60 |
| Multistage (1-degree)[e] | 3 | 1.06 | 0.79 | 0.00 | -0.93 | -0.93 | 29.22 | 18.28 | 9.48 |
| ***Multistage (2-degree)[e,f]*** | ***3*** | ***0.75*** | ***0.86*** | ***-0.60*** | ***0.60*** | ***0.60*** | ***28.43*** | ***32.80*** | ***10.43*** |
| Multistage (3-degree)[e] | 2 | 0.77 | 0.68 | -0.59 | 0.61 | 0.61 | 30.43 | 33.22 | 10.43 |
| Probit | 2 | 1.27 | 0.53 | -0.53 | 0.90 | 0.90 | 30.81 | 39.58 | 23.38 |
| Weibull[c] | 2 | 0.82 | 0.67 | -0.54 | 0.68 | 0.68 | 30.40 | 35.31 | 10.47 |
| **Oil Vehicle** | | | | | | | | | |
| ***Gamma[c,d]*** | ***3*** | ***0.92*** | ***0.82*** | ***-0.07*** | ***-0.75*** | ***-0.75*** | ***20.75*** | ***41.98*** | ***33.49*** |
| Logistic | 2 | 0.02 | 0.99 | -0.14 | 0.05 | -0.14 | 21.74 | 46.93 | 32.92 |
| LogLogistic[e] | 2 | 0 | 1.00 | -0.04 | 0.00 | -0.04 | 21.70 | 47.33 | 36.09 |
| LogProbit[e] | 2 | 0 | 1.00 | 0.00 | 0.00 | 0.00 | 21.70 | 47.79 | 37.41 |
| Multistage (1-degree)[f] | 3 | 13.59 | 0.00 | 0.00 | -1.74 | 2.73 | 36.93 | ND | ND |
| Multistage (2-degree)[f] | 3 | 7.7 | 0.05 | 0.00 | -1.18 | 1.83 | 29.82 | ND | ND |
| Multistage (3-degree)[f] | 3 | 4.34 | 0.23 | -0.76 | -1.50 | -1.50 | 25.37 | 27.01 | 16.74 |
| Probit | 2 | 0 | 1.00 | 0.01 | 0.00 | -0.04 | 21.70 | 47.25 | 33.90 |
| Weibull[c] | 2 | 0.01 | 1.00 | 0.02 | 0.00 | -0.08 | 21.71 | 46.71 | 33.32 |

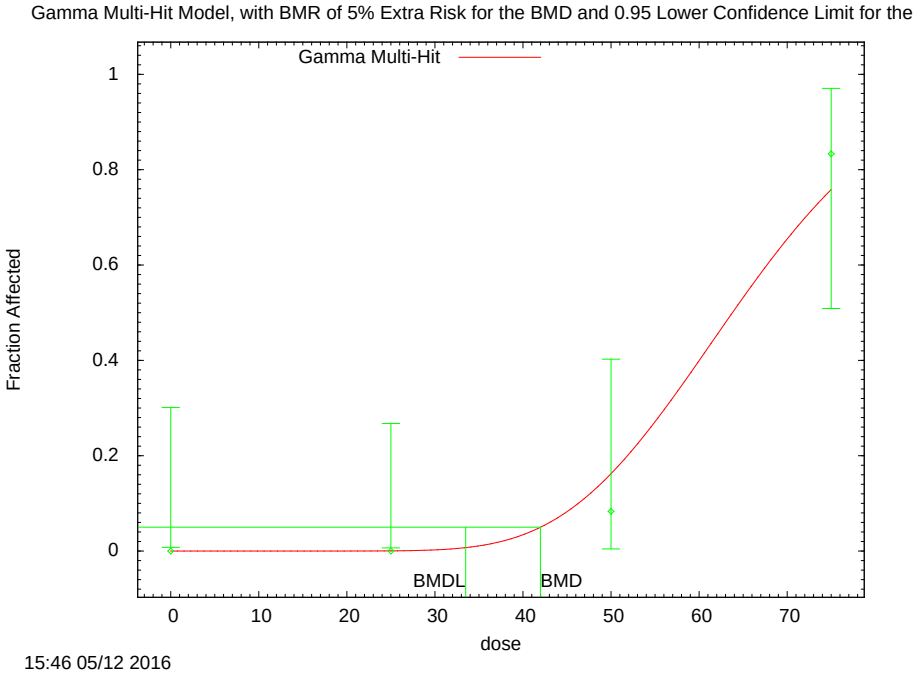
[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Betas restricted to ≥0.
[f]Selected model. All models provided adequate fit to the data. BMDLs for models providing adequate fit were sufficiently close (differed by <3 fold), so the model with the lowest AIC was selected (Multistage (2-degree).

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{050}$ = exposure concentration associated with 5% extra risk); DF = degrees of freedom

## Figure A-1. Fit of 2-Degree Multistage Model to Data on Incidence of Full-Litter Resorption in Rats Administered [Substance x] in Aqueous Vehicle (mg/kg/day)



## Figure A-2. Fit of Gamma Model to Data on Incidence of Full-Litter Resorption in Rats Administered [Substance x] in Oil Vehicle (mg/kg/day)




***Adjustment for Intermittent Exposure:*** Not applicable.

***Uncertainty Factor:*** The $BMDL_{05}$ is divided by a total uncertainty factor of 100

- 10 for extrapolation from animals to humans
- 10 for human variability

MRL = $BMDL_{05}$ ÷ UFs

10 mg/kg/day ÷ (10 x 10) = 0.1 mg/kg/day

***Other Additional Studies or Pertinent Information that Lend Support to this MRL:*** EPA (2005b) estimated that the average exposure of the general population to bromodichloromethane is 20 µg/person/day (0.0003 mg/kg/day assuming a reference body weight of 70 kg) from surface water systems and 8.1 µg/person/day (0.0001 mg/kg/day) from groundwater systems. These average intakes are approximately 1000-fold lower than the MRL.

***Agency Contacts (Chemical Managers):*** [Chemical Manager Name]

***MRL Summary Guidance:*** Provide a stand-alone summary of approximately four sentences suitable for copying and pasting into public health documents. It should include:

- Route, duration, MRL value, substance, critical effect, species, and short study reference(s).
- Values and types for point(s) of departure and uncertainty and modifying factors (if one is a modifying factor, say so; don't merely say uncertainty Factors).
- For BMDs, do not discuss selection criteria or other extended details. Just say what BMDL health effect endpoint(s) was used, and the type of numeric value derived (e.g., "$BMDL_{1SD}$ of 10 mg/kg/day for decreased body weight gain).
- The types of any additional numeric transformations should be stated briefly, without values. (HEC, PBPK, intermittent exposure, etc.)
- Avoid long decimal strings by changing units when possible (e.g., 0.8 ppb, not 0.0008 ppm), unless it overly complicates the Summary's text to have units changing.
- Include unusual but highly relevant information, such as the soluble particulate criterion for certain chromium MRLs, identity details when needed (PCB or PBB variants), or nature of radiation MRLs. This should only occur very rarely.
- The Summary should be a short, crafted statement that can be copied out and stand alone (thus the need for the substance name). The goal is three to four sentences, but can be exceeded for complex MRLs. Just provide highlights. Readers can always consult the Worksheet for details.

Mock Example:

A chronic inhalation MRL of $3x10^{-6}$ ppm for toluene diisocyanate was derived for decreased lung function in rats as seen in the Clark et al. (1998) study. It is based on a LOAEL of 1.2 ppb that was adjusted for intermittent exposure and converted to a HEC. A total uncertainty factor of 30 was applied (3 for use a minimal LOAEL and 10 for human variability).

# APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR [SUBSTANCE X]

***This appendix is required in every toxicological profile****. The pages that follow demonstrate much of the content for this appendix.*

The objective of the toxicological profile is to evaluate the potential for human exposure and the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to [Substance x].

## B.1 LITERATURE SEARCH AND SCREEN

A literature search and screen was conducted to identify studies examining health effects, toxicokinetics, mechanisms of action, susceptible populations, biomarkers, chemical interactions, physical and chemical properties, production, use, environmental fate, environmental releases, and environmental and biological monitoring data for [Substance x]. ATSDR primarily focused on peer-reviewed articles without publication date or language restrictions. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of [Substance x] have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest. The inclusion criteria used to identify relevant studies examining the health effects of [Substance x] are presented in Table B-1.

**Table B-1. Inclusion Criteria for the Literature Search and Screen**

| |
|---|
| Health Effects |
| Species |
| Human |
| Laboratory mammals |
| Route of exposure |
| Inhalation |
| Oral |
| Dermal (or ocular) |
| Parenteral (these studies will be considered supporting data) |
| Health outcome |
| Death |
| Systemic effects |
| Body weight effects |
| Respiratory effects |
| Cardiovascular effects |
| Gastrointestinal effects |
| Hematological effects |
| Musculoskeletal effects |
| Hepatic effects |
| Renal effects |
| Dermal effects |
| Ocular effects |
| Endocrine effects |
| Immunological effects |

**Table B-1. Inclusion Criteria for the Literature Search and Screen**

| |
|---|
| Neurological effects |
| Reproductive effects |
| Developmental effects |
| Other noncancer effects |
| Cancer |
| Toxicokinetics |
| Absorption |
| Distribution |
| Metabolism |
| Excretion |
| PBPK models |
| Biomarkers |
| Biomarkers of exposure |
| Biomarkers of effect |
| Interactions with other chemicals |
| Potential for human exposure |
| Releases to the environment |
| Air |
| Water |
| Soil |
| Environmental fate |
| Transport and partitioning |
| Transformation and degradation |
| Environmental monitoring |
| Air |
| Water |
| Sediment and soil |
| Other media |
| Biomonitoring |
| General populations |
| Occupation populations |

### B.1.1 Literature Search

*For new profiles:* The following main databases were searched in [Month YEAR]:

**OR**

*For update profiles:* The current literature search was intended to update the draft toxicological profile for [Substance x] released for public comment in [YEAR]. The following main databases were searched in [Month YEAR]:

The current literature search was intended to update the existing toxicological profile for substance x (ATSDR [####]), thus, the literature search was restricted to studies published between [Month YEAR] to [Month YEAR]. The following main databases were searched in [Month YEAR]:

The following main databases were searched in Month YEAR:

- PubMed
- National Library of Medicine's TOXLINE
- Scientific and Technical Information Network's TOXCENTER

The search strategy used the chemical names, Chemical Abstracts Service (CAS) numbers, synonyms, Medical Subject Headings (MeSH) headings, and keywords for [Substance x]. The query strings used for the literature search are presented in Table B-2.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), NTP website, and National Institute of Health Research Portfolio Online Reporting Tools Expenditures and Results (NIH RePORTER) databases using the queries presented in Table B-3. Additional databases were searched in the creation of various tables and figures, such as the TRI Explorer, the Substance Priority List (SPL) resource page, and other items as needed. Regulations applicable to [Substance x] were identified by searching international and U.S. agency websites and documents.

