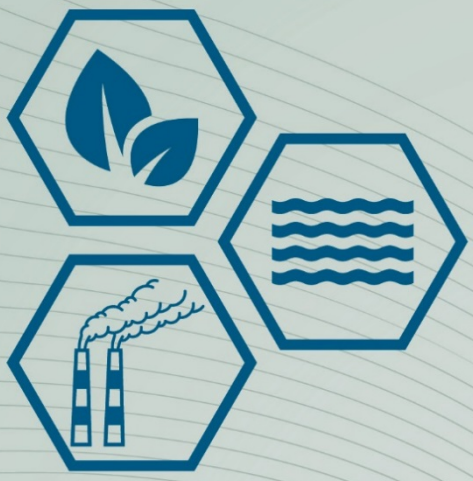

# Toxicological Profile for 4,4’-Methylenebis(2-chloroaniline) (MBOCA)

**October 2017**

ATSDR
U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

CS274127-A

# FOREWORD

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

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for 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.

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 due to associated acute, intermediate, and chronic exposures;

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

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. 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 was 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
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*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.

# VERSION HISTORY

| Date | Description |
|---|---|
| May 1994 | Final toxicological profile released |
| October 2017 | Update of data in Chapters 2, 3, and 7 |

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# CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

## 1.1 OVERVIEW AND U.S. EXPOSURES

ATSDR's *Toxicological Profile for 4,4'-Methylenebis(2-chloroaniline) (MBOCA)* was released in 1994. 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 and 3 were revised to reflect the most current health effects 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.

4,4'-Methylenebis(2-chloroaniline) (MBOCA) is a synthetic chemical used in industry primarily to produce castable polyurethane parts. It also has a coating application in chemical reactions to "set" glues, plastics, and adhesives. Since plastics have many uses, MBOCA is used very widely. Pure MBOCA is a colorless solid, but MBOCA is usually made and used as yellow, tan, or brown pellets. If MBOCA is heated above 205°C, it may decompose. MBOCA has no odor or taste.

Most exposure to MBOCA occurs in the workplace. If you work with MBOCA, you may breathe small particles of it in the air or get it on your skin if you brush against a surface covered by MBOCA dust. There are several ways to be exposed to MBOCA outside of the workplace. For example, you may be exposed to MBOCA if you live in an area where the soil is contaminated with MBOCA. You may also be exposed if you eat foods grown in soils that contain MBOCA. However, you are unlikely to drink water contaminated with MBOCA because it does not dissolve in water.