Review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature, which included unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

**Table B-2. Database Query Strings**

| Database search date | Query string |
|---|---|
| **PubMed** | |
| [mo/year] | |
| **Toxline** | |
| [mo/year] | |
| **Toxcenter** | |
| [mo/year] | |

**Table B-3. Strategies to Augment the Literature Search**

| Source | Query and number screened when available |
|---|---|
| **TSCATS[a]** | |
| [mo/year] | Compounds searched: [xx-xx-x; xx-xx-x; xx-xx-x] |
| **NTP** | |
| [mo/year] | xxx" OR "xxx" |
| **NPIRS** | |

**Table B-3. Strategies to Augment the Literature Search**

| Source | Query and number screened when available |
|---|---|
| [mo/year] | PC Codes searched: [xxxxxx; xxxxxx] |
| **NIH RePORTER** | |
| | Active projects,<br>"xxx" OR "xxx" OR [xx-xx-x] |
| **Other** | Identified throughout the assessment process |

[a]Several versions of the TSCATS database were searched, as needed, by CASRN including TSCATS1 via Toxline (no date limit), TSCATS2 via https://yosemite.epa.gov/oppts/epatscat8.nsf/ReportSearch?OpenForm (date restricted by EPA receipt date), and TSCATS via CDAT (date restricted by 'Mail Received Date Range'), as well as google for recent TSCA submissions.

The [Year ####] results were:

- Number of records identified from PubMed, TOXLINE, and TOXCENTER (after duplicate removal): [####]
- Number of records identified from other strategies: [####]
- Total number of records to undergo literature screening: [####]

### B.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on [Substance x]:

- Title and abstract screen
- Full text screen

***Title and Abstract Screen.*** Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

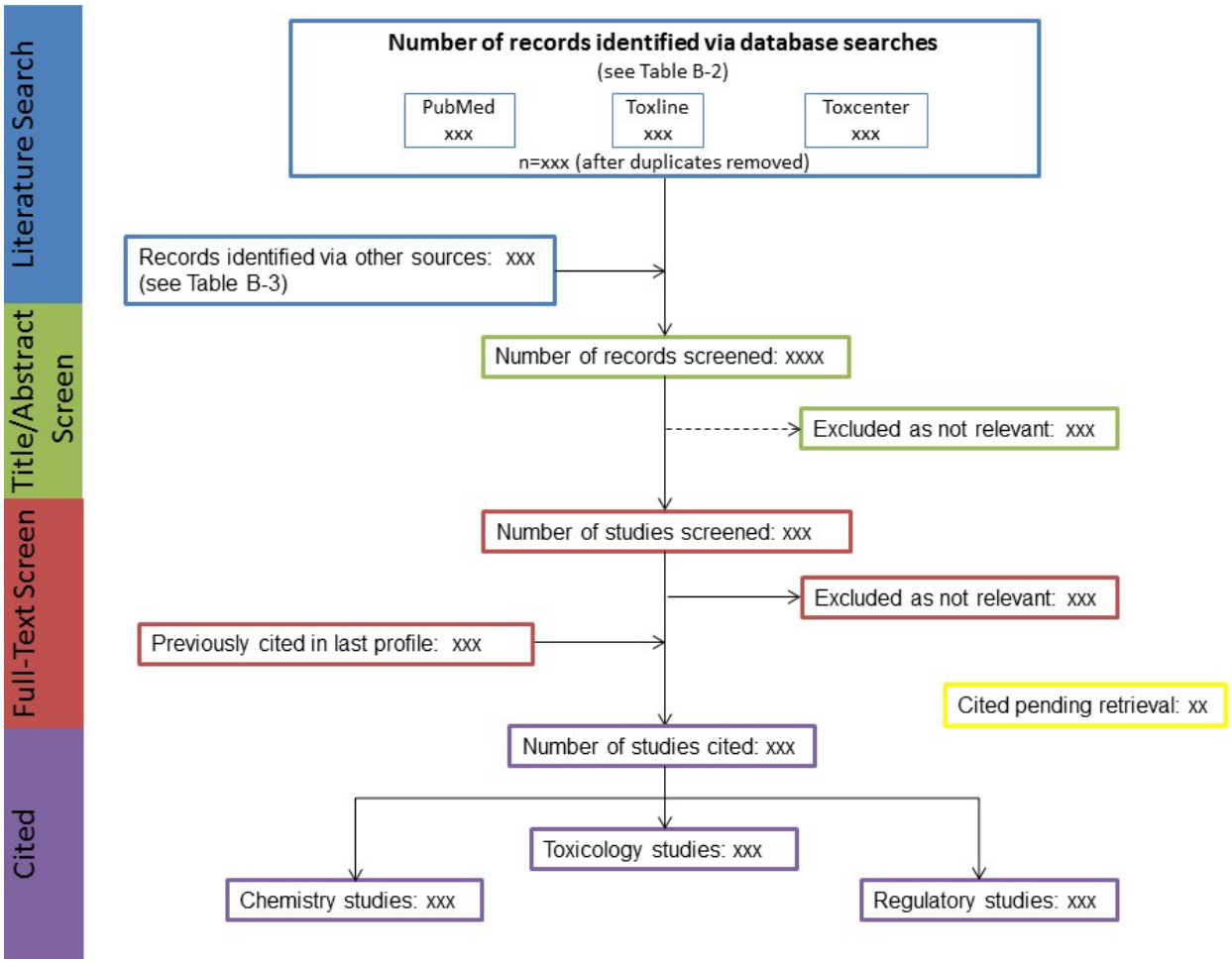
- Number of titles and abstracts screened: [#####]
- Number of studies considered relevant and moved to the next step: [####]

***Full Text Screen.*** The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: [####]
- Number of studies cited in the pre-public draft of the toxicological profile: [####]
- Total number of studies cited in the profile: [####]

Present a summary of the results of the literature search and screening in Figure B-1.

## Figure B-1. [Month YEAR] Literature Search Results and Screen for [Substance x]



# APPENDIX C. FRAMEWORK FOR ATSDR'S SYSTEMATIC REVIEW OF HEALTH EFFECTS DATA FOR [SUBSTANCE X]

*This appendix is to be included in a toxicological profile only when a systematic review has been completed for a chemical. The pages that follow demonstrate much of the content for this appendix.*

To increase the transparency of ATSDR's process of identifying, evaluating, synthesizing, and interpreting the scientific evidence on the health effects associated with exposure to [Substance x], ATSDR utilized a slight modification of NTP's Office of Health Assessment and Translation (OHAT) systematic review methodology (NTP 2013, 2015; Rooney et al. 2014). ATSDR's framework is an eight-step process for systematic review with the goal of identifying the potential health hazards of exposure to [Substance x]:

- Step 1. Problem Formulation
- Step 2. Literature Search and Screen for Health Effects Studies
- Step 3. Extract Data from Health Effects Studies
- Step 4. Identify Potential Health Effect Outcomes of Concern
- Step 5. Assess the Risk of Bias for Individual Studies
- Step 6. Rate the Confidence in the Body of Evidence for Each Relevant Outcome
- Step 7. Translate Confidence Rating into Level of Evidence of Health Effects
- Step 8. Integrate Evidence to Develop Hazard Identification Conclusions

## C.1 PROBLEM FORMULATION

The objective of the toxicological profile and this systematic review was to identify the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to [Substance x]. The inclusion criteria used to identify relevant studies examining the health effects of [Substance x] are presented in Table C-1.

Data from human and laboratory animal studies were considered relevant for addressing this objective. Human studies were divided into two broad categories: observational epidemiology studies and controlled exposure studies. The observational epidemiology studies were further divided: cohort studies (retrospective and prospective studies), population studies (with individual data or aggregate data), and case-control studies.

**Table C-1. Inclusion Criteria for Identifying Health Effects Studies**

| |
|---|
| Species |
| Human |
| Laboratory mammals |
| Route of exposure |
| Inhalation |
| Oral |
| Dermal (or ocular) |
| Parenteral (these studies will be considered supporting data) |
| Health outcome |
| Death |
| Systemic effects |

**Table C-1. Inclusion Criteria for Identifying Health Effects Studies**

| |
|---|
| Body weight effects |
| Respiratory effects |
| Cardiovascular effects |
| Gastrointestinal effects |
| Hematological effects |
| Musculoskeletal effects |
| Hepatic effects |
| Renal effects |
| Dermal effects |
| Ocular effects |
| Endocrine effects |
| Immunological effects |
| Neurological effects |
| Reproductive effects |
| Developmental effects |
| Other noncancer effects |
| Cancer |

## C.2 LITERATURE SEARCH AND SCREEN FOR HEALTH EFFECTS STUDIES

A literature search and screen was conducted to identify studies examining the health effects of [Substance x]. The literature search framework for the toxicological profile is discussed in detail in Appendix B.

### C.2.1 Literature Search

*If new profile:* As noted in Appendix B, the literature search for the toxicological profile for [Substance x] was conducted without date restriction. See Appendix B for the databases searched and the search strategy.

**OR**

*If update profile:* As noted in Appendix B, the literature search to update the existing toxicological profile for [Substance x] (ATSDR ####) was restricted to studies published between [year] to [year]. See Appendix B for the databases searched and the search strategy.]

A total of [####] records relevant to all sections of the toxicological profile were identified (after duplicate removal).

### C.2.2 Literature Screening

As described in Appendix B, a two-step process was used to screen the literature search to identify relevant studies examining the health effects of [Substance x].

***Title and Abstract Screen.*** In the Title and Abstract Screen step, [XXX] records were reviewed; [XX] studies were considered to meet the health effects inclusion criteria in Table C-1 and were moved to the next step in the process.

***Full Text Screen.*** In the second step in the literature screening process for the systematic review, a full text review of the [XX] health effects studies identified in the update literature was performed. Additionally, 14 studies cited in the LSE tables for the existing profile were included in the full study screen bringing the total number of studies for the qualitative review to [XX]. Of the [XX] studies undergoing Full Text Screen, [XX] studies did not meet the inclusion criteria; some of the excluded studies were used as background information on toxicokinetics or mechanisms of action or were relevant to other sections of the toxicological profile.

## C.3 EXTRACT DATA FROM HEALTH EFFECTS STUDIES

Relevant data extracted from the individual studies selected for inclusion in the systematic review were collected in customized data forms. A summary of the type of data extracted from each study is presented in Table C-2. For references that included more than one experiment or species, data extraction records were created for each experiment or species.

A summary of the extracted data for each study is presented in the Supplemental Document for [Substance x] and overviews of the [results of the inhalation and oral exposure studies (no dermal exposure studies were identified)] are presented in Sections 2.2–2.18 of the profile and in the Levels Significant Exposures tables in Section 2.1 of the profile ([Tables 2-2 and 2-3], respectively).

**Table C-2. Data Extracted From Individual Studies**

| |
|---|
| Citation |
| Chemical form |
| Route of exposure (e.g., inhalation, oral, dermal) |
| Specific route (e.g., gavage in oil, drinking water) |
| Species |
| Strain |
| Exposure duration category (e.g., acute, intermediate, chronic) |
| Exposure duration |
| Frequency of exposure (e.g., 6 hours/day, 5 days/week) |
| Exposure length |
| Number of animals or subjects per sex per group |
| Dose/exposure levels |
| Parameters monitored |
| Description of the study design and method |
| Summary of calculations used to estimate doses (if applicable) |
| Summary of the study results |
| Reviewer's comments on the study |
| Outcome summary (one entry for each examined outcome) |
| No-observed-adverse-effect level (NOAEL) value |
| Lowest-observed-adverse-effect level (LOAEL) value |
| Effect observed at the LOAEL value |

## C.4 IDENTIFY POTENTIAL HEALTH EFFECT OUTCOMES OF CONCERN

Overviews of the potential health effect outcomes for [Substance x] identified in human and animal studies are presented in Tables C-3 and C-4, respectively. Studies examining these potential outcomes were carried through to Steps 4–8 of the systematic review.

*Provide a brief overview of the studies identified and identify the specific health effect endpoint categories that will undergo the full systematic review. Work with the chemical manager to identify which endpoints will undergo the full systematic review and how to briefly summarize. Two examples follow.*

**Example 1.** The only available human studies evaluating noncancer effects are limited to case reports of accidental or intentional exposure. However, when evaluated together, these studies indicate that hematological, hepatic, renal, and neurological systems are susceptible to 1,2-dichloropropane toxicity. Animal studies examined a comprehensive set of endpoints following inhalation or oral exposure, but dermal studies were limited to acute lethality, skin irritation, and skin sensitization. Respiratory, hematological, hepatic, renal, neurological, and developmental effects were considered sensitive outcomes, i.e., effects were observed at low concentrations or doses. Studies examining these potential outcomes were carried through to Steps 4–8 of the systematic review.

**OR**

**Example 2.** The available human studies examined a limited number of endpoints and reported respiratory, cardiovascular, gastrointestinal, musculoskeletal, immunological, reproductive, and developmental effects. Animal studies examined a number of endpoints following inhalation, oral, or dermal exposure. These studies examined most systemic endpoints and reported respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, body weight, and metabolic effects. Additionally, animal studies have reported immunological, reproductive, and developmental effects.

Respiratory, cardiovascular (damage to the myocardium and/or EKG alterations), gastrointestinal, metabolic (alterations in blood glucose levels), and developmental effects were considered sensitive outcomes, i.e., effects were observed at low concentrations or doses. Studies examining these potential outcomes were carried through to Step 4 of the systematic review.

APPENDIX C

## Table C-3. Overview of the Health Outcomes for [Substance X] Evaluated in Human Studies

| | Body weight | Respiratory | Cardiovascular | Gastrointestinal | Hematological | Musculoskeletal | Hepatic | Renal | Dermal | Ocular | Endocrine | Immunological | Neurological | Reproductive | Developmental | Other Noncancer | Caner |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Inhalation studies | | | | | | | | | | | | | | | | | |
| Cohort | | | | | | | | | | | | | | | | | |
| Case control | | | | | | | | | | | | | | | | | |
| Population | | | | | | | | | | | | | | | | | |
| Case series | | | | | | | | | | | | | | | | | |
| Oral studies | | | | | | | | | | | | | | | | | |
| Cohort | | | | | | | | | | | | | | 2<br>2 | 8<br>7 | | |
| Case control | | | | | | | | | | | | | | | 2<br>1 | | 1<br>1 |
| Population | | | | | | | 1<br>0 | | | | | | | 1<br>0 | | | |
| Case series | | | | | | | | | | | | | | | | | |
| Dermal studies | | | | | | | | | | | | | | | | | |
| Cohort | | | | | | | | | | | | | | | | | |
| Case control | | | | | | | | | | | | | | | | | |
| Population | | | | | | | | | | | | | | | | | |
| Case series | | | | | | | | | | | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Number of studies examining endpoint | 0 | 1 | 2 | 3 | 4 | 5-9 | ≥10 |
| Number of studies reporting outcome | 0 | 1 | 2 | 3 | 4 | 5-9 | ≥10 |

## Table C-4. Overview of the Health Outcomes for [Substance X] Evaluated in Experimental Animal Studies

| | Body weight | Respiratory | Cardiovascular | Gastrointestinal | Hematological | Musculoskeletal | Hepatic | Renal | Dermal | Ocular | Endocrine | Immunological[a] | Neurological[a] | Reproductive[a] | Developmental | Other Noncancer | Caner |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Inhalation studies | | | | | | | | | | | | | | | | | |
| Acute-duration | 2 | | | | | | 2 | 2 | | 2 | | | | | | 2 | |
| | 2 | | | | | | 2 | 2 | | 2 | | | | | | 0 | |
| Intermediate-duration | 3 | | | | | | 2 | 2 | | | | | | | | 3 | |
| | 0 | | | | | | 0 | 2 | | | | | | | | 0 | |
| Chronic-duration | | | | | | | | | | | | | | | | | |
| Oral studies | | | | | | | | | | | | | | | | | |
| Acute-duration | 16 | 1 | 1 | 1 | 3 | 1 | 9 | 8 | | | 1 | 3 | 3 | 4 | 7 | 5 | |
| | 12 | 0 | 0 | 0 | 2 | 0 | 7 | 6 | | | 0 | 2 | 2 | 3 | 7 | 1 | |
| Intermediate-duration | 11 | 7 | 7 | 9 | 6 | | 9 | 11 | | | 7 | 2 | 8 | 6 | 3 | 2 | |
| | 5 | 0 | 0 | 1 | 0 | | 6 | 2 | | | 0 | 1 | 1 | 2 | 1 | 1 | |
| Chronic-duration | 8 | 7 | 7 | 7 | 1 | | 8 | 7 | | | 7 | | 1 | 6 | | 1 | 8 |
| | 4 | 0 | 0 | 0 | 0 | | 4 | 3 | | | 1 | | 0 | 1 | | 1 | 3 |
| Dermal studies | | | | | | | | | | | | | | | | | |
| Acute-duration | | | | | | | | | | | | | | | | | |
| Intermediate-duration | | | | | | | | | | | | | | | | | |
| Chronic-duration | | | | | | | | | | | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Number of studies examining endpoint | 0 | 1 | 2 | 3 | 4 | 5-9 | ≥10 |
| Number of studies reporting outcome | 0 | 1 | 2 | 3 | 4 | 5-9 | ≥10 |

[a]Number of studies examining endpoint includes study evaluating histopathology, but not evaluating function.

## C.5 ASSESS THE RISK OF BIAS FOR INDIVIDUAL STUDIES

### C.5.1 Risk of Bias Assessment

The risk of bias of individual studies was assessed using OHAT's Risk of Bias Tool (NTP 2015). The risk of bias questions for observational epidemiology studies, human-controlled exposure studies, and animal experimental studies are presented in Tables C-5, C-6, and C-7, respectively. Each risk of bias question was answered on a four-point scale:

- **Definitely low risk of bias (++)**
- **Probably low risk of bias (+)**
- **Probably high risk of bias (-)**
- **Definitely high risk of bias (– –)**

In general, "definitely low risk of bias" or "definitely high risk of bias" were used if the question could be answered with information explicitly stated in the study report. If the response to the question could be inferred, then "probably low risk of bias" or "probably high risk of bias" responses were typically used.

**Table C-5. Risk of Bias Questionnaire for Observational Epidemiology Studies**

| |
|---|
| **Selection bias** |
| Were the comparison groups appropriate? |
| **Confounding bias** |
| Did the study design or analysis account for important confounding and modifying variables? |
| **Attrition/exclusion bias** |
| Were outcome data complete without attrition or exclusion from analysis? |
| **Detection bias** |
| Is there confidence in the exposure characterization? |
| Is there confidence in outcome assessment? |
| **Selective reporting bias** |
| Were all measured outcomes reported? |

**Table C-6. Risk of Bias Questionnaire for Human-Controlled Exposure Studies**

| |
|---|
| **Selection bias** |
| Was administered dose or exposure level adequately randomized? |
| Was the allocation to study groups adequately concealed? |
| **Performance bias** |
| Were the research personnel and human subjects blinded to the study group during the study? |
| **Attrition/exclusion bias** |
| Were outcome data complete without attrition or exclusion from analysis? |
| **Detection bias** |
| Is there confidence in the exposure characterization? |
| Is there confidence in outcome assessment? |
| **Selective reporting bias** |
| Were all measured outcomes reported? |

## Table C-7. Risk of Bias Questionnaire for Experimental Animal Studies

| |
|---|
| **Selection bias** |
| Was administered dose or exposure level adequately randomized? |
| Was the allocation to study groups adequately concealed? |
| **Performance bias** |
| Were experimental conditions identical across study groups? |
| Were the research personnel blinded to the study group during the study? |
| **Attrition/exclusion bias** |
| Were outcome data complete without attrition or exclusion from analysis? |
| **Detection bias** |
| Is there confidence in the exposure characterization? |
| Is there confidence in outcome assessment? |
| **Selective reporting bias** |
| Were all measured outcomes reported? |

After the risk of bias questionnaires were completed for the health effects studies, the studies were assigned to one of three risk of bias tiers based on the responses to the key questions listed below and the responses to the remaining questions.