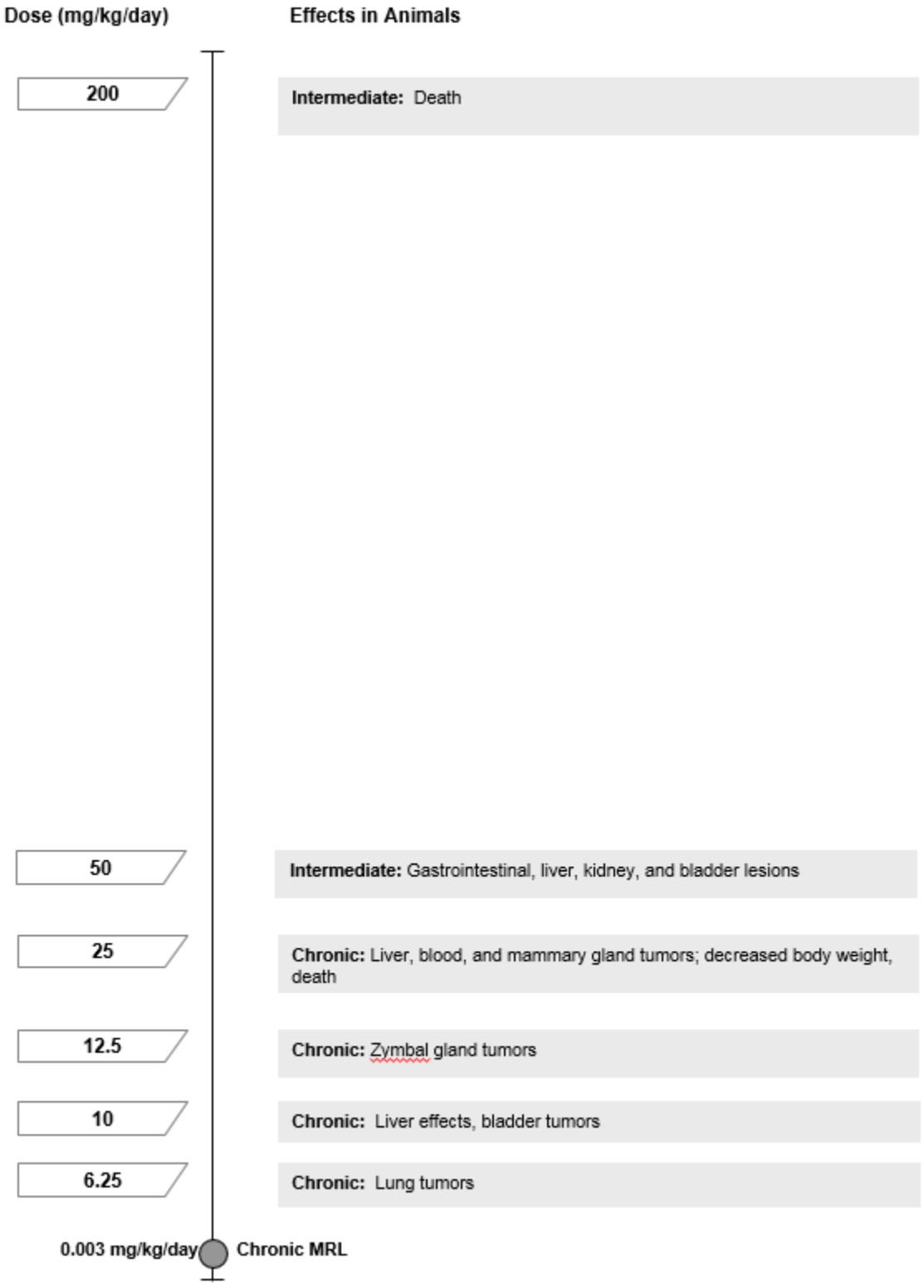
## 1.2 SUMMARY OF HEALTH EFFECTS

The health effects of MBOCA have been evaluated in two human occupational retrospective cohort studies of cancer, an occupational health survey, a limited number of case studies, a single intermediate-duration study evaluating oral and dermal exposure, a dermal initiation-promotion cancer study, and a limited number of chronic oral animal studies predominately focused on carcinogenicity. No animals studies evaluating potential health effects following inhalation exposure to MBOCA were identified.

As illustrated in Figure 1-1, cancer and the liver are the most sensitive targets of MBOCA toxicity in animals following oral exposure, followed by the gastrointestinal tract, kidney, and urinary bladder. Renal, dermal, ocular, and carcinogenic effects have also been described in a limited number of

occupational exposure studies with potential for exposure via multiple routes. Data regarding these effects are discussed briefly below. Available data following exposure to MBOCA in humans and animals are inadequate to determine the potential for adverse effects in the respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, endocrine, immune, nervous, or reproductive systems. It is unknown whether or not MBOCA can damage a developing fetus because no developmental exposure studies are available.

## Figure 1-1. Health Effects Found in Animals Following Oral Exposure to MBOCA



***Gastrointestinal Effects.*** Stomach upset was reported in a worker who was accidently sprayed in the face with molten MBOCA (some entered his mouth) (Hosein and Van Roosmalen 1978). In laboratory animals, stomach and intestinal degeneration and dysplasia were observed in mice following intermediate-duration oral exposure to doses ≥50 mg/kg/day or dermal exposure ≥100 mg/kg/day (Chen et al. 2014).

***Hepatic Effects.*** No information was located regarding adverse hepatic effects in humans following exposure to MBOCA. Hepatic effects such as elevated liver enzyme levels, nodular hepatic hyperplasia, fatty changes, necrosis, fibrosis, and bile duct proliferation were observed in rats and dogs following chronic oral exposure to MBOCA to doses as low as 10 mg/kg/day (Stula et al. 1975, 1977). Hepatic degeneration and dysplasia were also observed in mice following intermediate-duration oral exposure to doses ≥50 mg/kg/day or dermal exposure ≥100 mg/kg/day (Chen et al. 2014). Neoplastic lesions associated with MBOCA exposure are discussed below in the ***Cancer*** section.