- Is there confidence in the exposure characterization? (only relevant for observational studies)
- Is there confidence in the outcome assessment?
- Does the study design or analysis account for important confounding and modifying variables? (only relevant for observational studies)

***First Tier.*** Studies placed in the first tier received ratings of "definitely low" or "probably low" risk of bias on the key questions **AND** received a rating of "definitely low" or "probably low" risk of bias on the responses to at least 50% of the other applicable questions.

***Second Tier.*** A study was placed in the second tier if it did not meet the criteria for the first or third tiers.

***Third Tier.*** Studies placed in the third tier received ratings of "definitely high" or "probably high" risk of bias for the key questions **AND** received a rating of "definitely high" or "probably high" risk of bias on the response to at least 50% of the other applicable questions.

The results of the risk of bias assessment for the different types of [Substance x] health effects studies (observational epidemiology and animal experimental studies) are presented in Tables C-8 and C-9, respectively.

## Table C-8. Summary of Risk of Bias Assessment for [Substance x]—Observational Epidemiology Studies

| Reference | Risk of bias criteria and ratings | | | | | | |
|---|---|---|---|---|---|---|---|
| | Selection bias | Confounding bias | Attrition / exclusion bias | Detection bias | | Selective reporting bias | |
| | Were the comparison groups appropriate? | Did the study design or analysis account for important confounding and modifying variables?* | Were outcome data complete without attrition or exclusion from analysis? | Is there confidence in the exposure characterization?* | Is there confidence in the outcome assessment?* | Were all measured outcomes reported? | Risk of bias tier |
| ***Outcome: Respiratory effects*** | | | | | | | |
| *Cohort studies* | | | | | | | |
| Jones 1994 | – | – | + | NA | – | + | Second |
| Renes 1953 | NA | – | + | + | + | + | Second |
| *Cross-sectional studies* | | | | | | | |
| Brieger et al. 1954 | NA | – | + | + | + | + | Second |
| *Case series* | | | | | | | |
| Taylor 1966 | NA | – | + | – | – | + | Third |

***Continue this table, like above, with other health outcomes and identify bias by answering the questions.***

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias; NA = not applicable;
*Key question used to assign risk of bias tier

## Table C-9. Summary of Risk of Bias Assessment for [Substance x]—Experimental Animal Studies

| Reference | Risk of bias criteria and ratings: Selection bias — Was administered dose or exposure level adequately randomized? | Selection bias — Was the allocation to study groups adequately concealed? | Performance bias — Were experimental conditions identical across study groups? | Performance bias — Were the research personnel blinded to the study group during the study? | Attrition/ exclusion bias — Were outcome data complete without attrition or exclusion from analysis? | Detection bias — Is there confidence in the exposure characterization? | Detection bias — Is there confidence in the outcome assessment?* | Selective reporting bias — Were all measured outcomes reported? | Other bias — Did the study design or analysis account for important confounding and modifying variables? | Risk of bias tier |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Outcome: Respiratory effects (inhalation only)*** | | | | | | | | | | |
| *Inhalation acute exposure* | | | | | | | | | | |
| NTP 2016 (rat) | ++ | + | ++ | + | ++ | ++ | **++** | ++ | NA | First |
| *Inhalation intermediate exposure* | | | | | | | | | | |
| Belyaeva 1967 (rat) | + | + | + | – | + | – | **–** | + | NA | Second |
| Newton et al. 1994 (rat) | – | + | + | – | ++ | ++ | **+** | + | NA | First |
| *Inhalation chronic exposure* | | | | | | | | | | |
| Gross et al. 1952 (rat) | – | + | + | – | + | – | **+** | + | NA | First |
| *Oral acute exposure* | | | | | | | | | | |
| NTP 1992 (rat) | + | + | ++ | + | ++ | ++ | **++** | ++ | NA | First |

***Continue this table, like above, with other health outcomes and identify bias by answering the questions.***

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias; NA = not applicable;
*Key question used to assign risk of bias tier

## C.6 RATE THE CONFIDENCE IN THE BODY OF EVIDENCE FOR EACH RELEVANT OUTCOME

Confidences in the bodies of human and animal evidence were evaluated independently for each potential outcome. ATSDR did not evaluate the confidence in the body of evidence for carcinogenicity; rather, the Agency defaulted to the cancer weight-of-evidence assessment of other agencies including DHHS, EPA, and IARC. The confidence in the body of evidence for an association or no association between exposure to [Substance x] and a particular outcome was based on the strengths and weaknesses of individual studies. Four descriptors were used to describe the confidence in the body of evidence for effects or when no effect was found:

- **High confidence:** the true effect is highly likely to be reflected in the apparent relationship
- **Moderate confidence:** the true effect may be reflected in the apparent relationship
- **Low confidence:** the true effect may be different from the apparent relationship
- **Very low confidence:** the true effect is highly likely to be different from the apparent relationship

Confidence in the body of evidence for a particular outcome was rated for each type of study: case-control, case series, cohort, population, human-controlled exposure, and experimental animal. In the absence of data to the contrary, data for a particular outcome were collapsed across animal species, routes of exposure, and exposure durations. If species (or strain), route, or exposure duration differences were noted, then the data were treated as separate outcomes.

### C.6.1 Initial Confidence Rating

In ATSDR's modification to the OHAT approach, the body of evidence for an association (or no association) between exposure to [Substance x] and a particular outcome was given an initial confidence rating based on the key features of the individual studies examining that outcome. The presence of these key features of study design was determined for individual studies using four "yes or no" questions in Distiller, which were customized for epidemiology, human controlled exposure, or experimental animal study designs. Separate questionnaires were completed for each outcome assessed in a study. The key features for observational epidemiology (cohort, population, and case-control) studies, human controlled exposure, and experimental animal studies are presented in Tables C-10, C-11, and C-12, respectively. The initial confidence in the study was determined based on the number of key features present in the study design:

- **High Initial Confidence:** Studies in which the responses to the four questions were "yes".
- **Moderate Initial Confidence:** Studies in which the responses to only three of the questions were "yes".
- **Low Initial Confidence:** Studies in which the responses to only two of the questions were "yes".
- **Very Low Initial Confidence:** Studies in which the response to one or none of the questions was "yes".

## Table C-10. Key Features of Study Design for Observational Epidemiology Studies

| |
|---|
| Exposure was experimentally controlled |
| Exposure occurred prior to the outcome |
| Outcome was assessed on individual level rather than at the population level |
| A comparison group was used |

## Table C-11. Key Features of Study Design for Human-Controlled Exposure Studies

| |
|---|
| A comparison group was used or the subjects served as their own control |
| A sufficient number of subjects were tested |
| Appropriate methods were used to measure outcomes (i.e., clinically-confirmed outcome versus self-reported) |
| Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis |

## Table C-12. Key Features of Study Design for Experimental Animal Studies

| |
|---|
| A concurrent control group was used |
| A sufficient number of animals per group were tested |
| Appropriate parameters were used to assess a potential adverse effect |
| Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis |

The presence or absence of the key features and the initial confidence levels for studies examining *[insert health effect categories going through the full systemic review; i.e., respiratory, hepatic, renal, neurological, and development]* observed in the observational epidemiology and animal experimental studies are presented in Tables C-13 and C-14, respectively.

A summary of the initial confidence ratings for each outcome is presented in Table C-15. If individual studies for a particular outcome and study type had different study quality ratings, then the highest confidence rating for the group of studies was used to determine the initial confidence rating for the body of evidence; any exceptions were noted in Table C-15.

## Table C-13. Presence of Key Features of Study Design for [Substance X]—Observational Epidemiology Studies

| Reference | Key features | | | | Initial study confidence |
|---|---|---|---|---|---|
| | Controlled exposure | Exposure prior to outcome | Outcomes assessed on an individual level | Comparison group | |
| ***Outcome: Hepatic effects*** | | | | | |
| *Cross-sectional studies* | | | | | |
| Burch et al. 2015 | No | No | Yes | Yes | Low |
| ***Outcome: Reproductive effects*** | | | | | |
| *Cohort studies* | | | | | |
| MacLehose et al. 2008 | No | No | Yes | Yes | Low |
| Windham et al. 2003 | No | No | Yes | Yes | Low |
| *Cross-sectional studies* | | | | | |
| Zeng et al. 2013 | No | No | Yes | Yes | Low |
| ***Outcome: Developmental effects*** | | | | | |
| *Cohort studies* | | | | | |
| Cao et al. 2016 | No | No | Yes | Yes | Low |
| Dodds and King 2001 | No | No | Yes | Yes | Low |
| Grazuleviciene et al. 2013 | No | No | Yes | Yes | Low |
| King et al. 2000 | No | No | Yes | Yes | Low |
| Rivera-Nuñez and Wright 2013 | No | No | Yes | Yes | Low |
| Summerhayes et al. 2012 | No | No | Yes | Yes | Low |
| Waller et al. 1998 | No | No | Yes | Yes | Low |
| Wright et al. 2004 | No | No | Yes | Yes | Low |
| *Case-control studies* | | | | | |
| Danilevicuiute et al. 2012 | No | No | Yes | Yes | Low |
| Iszatt et al. 2011 | No | No | Yes | Yes | Low |