***Renal and Urinary Bladder Effects.*** Information on the potential for renal effects following MBOCA exposure in humans is limited to evidence of altered urinalysis parameters in occupationally exposed workers, including heme and atypical cells in urine sediment in workers exposed to MBOCA for a median duration of 3.2 months (Ward et al. 1990) and urinalysis findings suggestive of transient renal tubule damage in a worker involved in a high-exposure occupational accident (Hosein and Van Roosmalen 1978). However, no atypical cells were found in another group of workers exposed for up to 16 years (Linch et al. 1971). Renal system effects in laboratory animals include renal and urinary bladder degeneration and dysplasia in mice following intermediate-duration oral exposure to doses ≥50 mg/kg/day or dermal exposure ≥100 mg/kg/day (Chen et al. 2014) and abnormal cytology in urine sediment in dogs following chronic exposure to 10 mg/kg/day (Stula et al. 1977). Abnormal cytology is considered a potential biomarker for urinary tract lesions and neoplasias; neoplastic lesions in the bladder associated with MBOCA exposure are discussed below in the ***Cancer*** section.

***Cancer.*** A small number of retrospective cohort studies and case reports found increases in urinary bladder cancer following occupational exposure to MBOCA (Dost et al. 2009; Liu et al. 2005; Ward et al. 1988, 1990); however, these studies are limited by lack of control for confounding variables and concurrent exposures, lack of exposure levels and route information, small sample size, and/or low incidences. Chronic oral exposure studies in animals have found increases in neoplastic tumors in various organs in rodents and dogs, including the urinary bladder, lung, liver, mammary gland, Zymbal gland, and

vascular system (Grundmann and Steinhoff 1970; Kommineni et al. 1979; Russfield et al. 1975; Stula et al. 1975, 1977). Tumor type was affected by species, sex, and protein levels in the diet.

The U.S. Department of Health and Human Services (NTP 2016) has classified MBOCA as reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in laboratory animals. The U.S. Environmental Protection Agency (EPA) has not categorized the carcinogenicity of MBOCA (IRIS 2017). The International Agency for Research on Cancer (IARC 2012) has categorized MBOCA as a Group 1 carcinogen (carcinogenic to humans) based on inadequate evidence in humans, sufficient evidence in laboratory animals, and strong mechanistic evidence indicating that carcinogenicity of MBOCA is mediated via a genotoxic mechanism of action (MOA) similar to other known cancer-causing aromatic amines.

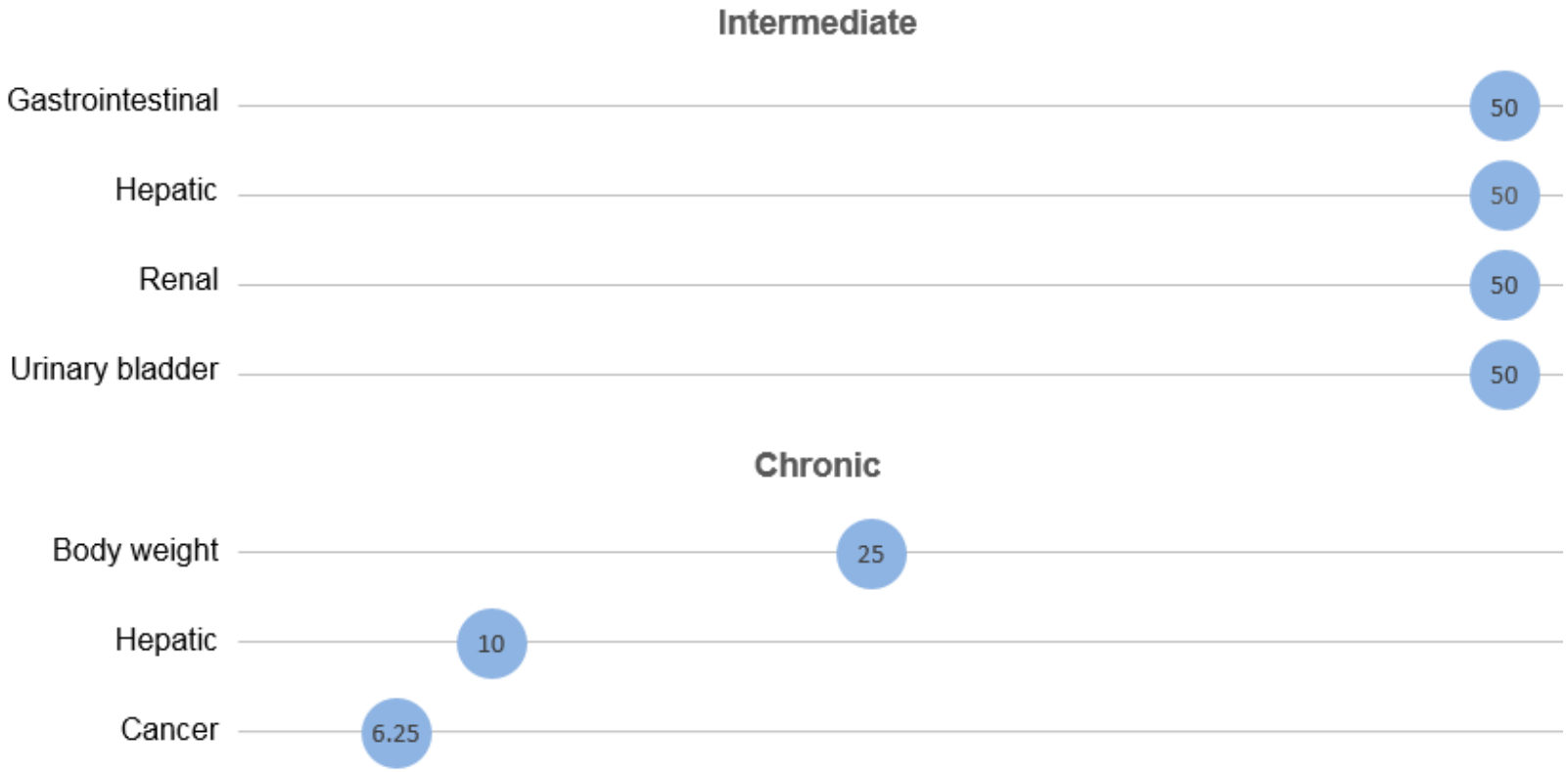
## 1.3 MINIMAL RISK LEVELS (MRLs)