## Table C-14. Presence of Key Features of Study Design for [Substance X]—Experimental Animal Studies

| Reference | Key feature | | | | Initial study confidence |
|---|---|---|---|---|---|
| | Concurrent control group | Sufficient number of animals per group | Appropriate parameters to assess potential effect | Adequate data for statistical analysis | |
| ***Outcome: Hepatic Effects*** | | | | | |
| *Inhalation acute exposure* | | | | | |
| Torti et al. 2001 (C57BL/6 mouse) | Yes | No | Yes | Yes | Moderate |
| Torti et al. 2001 (FVN mouse) | Yes | No | Yes | Yes | Moderate |
| *Inhalation intermediate exposure* | | | | | |
| Torti et al. 2001 (C57BL/6 mouse) | Yes | No | Yes | Yes | Moderate |
| Torti et al. 2001 (FVN mouse) | Yes | No | Yes | Yes | Moderate |
| *Oral acute exposure* | | | | | |
| Condie et al. 1983 (mouse) | Yes | No | Yes | Yes | Moderate |
| Keegan et al. 1998 (rat) | Yes | No | No | Yes | Low |
| Thornton-Manning et al. 1994 (mouse) | Yes | No | Yes | Yes | Moderate |
| *Oral intermediate exposure* | | | | | |
| Aida et al. 1989 (rat, F) | Yes | No | Yes | Yes | Moderate |
| Aida et al. 1989 (rat, W) | Yes | No | Yes | Yes | Moderate |
| Aida et al. 1992 (rat) | Yes | Yes | Yes | Yes | High |
| Chu et al. 1982 (rat) | Yes | No | Yes | Yes | Moderate |
| Hooth et al. 2002 (rat) | Yes | No | Yes | Yes | Moderate |
| NTP 1987 (rat) | Yes | Yes | Yes | Yes | High |
| NTP 1987 (mouse) | Yes | Yes | Yes | Yes | High |
| NTP 2006 (rat) | Yes | No | Yes | Yes | Moderate |
| NTP 2006 (mouse) | Yes | No | Yes | Yes | Moderate |
| *Oral chronic exposure* | | | | | |
| Aida et al. 1992 (rat) | Yes | Yes | Yes | Yes | High |
| George et al. 2002 (rat) | Yes | No | Yes | Yes | Moderate |
| George et al. 2002 (mouse) | Yes | No | Yes | Yes | Moderate |
| NTP 1987 (rat) | Yes | Yes | Yes | Yes | High |
| NTP 1987 (mouse) | Yes | Yes | Yes | Yes | High |
| NTP 2006 (rat) | Yes | No | Yes | Yes | Moderate |
| NTP 2006 (mouse) | Yes | No | Yes | Yes | Moderate |
| Tumasonis et al. 1985 (rat) | Yes | Yes | Yes | Yes | High |

## Table C-15. Initial Confidence Rating for [Substance X] Health Effects Studies

| | Initial study confidence | Initial confidence rating |
|---|---|---|
| ***Outcome: Hepatic Effects*** | | |
| *Inhalation acute exposure* | | |
| Animal studies | | |
| Torti et al. 2001 (C57BL/6 mouse) | Moderate | Moderate |
| Torti et al. 2001 (FVN mouse) | Moderate | |
| *Inhalation intermediate exposure* | | |
| Animal studies | | |
| Torti et al. 2001 (C57BL/6 mouse) | Moderate | Moderate |
| Torti et al. 2001 (FVN mouse) | Moderate | |
| *Oral acute exposure* | | |
| Animal studies | | |
| Condie et al. 1983 (mouse) | Moderate | Moderate |
| Keegan et al. 1998 (rat) | Low | |
| Thornton-Manning et al. 1994 (mouse) | Moderate | |
| *Oral intermediate exposure* | | |
| Animal studies | | |
| Aida et al. 1989 (rat, F) | Moderate | High |
| Aida et al. 1989 (rat, W) | Moderate | |
| Aida et al. 1992 (rat) | High | |
| Chu et al. 1982 (rat) | Moderate | |
| Hooth et al. 2002 (rat) | Moderate | |
| NTP 1987 (rat) | High | |
| NTP 1987 (mouse) | High | |
| NTP 2006 (rat) | Moderate | |
| NTP 2006 (mouse) | Moderate | |
| *Oral chronic exposure* | | |
| Human studies | | |
| Burch et al. 2015 | Low | Low |
| Animal studies | | |
| Aida et al. 1992 (rat) | High | High |
| George et al. 2002 (rat) | Moderate | |
| George et al. 2002 (mouse) | Moderate | |
| NTP 1987 (rat) | High | |
| NTP 1987 (mouse) | High | |
| NTP 2006 (rat) | Moderate | |
| NTP 2006 (mouse) | Moderate | |
| Tumasonis et al. 1985 (rat) | High | |

### C.6.2 Adjustment of the Confidence Rating

The initial confidence rating was then downgraded or upgraded depending on whether there were substantial issues that would decrease or increase confidence in the body of evidence. The nine properties of the body of evidence that were considered are listed below. The summaries of the assessment of the confidence in the body of evidence for *[insert health effect categories going through the full systemic review; i.e., respiratory, hepatic, renal, neurological, and development]* effects are presented in Table C-16. If the confidence ratings for a particular outcome were based on more than one type of human study, then the highest confidence rating was used for subsequent analyses. An overview of the confidence in the body of evidence for all health effects associated with [Substance x] exposure is presented in Table C-17.

Five properties of the body of evidence were considered to determine whether the confidence rating should be downgraded:

- **Risk of bias.** Evaluation of whether there is substantial risk of bias across most of the studies examining the outcome. This evaluation used the risk of bias tier groupings for individual studies examining a particular outcome (Tables C-8 and C-9). Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for risk of bias:
    - No downgrade if most studies are in the risk of bias first tier
    - Downgrade one confidence level if most studies are in the risk of bias second tier
    - Downgrade two confidence levels if most studies are in the risk of bias third tier

- **Unexplained inconsistency.** Evaluation of whether there is inconsistency or large variability in the magnitude or direction of estimates of effect across studies that cannot be explained. Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for unexplained inconsistency:
    - No downgrade if there is little inconsistency across studies or if only one study evaluated the outcome
    - Downgrade one confidence level if there is variability across studies in the magnitude or direction of the effect
    - Downgrade two confidence levels if there is substantial variability across studies in the magnitude or direct of the effect

- **Indirectness.** Evaluation of four factors that can affect the applicability, generalizability, and relevance of the studies:
    - Relevance of the animal model to human health—unless otherwise indicated, studies in rats, mice, and other mammalian species are considered relevant to humans
    - Directness of the endpoints to the primary health outcome—examples of secondary outcomes or nonspecific outcomes include organ weight in the absence of histopathology or clinical chemistry findings in the absence of target tissue effects
    - Nature of the exposure in human studies and route of administration in animal studies—inhalation, oral, and dermal exposure routes are considered relevant unless there are compelling data to the contrary
    - Duration of treatment in animal studies and length of time between exposure and outcome assessment in animal and prospective human studies—this should be considered on an outcome-specific basis

Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for indirectness:

- No downgrade if none of the factors are considered indirect
- Downgrade one confidence level if one of the factors is considered indirect
- Downgrade two confidence levels if two or more of the factors are considered indirect

- **Imprecision.** Evaluation of the narrowness of the effect size estimates and whether the studies have adequate statistical power. Data are considered imprecise when the ratio of the upper to lower 95% CIs for most studies is ≥10 for tests of ratio measures (e.g., odds ratios) and ≥100 for absolute measures (e.g., percent control response). Adequate statistical power is determined if the study can detect a potentially biologically meaningful difference between groups (20% change from control response for categorical data or risk ratio of 1.5 for continuous data). Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for imprecision:
  - No downgrade if there are no serious imprecisions
  - Downgrade one confidence level for serious imprecisions
  - Downgrade two confidence levels for very serious imprecisions

- **Publication bias.** Evaluation of the concern that studies with statistically significant results are more likely to be published than studies without statistically significant results.
  - Downgrade one level of confidence for cases where there is serious concern with publication bias

Four properties of the body of evidence were considered to determine whether the confidence rating should be upgraded:

- **Large magnitude of effect.** Evaluation of whether the magnitude of effect is sufficiently large so that it is unlikely to have occurred as a result of bias from potential confounding factors.
  - Upgrade one confidence level if there is evidence of a large magnitude of effect in a few studies, provided that the studies have an overall low risk of bias and there is no serious unexplained inconsistency among the studies of similar dose or exposure levels; confidence can also be upgraded if there is one study examining the outcome, provided that the study has an overall low risk of bias

- **Dose response.** Evaluation of the dose-response relationships measured within a study and across studies. Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
  - Upgrade one confidence level for evidence of a monotonic dose-response gradient
  - Upgrade one confidence level for evidence of a non-monotonic dose-response gradient where there is prior knowledge that supports a non-monotonic dose-response and a non-monotonic dose-response gradient is observed across studies

- **Plausible confounding or other residual biases.** This factor primarily applies to human studies and is an evaluation of unmeasured determinants of an outcome such as residual bias towards the null (e.g., "healthy worker" effect) or residual bias suggesting a spurious effect (e.g., recall bias). Below is the criterion used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:

- Upgrade one confidence level for evidence that residual confounding or bias would underestimate an apparent association or treatment effect (i.e., bias toward the null) or suggest a spurious effect when results suggest no effect

- **Consistency in the body of evidence.** Evaluation of consistency across animal models and species, consistency across independent studies of different human populations and exposure scenarios, and consistency across human study types. Below is the criterion used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
  - Upgrade one confidence level if there is a high degree of consistency in the database

*See the following page for the table.*

**Table C-16. Adjustments to the Initial Confidence in the Body of Evidence**

| | Initial confidence | Adjustments to the initial confidence rating | Final confidence |
|---|---|---|---|
| **Outcome: Hepatic Effects** | | | |
| Human studies | Low | -2 risk of bias | Very Low |
| Animal studies | High | +1 large magnitude of effect | High |
| **Outcome: Renal Effects** | | | |
| Animal studies | High | -1 inconsistency | Moderate |
| **Outcome: Immunological Effects** | | | |
| Animal studies | Moderate | None | Moderate |
| **Outcome: Reproductive Effects** | | | |
| Human studies | Low | -2 risk of bias | Very Low |
| Animal studies | High | -1 inconsistency. -1 imprecision | Low |
| **Outcome: Developmental Effects** | | | |
| Human studies | Low | -2 risk of bias | Very Low |
| Animal studies | High | +1 large magnitude of effect | High |

**Table C-17. Confidence in the Body of Evidence for [Substance X]**

| | Confidence in body of evidence | |
|---|---|---|
| Outcome | Human studies | Animal studies |
| Hepatic effects | Very Low | High |
| Renal effects | No data | Moderate |
| Immunological effects | No data | Moderate |
| Reproductive effects | Very Low | Low |
| Developmental effects | Very Low | High |

## C.7 TRANSLATE CONFIDENCE RATING INTO LEVEL OF EVIDENCE OF HEALTH EFFECTS

In the seventh step of the systematic review of the health effects data for [Substance x], the confidence in the body of evidence for specific outcomes was translated to a level of evidence rating. The level of evidence rating reflected the confidence in the body of evidence and the direction of the effect (i.e., toxicity or no toxicity); route-specific differences were noted. The level of evidence for health effects was rated on a five-point scale:

- **High level of evidence:** High confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Moderate level of evidence:** Moderate confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Low level of evidence:** Low confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Evidence of no health effect:** High confidence in the body of evidence that exposure to the substance is not associated with the health outcome
- **Inadequate evidence:** Low or moderate confidence in the body of evidence that exposure to the substance is not associated with the health outcome OR very low confidence in the body of evidence for an association between exposure to the substance and the health outcome

A summary of the level of evidence of health effects for [Substance x] is presented in Table C-18.