As presented in Figure 1-2, the limited available oral data for MBOCA suggest that the liver is the most sensitive target of toxicity. The inhalation database was considered inadequate for deriving MRLs (no animal inhalation studies identified). The MRL values are summarized in Table 1-1 and discussed in greater detail in Appendix A.

## Figure 1-2. Summary of Sensitive Targets of MBOCA -- Oral

**Cancer and the liver are the most sensitive targets of MBOCA.**

Numbers in circles are the lowest LOAELs (mg/kg/day) for all health effects in animals; no reliable dose response data were available for humans.



## Table 1-1. Minimal Risk Levels (MRLs) for MBOCA[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 | Insufficient data for MRL derivation | | | | |
| Intermediate | Insufficient data for MRL derivation | | | | |
| Chronic | 0.003 | Increased ALT, nodular hepatic hyperplasia | 10 (LOAEL) | 3,000 | Stula et al. 1977 |

[a]See Appendix A for additional information.

ALT = alanine transaminase; LOAEL = lowest-observed-adverse-effect level

# CHAPTER 2. HEALTH EFFECTS

## 2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of MBOCA. 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.

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 MBOCA, but may not be inclusive of the entire body of literature.

Human occupational studies are presented in Table 2-1. Animal oral studies are presented in Table 2-2 and Figure 2-2 and animal dermal studies are presented in Table 2-3; no inhalation animal studies were identified for MBOCA.

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. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of MBOCA are indicated in Tables 2-2 and 2-3 and Figure 2-2.

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

The health effects of MBOCA have been evaluated in a limited number of occupational cohort studies and case reports; exposure in these studies is expected to be predominantly via the inhalation and dermal route. Available animal studies include a single acute oral study, an intermediate-duration study evaluating oral and dermal exposure, a dermal initiation-promotion cancer study, and eight chronic oral animal studies predominantly focused on carcinogenicity. No animals studies evaluating potential health effects following inhalation exposure to MBOCA were identified. As illustrated in Figure 2-1, most of the health effects data come from oral studies in animals. Nonneoplastic toxicity data are available only for a limited number of health effect categories; no animal studies examined the cardiovascular, musculoskeletal, immune, neurological, or reproductive systems. It is also noted that no studies examined developmental toxicity.

The available human and animal studies suggest the following sensitive targets of toxicity:

- **Gastrointestinal Endpoint:** Stomach upset has been reported in a case report of accidental exposure to MBOCA (sprayed in face). Degeneration and dysplasia of the stomach and intestines have been observed in laboratory animals following intermediate-duration oral or dermal exposure.
- **Hepatic Endpoint:** Evidence of impaired hepatic function (elevated liver enzymes) and various nonneoplastic hepatic lesions have been observed in rats, mice, and dogs following intermediate- or chronic-duration oral exposure and intermediate-duration dermal exposure.

- **Renal and Urinary Bladder Endpoints:** Abnormal findings in urinalysis (protein, heme, abnormal cells) have been reported in some workers following occupational exposure to MBOCA. Abnormal cells in urine sediment have also been observed in dogs following chronic oral exposure. Degeneration and dysplasia of the kidney and urinary bladder have been observed in laboratory animals following intermediate-duration oral or dermal exposure.