**Table C-18. Level of Evidence of Health Effects for Bromodichloromethane**

| Outcome | Confidence in body of evidence | Direction of health effect | Level of evidence for health effect |
|---|---|---|---|
| **Human studies** | | | |
| Hepatic effects | Very Low | Health effect | Inadequate |
| Renal effects | No data | | No data |
| Immunological effects | No data | | No data |
| Reproductive effects | Very Low | Health effect | Inadequate |
| Developmental effect | Very Low | Health effect | Inadequate |
| **Animal studies** | | | |
| Hepatic effects | High | Health effect | High |
| Renal effects | Moderate | Health effect | Moderate |
| Immunological effects | Moderate | Health effect | Moderate |
| Reproductive effects | Low | Health effect | Low |
| Developmental effect | High | Health effect | High |

## C.8 INTEGRATE EVIDENCE TO DEVELOP HAZARD IDENTIFICATION CONCLUSIONS

The final step involved the integration of the evidence streams for the human studies and animal studies to allow for a determination of hazard identification conclusions. For health effects, there were four hazard identification conclusion categories:

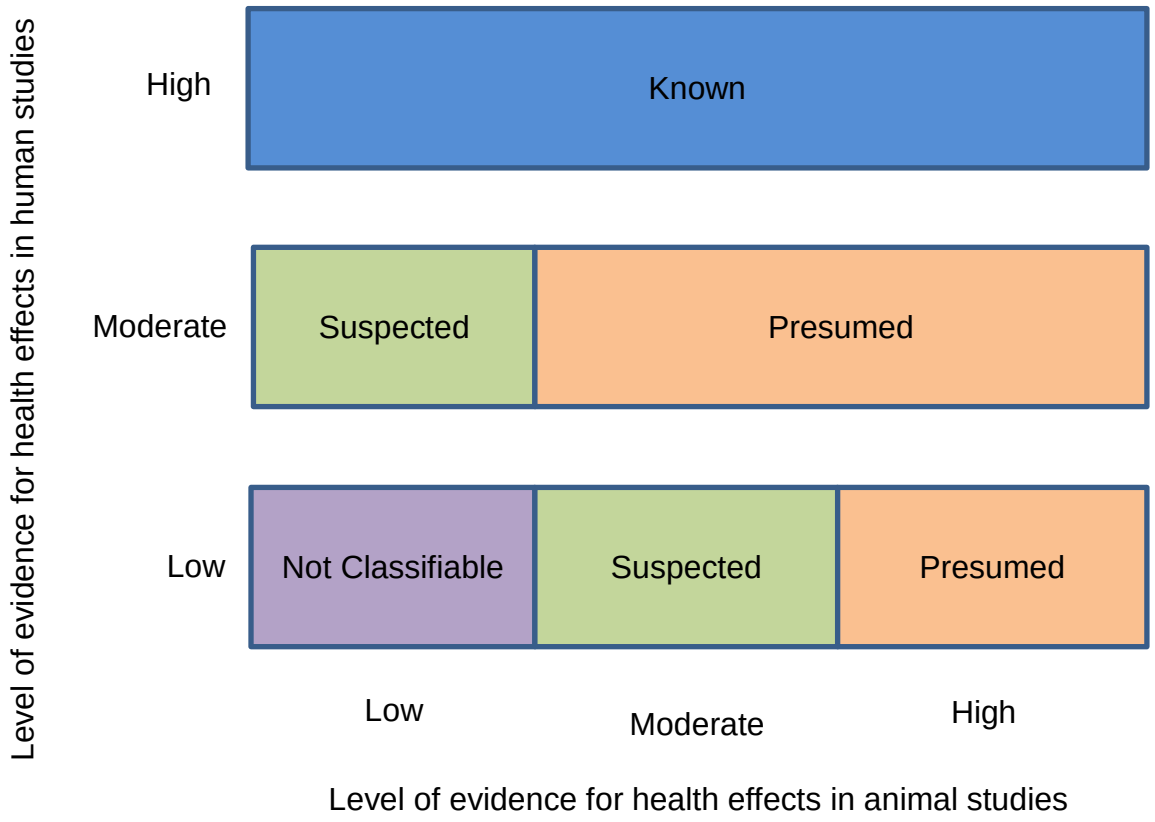
- **Known** to be a hazard to humans
- **Presumed** to be a hazard to humans
- **Suspected** to be a hazard to humans
- **Not classifiable** as to the hazard to humans

The initial hazard identification was based on the highest level of evidence in the human studies and the level of evidence in the animal studies; if there were no data for one evidence stream (human or animal), then the hazard identification was based on the one data stream (equivalent to treating the missing evidence stream as having low level of evidence). The hazard identification scheme is presented in Figure C-1 and described below:

- **Known:** A health effect in this category would have:
  - High level of evidence for health effects in human studies **AND** a high, moderate, or low level of evidence in animal studies.
- **Presumed:** A health effect in this category would have:
  - Moderate level of evidence in human studies **AND** high or moderate level of evidence in animal studies **OR**
  - Low level of evidence in human studies **AND** high level of evidence in animal studies
- **Suspected:** A health effect in this category would have:
  - Moderate level of evidence in human studies **AND** low level of evidence in animal studies **OR**
  - Low level of evidence in human studies **AND** moderate level of evidence in animal studies

- **Not classifiable:** A health effect in this category would have:
  - Low level of evidence in human studies **AND** low level of evidence in animal studies

**Figure C-1. Hazard Identification Scheme**




Other relevant data such as mechanistic or mode-of-action data were considered to raise or lower the level of the hazard identification conclusion by providing information that supported or opposed biological plausibility.

Two hazard identification conclusion categories were used when the data indicated that there may be no health effect in humans:

- **Not identified** to be a hazard in humans
- **Inadequate** to determine hazard to humans

If the human level of evidence conclusion of no health effect was supported by the animal evidence of no health effect, then the hazard identification conclusion category of "not identified" was used. If the human or animal level of evidence was considered inadequate, then a hazard identification conclusion category of "inadequate" was used. As with the hazard identification for health effects, the impact of other relevant data was also considered for no health effect data.

The hazard identification conclusions for [Substance x] are listed below and summarized in Table C-19.

*Summarize the different health effects and their final hazard identification conclusions; an example is provided below.*

**Presumed Health Effects**

- Respiratory effects following inhalation exposure
    - Low evidence from studies of antimony workers (Cooper et al. 1968; Potkonjak and Pavlovich 1983; Renes 1953; Schnorr et al. 1995; Taylor 1966).
    - High level of evidence in rats, mice, rabbits, guinea pigs, and pigs from acute exposure to antimony trisulfide, antimony trioxide, and stibine (Brieger et al. 1954; NTP 2016; Price et al. 1979), intermediate exposure to antimony trisulfide and antimony trioxide (Belyaeva 1967; Brieger et al. 1954; Dernehl et al. 1945; Newton et al. 1994), and chronic exposure to antimony trisulfide, antimony trioxide, and antimony ore (Gross et al. 1952; Groth et al. 1986; Newton et al. 1994; NTP 2016; Watt 1983).
- *Continue with health endpoints that are presumed health effects in the same manner as above.*

**Suspected Health Effects**

- Metabolic effect (decreases in blood glucose levels)
    - No data are available on whether inhalation, oral, or dermal exposure to antimony alters blood glucose levels in humans.
    - High evidence in animal studies based on two studies that found decreases in blood glucose levels following intermediate (Poon et al. 1998) or chronic (Schroeder et al. 1970) oral exposure. Decreases in blood glucose levels were also found in rats following repeated intramuscular injection of two organic pentavalent compounds (Alkhawajah et al. 1992b).
    - Based on the high evidence found in the two animal studies, decreases in blood glucose levels should be classified as a presumed health effect. However, because blood glucose levels have only been assessed in two studies administering antimony via environmentally relevant routes of exposure, the hazard identification was downgraded to suspected health effect.
- *Continue with health endpoints that are suspected and the final category of "known" (if identified) in the same manner as above.*

**Table C-19. Hazard Identification Conclusions for [Substance x]**

| Outcome | Hazard identification |
|---|---|
| Respiratory effects | Presumed health effect following inhalation exposure |
| Metabolic effects (decreased serum glucose levels) | Suspected health effect |
| Developmental effects | Known health effect |

# APPENDIX D. USER'S GUIDE

*All profiles shall have the User's Guide as an appendix.*

## Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints 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?

### Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives 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 hazardous substance 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. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, 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 endpoint 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 endpoint 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 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 that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

**Chapter 2. 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 and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used 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 tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

**TABLE LEGEND**

**See Sample LSE Table (page [C OR D]-5)**

(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 figures are limited to the inhalation and oral routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures. Profiles with more than one chemical may have more LSE tables and figures.

(2) Exposure period. Three exposure periods—acute (<15 days), intermediate (15–364 days), and chronic (≥365 days)—are presented within each relevant route of exposure. In this example, two oral studies of chronic-duration exposure are 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) Figure key. 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 51 identified NOAELs and less serious LOAELs (also see the three "51R" data points in sample LSE Figure 2-X).

(4) Species (strain) No./group. The test species (and strain), whether animal or human, are identified in this column. The column also contains information on the number of subjects and sex per group. Chapter 1, Relevance to Public Health, covers the relevance of animal data to human toxicity and Section 3.1, 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.

(5) Exposure parameters/doses. The duration of the study and exposure regimens are provided in these columns. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 51), rats were orally exposed to “Chemical X” via feed for 2 years. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992).