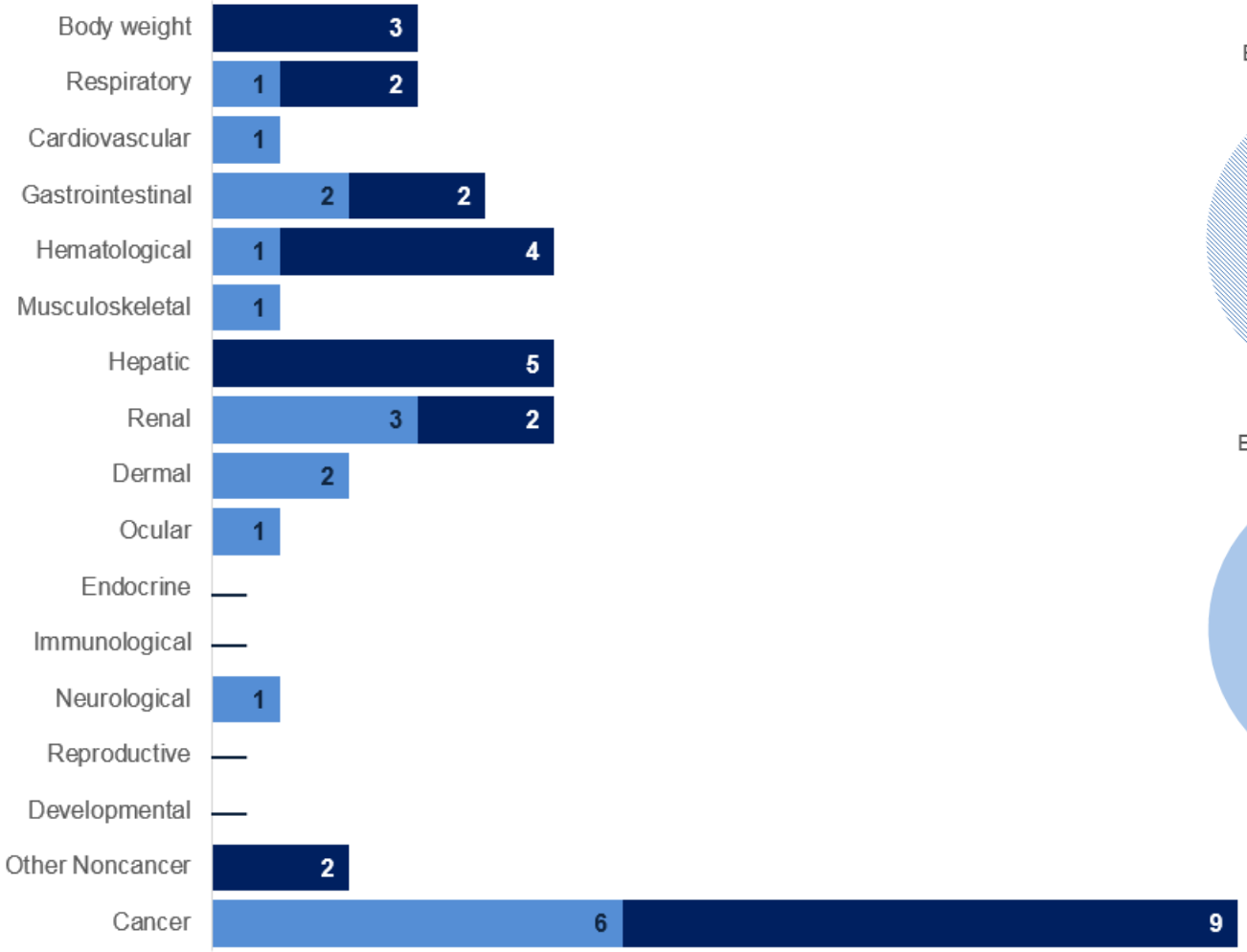
- **Body Weight Endpoint:** Mild decreases in body weight were observed in rats following chronic oral exposure, but not mice or dogs.

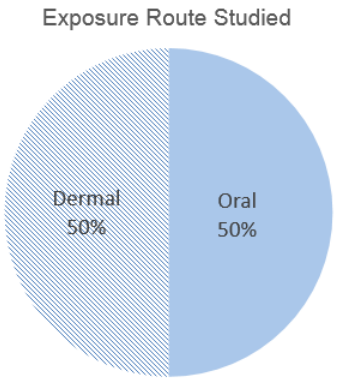
- **Cancer:** A small number of retrospective occupational cohort studies and case reports report bladder cancer in humans following occupational exposure to MBOCA. Chronic oral studies in animals indicate that MBOCA is a multi-site carcinogen in rats, mice, and dogs. Observed tumors include lung, liver, urinary bladder, mammary gland, Zymbal gland, and vascular system.

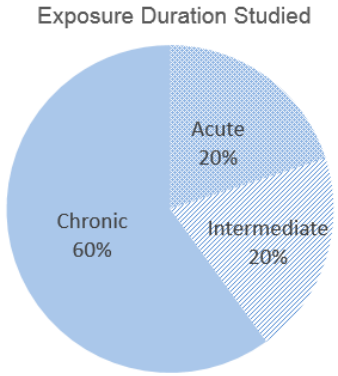
## Figure 2-1. Overview of the Number of Studies Examining MBOCA Health Effects

**Most studies examined the potential carcinogenic effects of MBOCA**

More studies evaluated health effects in **animals** than **humans** (counts represent studies examining endpoint)




*Includes studies discussed in Chapter 2. A total of 20 studies include those finding no effect. Most studies examined multiple endpoints. All human studies were classified as dermal studies; however, occupational exposure is expected to be via inhalation and dermal routes.

## Table 2-1. Health Effects in Humans Occupationally Exposed to MBOCA

| Reference and study population | Exposure | Outcomes |
|---|---|---|
| **Epidemiological studies** | | |
| **Linch et al. 1971**<br><br>Exposure groups: 31 current workers exposed to MBOCA (6 months–16 years); 172 workers formerly exposed to MBOCA<br><br>Referent groups: 31 never-exposed referents (current employees); all former workers (number not reported) | Current maximum exposure levels:<br>Personal air monitoring level: 0.02 mg/m$^3$ (0.002 ppm)<br>Air levels in close proximity to manufacturing equipment: 0.25 mg/m$^3$ (0.02 ppm)<br><br>Note: urinary biomonitoring indicated higher exposure levels, indicating that dermal exposure was primary route of exposure. | No increase in disability rate attributed to dysfunction in the respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, or central nervous systems.<br><br>No abnormal class IV or V cells were identified in the urine of current or former workers using the Papanicolaou technique[a].<br><br>No increase in incidence of cancer (any kind). |
| **Dost et al. 2009**<br><br>Exposure group: 308 MBOCA-exposed workers (minimum employment of 1 year between 1973 and 2000)<br><br>Referent group: general population (national cancer rates between 1979 and 2007) | No exposure levels reported; most likely exposed via dermal and inhalation routes. | **Bladder cancer mortality:**<br>Observed: 1/308<br>Expected: 0.18/308<br>SMR (95% CI): 560 (14–3,122)<br><br>**Bladder cancer registrations:**<br>Observed: 2/308<br>Expected: 0.61/308<br>SMR (95% CI): 328 (40–1,184)<br><br>Cases were employed for 6–10 years; one case was a smoker (smoking was not controlled for in analysis).<br><br>Other cancer incidences were at or below expected incidences. |
| **Ward et al. 1990**<br><br>Exposure group: 385 MBOCA-exposed workers (mean employment 3.2 months)<br><br>Referent group: none | No exposure levels reported; most likely exposed via dermal and inhalation routes.<br><br>Interval between time of first exposure and study initiation was an average of 11.5 years.<br><br>Additional co-exposures may have included 4,4'-methylenedianiline, 4-chloro-*ortho*-toluidine, aniline, and *ortho*-toluidine (Hogan 1993; Ward 1993). | **Urinalysis:**<br>Suspicious or positive cytology: 0/385<br>Evidence of atypical cells: 21/385<br>Heme: 60/385<br><br>**Bladder cystoscopy:**<br>Low-grade papillary tumors: 2/200<br>Full-blown papillary tumors: 1/200 |