(6) Parameters monitored. This column lists the parameters used to assess health effects. Parameters monitored could include serum (blood) chemistry (BC), behavioral (BH), biochemical changes (BI), body weight (BW), clinical signs (CS), developmental toxicity (DX), enzyme activity (EA), food intake (FI), fetal toxicity (FX), gross necropsy (GN), hematology (HE), histopathology (HP), lethality (LE), maternal toxicity (MX), organ function (OF), ophthalmology (OP), organ weight (OW), teratogenicity (TG), urinalysis (UR), and water intake (WI).

(7) Endpoint. This column lists the endpoint examined. The major categories of health endpoints included in LSE tables and figures are death, body weight, respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, endocrine, immunological, neurological, reproductive, developmental, other noncancer, and cancer. "Other noncancer" refers to any effect (e.g., alterations in blood glucose levels) not covered in these systems. In the example of key number 51, three endpoints (body weight, hematological, and hepatic) were investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. The body weight effect reported in key number 51 is a NOAEL at 25.5 mg/kg/day. NOAELs are not reported for cancer and death; with the exception of these two endpoints, this field is left blank if no NOAEL was identified in the study.

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse 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 endpoint used to quantify the adverse effect accompanies the LOAEL. Key number 51 reports a less serious LOAEL of 6.1 mg/kg/day for the hepatic system, which was used to derive a chronic exposure, oral MRL of 0.008 mg/kg/day (see footnote "c"). MRLs are not derived from serious LOAELs. A cancer effect level (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. If no LOAEL/CEL values were identified in the study, this field is left blank.

(10) Reference. The complete reference citation is provided in Chapter 8 of the profile.

(11) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. For example, footnote "c" indicates that the LOAEL of 6.1 mg/kg/day in key number 51 was used to derive an oral MRL of 0.008 mg/kg/day.

**FIGURE LEGEND**

**See Sample LSE Figure (page [COR D]-6)**

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 chronic exposure period are illustrated.

(14) Endpoint. These are the categories of health effects for which reliable quantitative data exist. The same health effect endpoints 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) LOAEL. In this example, the half-shaded circle that is designated 51R identifies a LOAEL critical endpoint in the rat upon which a chronic oral exposure MRL is based. The key number 51 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 6.1 mg/kg/day (see entry 51 in the sample LSE table) to the MRL of 0.008 mg/kg/day (see footnote "c" in the sample LSE table).

(17) CEL. Key number 59R is one of studies for which CELs were derived. The diamond symbol refers to a CEL for the test species (rat). The number 59 corresponds to the entry in the LSE table.

(18) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

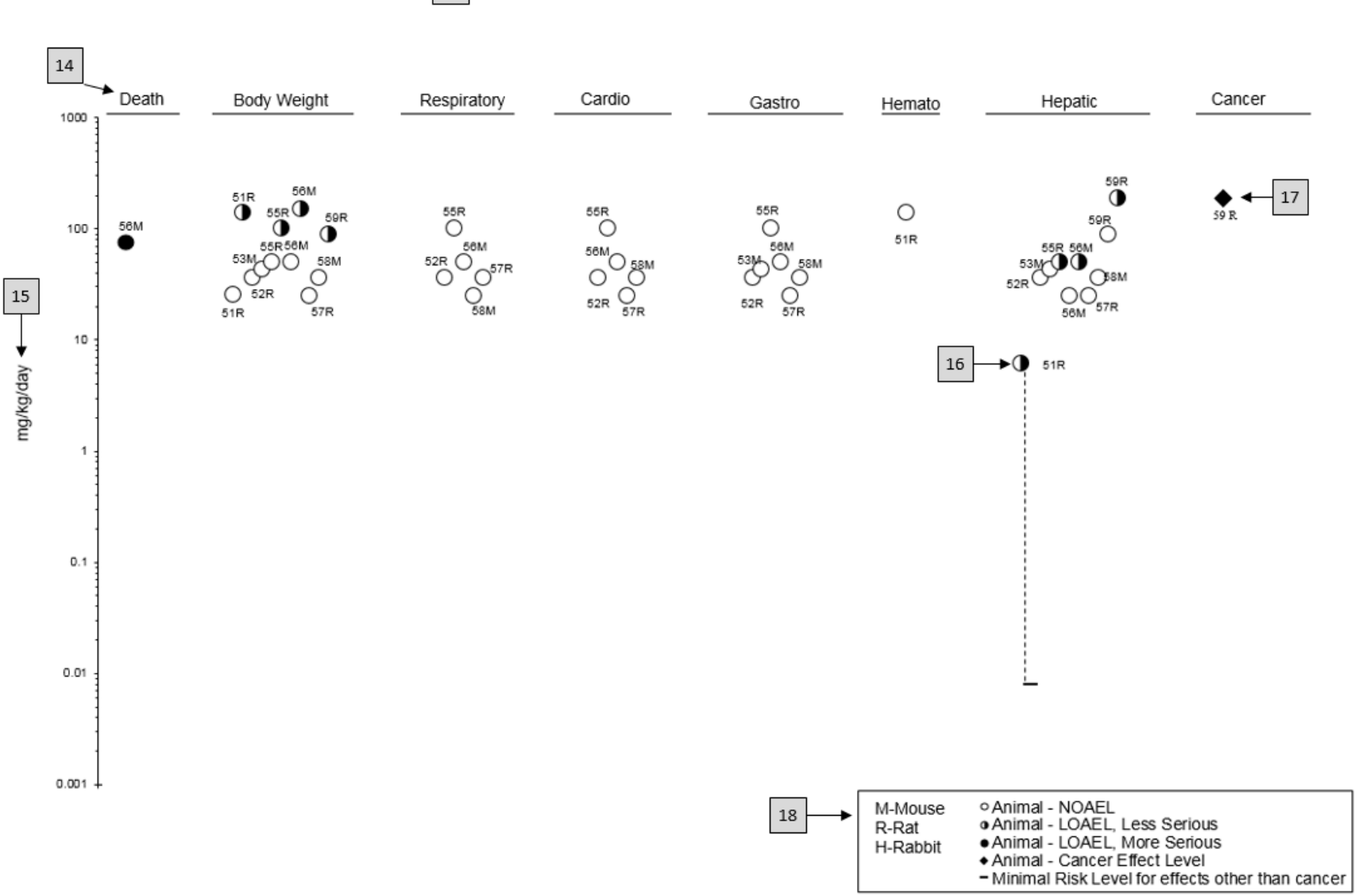
**Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral** (1)

| Figure key[a] (3) | Species (strain) No./group (4) | Exposure parameters (5) | Doses (mg/kg/day) (5) | Parameters monitored (6) | Endpoint (7) | NOAEL (mg/kg/day) (8) | Less serious LOAEL (mg/kg/day) (9) | Serious LOAEL (mg/kg/day) (9) | Effect |
|---|---|---|---|---|---|---|---|---|---|
| **CHRONIC EXPOSURE** (2) | | | | | | | | | |
| 51 | Rat (Wistar) 40 M, 40 F | 2 years (F) | M: 0, 6.1, 25.5, 138.0 F: 0, 8.0, 31.7, 168.4 | CS, WI, BW, OW, HE, BC, HP | Bd wt | 25.5 | 138.0 | | Decreased body weight gain in males (23–25%) and females (31–39%) |
| | | | | | Hemato | 138.0 | | | |
| | | | | | Hepatic | | 6.1[c] | | Increases in absolute and relative weights at ≥6.1/8.0 mg/kg/day after 12 months of exposure; fatty generation at ≥6.1 mg/kg/day in males and at ≥31.7 mg/kg/day in females, and granulomas in females at 31.7 and 168.4 mg/kg/day after 12, 18, or 24 months of exposure and in males at ≥6.1 mg/kg/day only after 24 months of exposure |
| **Aida et al. 1992** (10) | | | | | | | | | |
| 52 | Rat (F344) 78 M | 104 weeks (W) | 0, 3.9, 20.6, 36.3 | CS, BW, FI, BC, OW, HP | Hepatic | 36.3 | | | |
| | | | | | Renal | 20.6 | 36.3 | | Increased incidence of renal tubular cell hyperplasia |
| | | | | | Endocr | 36.3 | | | |
| **George et al. 2002** | | | | | | | | | |
| 59 | Rat (Wistar) 58M, 58F | Lifetime (W) | M: 0, 90 F: 0, 190 | BW, HP | Cancer | | 190 F | | Increased incidence of hepatic neoplastic nodules in females only; no additional description of the tumors was provided |
| **Tumasonis et al. 1985** | | | | | | | | | |

[a]The number corresponds to entries in Figure 2-x.
[b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on the $BMDL_{05}$ of 10 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). (11)
[c]Used to derive a chronic-duration oral MRL of 0.008 mg/kg/day based on the $BMDL_{10}$ of 0.78 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

APPENDIX D

**Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral**



# APPENDIX E. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

*All profiles shall have the Quick Reference for Health Care Providers as an appendix.*

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 may find the following information helpful for fast answers to often-asked questions.

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***Primary Chapters/Sections of Interest***

**Chapter 1: Relevance to Public Health**: The Relevance to Public Health Section provides an overview of exposure and health effects and evaluates, interprets, and assesses the significance of toxicity data to human health. A table listing minimal risk levels (MRLs) is also included in this chapter.

**Chapter 2: Health Effects**: Specific health effects identified in both human and animal studies are reported by type of health effect (e.g., death, hepatic, renal, immune, reproductive), route of exposure (e.g., inhalation, oral, dermal), and length of exposure (e.g., acute, intermediate, and chronic).
***NOTE***: Not all health effects reported in this section are necessarily observed in the clinical setting.

**Pediatrics**:

**Section 3.2 Children and Other Populations that are Unusually Susceptible**
**Section 3.3 Biomarkers of Exposure and Effect**

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***ATSDR Information Center***

***Phone:*** 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
***Internet***: http://www.atsdr.cdc.gov

The following additional materials are available online:

*Case Studies in Environmental Medicine* are self-instructional publications designed to increase primary health care providers' knowledge of a hazardous substance in the environment and to aid in the evaluation of potentially exposed patients (see https://www.atsdr.cdc.gov/csem/csem.html).