## Table 2-1. Health Effects in Humans Occupationally Exposed to MBOCA

| Reference and study population | Exposure | Outcomes |
|---|---|---|
| **Hosein and Van Roosmalen 1978**<br><br>Case report of occupational accident; one male worker | Routes of exposure: dermal, oral, and inhalation; exposure level high judged by urinary biomarkers.<br><br>Worker was accidently sprayed in face with molten MBOCA. Worker was wearing gloves and safety glasses, but no respiratory or face shield. Some of the compound entered his mouth. | Reported symptoms included burning sensation of skin and eyes, stomach upset, and evidence of transient damage to the renal tubules (increased urinary protein, low specific gravity of urine).<br><br>Note: unclear if the burning sensations were a thermal or chemical effect of exposure to molten MBOCA. |
| **Liu et al. 2005**<br><br>Case report; 52-year-old male worker exposed to MBOCA for 14 years | No exposure levels reported; most likely exposed via dermal and inhalation routes. | Grade 3 invasive transitional cell carcinoma of the bladder. |
| **NIOSH 1986a; Osorio et al. 1990**<br><br>Case report of occupational accident; one male worker | Routes of exposure: dermal and inhalation; exposure level high judged by urinary biomarkers.<br><br>Worker was sprayed over chest, abdomen, and extremities with molten MBOCA. | Worker reported burning sensation of the skin.<br><br>Note: unclear if the burning sensation was a thermal or chemical effect of exposure to molten MBOCA. |
| **Ward et al. 1988**<br><br>Case report; 29-year-old male worker exposed to MBOCA for 9 months | No exposure levels reported; most likely exposed via dermal and inhalation routes. | Grade 1 papillary urothelial neoplasm in the bladder; diagnosed 11 years post-exposure. |
| **Ward et al. 1988**<br><br>Case report; 28-year-old male worker exposed to MBOCA for 1 year | No exposure levels reported; most likely exposed via dermal and inhalation routes. | Grade 1–2 noninvasive papillary transitional cell tumor in the bladder; diagnosed 8 years post-exposure. |

[a]Papanicolaou technique is utilized to evaluated potential urinary tract pathologies in MBOCA-exposed workers. Using this technique, urinary sediment is graded on a scale of I–V, with I and II indicating no evidence of pathology, IV and V indicating unequivocal evidence of abnormal cells in the urinary tract, and III indicating inconclusive results.

CI = confidence interval; MBOCA = 4,4'-methylenebis(2-chloroaniline); SMR = standardized mortality ratio

## Table 2-2. Levels of Significant Exposure to MBOCA – 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** | | | | | | | | | |
| 1 | Mouse (ICR) 6–10 M | 3 months (W) | 0, 50, 100, 200 | BI, HP, LE | Death | | | 200 | 100% mortality |
| | | | | | Gastro | | 50 | | Degeneration and/or dysplasia of the stomach and intestine, swelling and distension of the intestine |
| | | | | | Hepatic | | 50 | | Liver degeneration and/or dysplasia |
| | | | | | Renal | | 50 | | Renal degeneration and/or dysplasia |
| | | | | | Other noncancer | | 50 | | Urinary bladder degeneration and/or dysplasia |
| **Chen et al. 2014** (Note: Histological exam conducted 6 months after final exposure.) | | | | | | | | | |
| **CHRONIC EXPOSURE** | | | | | | | | | |
| 2 | Rat (Wistar) 25 M, 25 F | 500 days (F; low protein diet) | 0, 54 | HP, LE | Death | | | 54 | Decreased survival |
| | | | | | Cancer | | | 54 | CEL: liver hepatocellular carcinoma in 88% of males and 72% of females; primary lung adenomatosis in 32% of males and 12% of females |
| **Grundmann and Steinhoff 1970** | | | | | | | | | |
| 3 | Rat (Sprague-Dawley) 50–100 M | 18 months (F; low-protein diet) | 0, 6.25 12.5, 25 | HE, HP, LE | Death | | | 25 | 23/50 males died after 72 weeks |
| | | | | | Hemato | 25 | | | |
| | | | | | Cancer | | | 6.25 | CEL: 6–26% incidence of lung adenocarcinomas at ≥6.25 mg/kg/day; 5–12% incidence of Zymbal's gland carcinoma at ≥12.5 mg/kg/day; 6% incidence of mammary adenocarcinomas, 18% incidence of hepatocellular carcinomas, and 8% incidence of hemangiosarcomas at 25 mg/kg/day |
| **Kommineni et al. 1979** | | | | | | | | | |