*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 (see https://www.atsdr.cdc.gov/MHMI/index.asp). 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 Exposures*—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 (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

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***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 • Web Page: https://www.cdc.gov/nceh/.

*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, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/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 • Web Page: https://www.niehs.nih.gov/.

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***Clinical Resources (Publicly Available Information)***

*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 • Web Page: http://www.acoem.org/.

*The American College of Medical Toxicology* (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard, Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

*The Pediatric Environmental Health Specialty Units* (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

*The American Association of Poison Control Centers* (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

# APPENDIX F. GLOSSARY

*The standard glossary should be included in every document. Substance-specific recommendations for revisions to the glossary can be made to the chemical manager, who can approve revisions without consulting the guidance committee. Revisions that are not substance-specific (i.e., that might apply to any other profile) should be brought to the attention of the guidance committee.*

**Absorption—**The process by which a substance crosses biological membranes and enters systemic circulation. Absorption can also refer to 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, 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) or Benchmark Concentration (BMC)—**is the dose/concentration corresponding to a specific response level estimate using a statistical dose-response model applied to either experimental toxicology or epidemiology data. For example, a $BMD_{10}$ would be the dose corresponding to a 10% benchmark response (BMR). The BMD is determined by modeling the dose-response curve in the region of the dose-response relationship where biologically observable data are feasible. The BMDL or BMCL is the 95% lower confidence limit on the BMD or BMC.

**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—**Indicators signaling events in biologic systems or samples, typically classified as markers of exposure, effect, and susceptibility.

**Cancer Effect Level (CEL)—**The lowest dose of a 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-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

**Case Report—**A report that describes a single individual with a particular disease or exposure. These reports may suggest some potential topics for scientific research, but are not actual research studies.

**Case Series—**Reports that describe the experience of a small number of individuals with the same disease or exposure. These reports may suggest potential topics for scientific research, but are not actual research studies.

**Ceiling Value—**A concentration that must not be exceeded.

**Chronic Exposure—**Exposure to a chemical for ≥365 days, as specified in the Toxicological Profiles.

**Clastogen—**A substance that causes breaks in chromosomes resulting in addition, deletion, or rearrangement of parts of the chromosome.

**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, and who are disease-free at start of follow-up. Often, at least one exposed group is compared to one unexposed group, while in other cohorts, exposure is a continuous variable and analyses are directed towards analyzing an exposure-response coefficient.

**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 a specific point in time.

**Data Needs—**Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk 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 response or amount of the response.

**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 effect occurs. Effects include malformations and variations, altered growth, and *in utero* death.

**Epidemiology—**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.

**Excretion—**The process by which metabolic waste products are removed from the body.

**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.

**Health Advisory—**An estimate of acceptable drinking water levels for a chemical substance derived by EPA and 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.

**Immediately Dangerous to Life or Health (IDLH)—**A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

**Immunotoxicity—**Adverse effect on the functioning of the immune system that may result from exposure to chemical substances.

**Incidence—**The ratio of new cases 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.

**Metabolism—**Process in which chemical substances are biotransformed in the body that could result in less toxic and/or readily excreted compounds or produce a biologically active intermediate.

**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—**The state of being diseased; the morbidity rate is the incidence or prevalence of a disease in a specific population.

**Mortality—**Death; the 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, which are changes 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 hazardous substance.

**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. Although effects may be produced at this dose, 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 odds ratio that is greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

**Permissible Exposure Limit (PEL)—**An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work 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 (insects or other organisms harmful to cultivated plants or animals).

**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 endpoints. 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—**A type of physiologically based dose-response model that is 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, including tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air 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 a group is followed over time and the pertinent observations are made on events occurring after the start of the study.

**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 RfC is 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 oral exposure of the human population to a potential hazard that is likely to be without risk of deleterious noncancer health effects during a lifetime. The oral RfD is expressed in units of mg/kg/day.

**Reportable Quantity (RQ)—**The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). RQs are (1) $\geq$1 pound 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 hazardous substance. 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 hazardous substance.

**Risk Factor—**An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

**Risk Ratio/Relative Risk—**The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio that is greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

**Short-Term Exposure Limit (STEL)—**A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

**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 it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time-Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

**Time-Weighted Average (TWA)—**An average exposure within a given time period.

**Toxicokinetic—**The absorption, distribution, metabolism, and elimination of toxic compounds in the living organism.

**Toxics Release Inventory (TRI)—**The TRI is an EPA program that tracks toxic chemical releases and pollution prevention activities reported by industrial and federal facilities.

**Uncertainty Factor (UF)—**A factor used in operationally deriving the Minimal Risk Level (MRL), 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 substance that is foreign to the biological system.

# APPENDIX G. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

*All profiles shall have the list of Acronyms, Abbreviations, and Symbols as an appendix.*

| | |
|---|---|
| AAPCC | American Association of Poison Control Centers |
| ACGIH | American Conference of Governmental Industrial Hygienists |
| ACOEM | American College of Occupational and Environmental Medicine |
| ACMT | American College of Medical Toxicology |
| ADI | acceptable daily intake |
| ADME | absorption, distribution, metabolism, and excretion |
| AEGL | Acute Exposure Guideline Level |
| AIC | Akaike's information criterion |
| AIHA | American Industrial Hygiene Association |
| ALT | alanine aminotransferase |
| AOEC | Association of Occupational and Environmental Clinics |
| AP | alkaline phosphatase |
| AST | aspartate aminotransferase |
| atm | atmosphere |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| AWQC | Ambient Water Quality Criteria |
| BCF | bioconcentration factor |
| BMD/C | benchmark dose or benchmark concentration |
| $BMD_X$ | dose that produces a X% change in response rate of an adverse effect |
| $BMDL_X$ | 95% lower confidence limit on the $BMD_X$ |
| BMDS | Benchmark Dose Software |
| BMR | benchmark response |
| BUN | blood urea nitrogen |
| C | centigrade |
| CAA | Clean Air Act |
| CAS | Chemical Abstract Services |
| CDC | Centers for Disease Control and Prevention |
| CEL | cancer effect level |
| CERCLA | Comprehensive Environmental Response, Compensation, and Liability Act |
| CFR | Code of Federal Regulations |
| Ci | curie |
| CI | confidence interval |
| cm | centimeter |
| CPSC | Consumer Products Safety Commission |
| CWA | Clean Water Act |
| DHHS | Department of Health and Human Services |
| DNA | deoxyribonucleic acid |
| DOD | Department of Defense |
| DOE | Department of Energy |
| DWEL | drinking water exposure level |
| EAFUS | Everything Added to Food in the United States |
| ECG/EKG | electrocardiogram |
| EEG | electroencephalogram |
| EPA | Environmental Protection Agency |
| ERPG | emergency response planning guidelines |
| F | Fahrenheit |

| | |
|---|---|
| F1 | first-filial generation |
| FDA | Food and Drug Administration |
| FIFRA | Federal Insecticide, Fungicide, and Rodenticide Act |
| FR | Federal Register |
| FSH | follicle stimulating hormone |
| g | gram |
| GC | gas chromatography |
| gd | gestational day |
| GGT | γ-glutamyl transferase |
| GRAS | generally recognized as safe |
| HEC | human equivalent concentration |
| HED | human equivalent dose |
| HHS | Department of Health and Human Services |
| HPLC | high-performance liquid chromatography |
| HSDB | Hazardous Substance Data Bank |
| IARC | International Agency for Research on Cancer |
| IDLH | immediately dangerous to life and health |
| IRIS | Integrated Risk Information System |
| Kd | adsorption ratio |
| kg | kilogram |
| kkg | kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 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 | Level of Significant Exposure |
| $LT_{50}$ | lethal time, 50% kill |
| m | meter |
| mCi | millicurie |
| MCL | maximum contaminant level |
| MCLG | maximum contaminant level goal |
| MF | modifying factor |
| mg | milligram |
| mL | milliliter |
| mm | millimeter |
| mmHg | millimeters of mercury |
| mmol | millimole |
| MRL | Minimal Risk Level |
| MS | mass spectrometry |
| MSHA | Mine Safety and Health Administration |
| Mt | metric ton |
| NAAQS | National Ambient Air Quality Standard |
| NAS | National Academy of Science |
| NCEH | National Center for Environmental Health |

| | |
|---|---|
| ND | not detected |
| ng | nanogram |
| NHANES | National Health and Nutrition Examination Survey |
| NIEHS | National Institute of Environmental Health Sciences |
| NIOSH | National Institute for Occupational Safety and Health |
| NLM | National Library of Medicine |
| nm | nanometer |
| nmol | nanomole |
| NOAEL | no-observed-adverse-effect level |
| NPL | National Priorities List |
| NR | not reported |
| NRC | National Research Council |
| NS | not specified |
| NTP | National Toxicology Program |
| OR | odds ratio |
| OSHA | Occupational Safety and Health Administration |
| PAC | Protective Action Criteria |
| PAH | polycyclic aromatic hydrocarbon |
| PBPD | physiologically based pharmacodynamic |
| PBPK | physiologically based pharmacokinetic |
| PEHSU | Pediatric Environmental Health Specialty Unit |
| PEL | permissible exposure limit |
| PEL-C | permissible exposure limit-ceiling value |
| pg | picogram |
| PND | postnatal day |
| POD | point of departure |
| ppb | parts per billion |
| ppbv | parts per billion by volume |
| ppm | parts per million |
| ppt | parts per trillion |
| REL | recommended exposure level/limit |
| REL-C | recommended exposure level-ceiling value |
| RfC | reference concentration |
| RfD | reference dose |
| RNA | ribonucleic acid |
| SARA | Superfund Amendments and Reauthorization Act |
| SCE | sister chromatid exchange |
| SD | standard deviation |
| SE | standard error |
| SGOT | serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST) |
| SGPT | serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT) |
| SIC | standard industrial classification |
| SMR | standardized mortality ratio |
| sRBC | sheep red blood cell |
| STEL | short term exposure limit |
| TLV | threshold limit value |
| TLV-C | threshold limit value-ceiling value |
| 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 |
| USNRC | U.S. Nuclear Regulatory Commission |
| VOC | volatile organic compound |
| WBC | white blood cell |
| WHO | World Health Organization |

| | |
|---|---|
| > | greater than |
| ≥ | greater than or equal to |
| = | equal to |
| < | less than |
| ≤ | 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 |