## Table 2-2. Levels of Significant Exposure to MBOCA – 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 |
|---|---|---|---|---|---|---|---|---|---|
| 4 | Rat (Sprague-Dawley) 50–100 M | 18 months (F; standard diet) | 0, 12.5, 25, 50 | HE, HP, LE | Death | | | 25 | Significant increase in mortality |
| | | | | | Hemato | 50 | | | |
| | | | | | Cancer | | | 12.5 | CEL: 23–70% incidence of lung adenocarcinoma tumors at ≥12.5 mg/kg/day; 8–22% incidence of Zymbal's gland carcinoma at ≥12.5 mg/kg/day; 11–28% incidence of mammary adenocarcinomas at ≥25 mg/kg/day; 36% incidence in hepatic carcinomas and 8% incidence of skin hemangiosarcomas at 50 mg/kg/day |
| **Kommineni et al. 1979** | | | | | | | | | |
| 5 | Rat (CD) 25 M | 18 months (F; standard diet) | 0, 25, 50 | BW, HP, LE | Bd Wt | | 25 | | 6–13% decrease in body weight at ≥25 mg/kg/day |
| | | | | | Cancer | | | 25 | CEL: 14–21% incidence of lung adenomatosis at ≥25 mg/kg/day; 20% incidence of hepatoma at 50 mg/kg/day |
| **Russfield et al. 1975** (Note: Histological exam conducted 6 months after final exposure) | | | | | | | | | |
| 6 | Rat (Sprague-Dawley) 50 M, 50 F | 2 years (F; standard diet) | 0, 50 | HP, LE | Death | | | 50 | Decreased survival |
| | | | | | Hepatic | | | 50 | Hepatocytomegaly, fatty change, necrosis, fibrosis, bile duct proliferation |
| | | | | | Cancer | | | 50 | CEL: lung adenocarcinoma in 21/44 males and 27/44 females (0% incidence in controls); lung adenomatosis in 14/44 males and 11/44 females (control incidence 1/44 males, 1/44 females) |
| **Stula et al. 1975** | | | | | | | | | |

## Table 2-2. Levels of Significant Exposure to MBOCA – 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 |
|---|---|---|---|---|---|---|---|---|---|
| 7 | Rat (Sprague-Dawley) 25 M, 25 F | 2 years (F; low-protein diet) | 0, 50 | HP, LE | Death | | | 50 | Decreased survival |
| | | | | | Hepatic | | | 50 | Hepatocytomegaly, fatty change, necrosis, fibrosis, bile duct proliferation |
| | | | | | Cancer | | | 50 | CEL: lung adenocarcinoma in 5/21 males and 6/21 females; lung adenomatosis in 8/21 males and 14/21 females; liver hepatocellular adenomas (5/21) and carcinomas (11/21) in males; mammary adenocarcinomas in 6/21 females (0% control incidence for all tumors except lung adenomatosis [1/21 males, 1/21 females]) |
| **Stula et al. 1975** | | | | | | | | | |
| 8 | Mouse (CD) 25 M, 25 F | 18 months (F; standard diet) | 0, 130, 260 | BW, HP, LE | Death | | | 260 F | Increased early mortality in females |
| | | | | | Bd wt | 260 | | | |
| | | | | | Cancer | | | 130 | CEL: subcutaneous hemangiomas and hemangiosarcomas in 23–40% of males at ≥130 mg/kg/day and 43% of females at 260 mg/kg/day; hepatomas in 43–50% of females at ≥130 mg/kg/day |
| **Russfield et al. 1975** (Note: Histological exam conducted 6 months after final exposure.) | | | | | | | | | |

## Table 2-2. Levels of Significant Exposure to MBOCA – 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 |
|---|---|---|---|---|---|---|---|---|---|
| 9 | Dog (Beagle) 6 F | 9 years 3–5 days/week 1 time/day (C) | 0, 10 | BC, BW, GN, HE, HP, UR | Bd wt | 10 | | | |
| | | | | | Hepatic | | 10[b] | | Increased ALT, nodular hepatic hyperplasia |
| | | | | | Hemato | 10 | | | |
| | | | | | Cancer | | | 10 | CEL: neoplasms of the genitourinary system (3/5 papillary transitional cell carcinoma of the urinary bladder; 1/5 combined transitional cell carcinoma and adenocarcinoma of the urethra) |
| **Stula et al. 1977** | | | | | | | | | |

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive a chronic oral Minimal Risk Level (MRL) of 0.003 mg/kg/day; dose divided by an uncertainty factor of 3,000 (10 for use of a LOAEL, 10 for interspecies extrapolation, 10 for human variability, and 3 for limitations in the database).

ALT = alanine aminotransferase; BC = blood chemistry; Bd wt or BW = body weight; BI = biochemical analysis; (C) = capsule; CEL = cancer effect level; F = female(s); (F) = feed; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; LE = lethality; LOAEL = lowest-observed-adverse=effect level; M = male(s); MBOCA = 4,4'-methylenebis(2-chloroaniline); NOAEL = no-observed-adverse-effect level; UR = urinalysis

**Figure 2-2. Levels of Significant Exposure to MBOCA – Oral**
Intermediate (15-364 days)

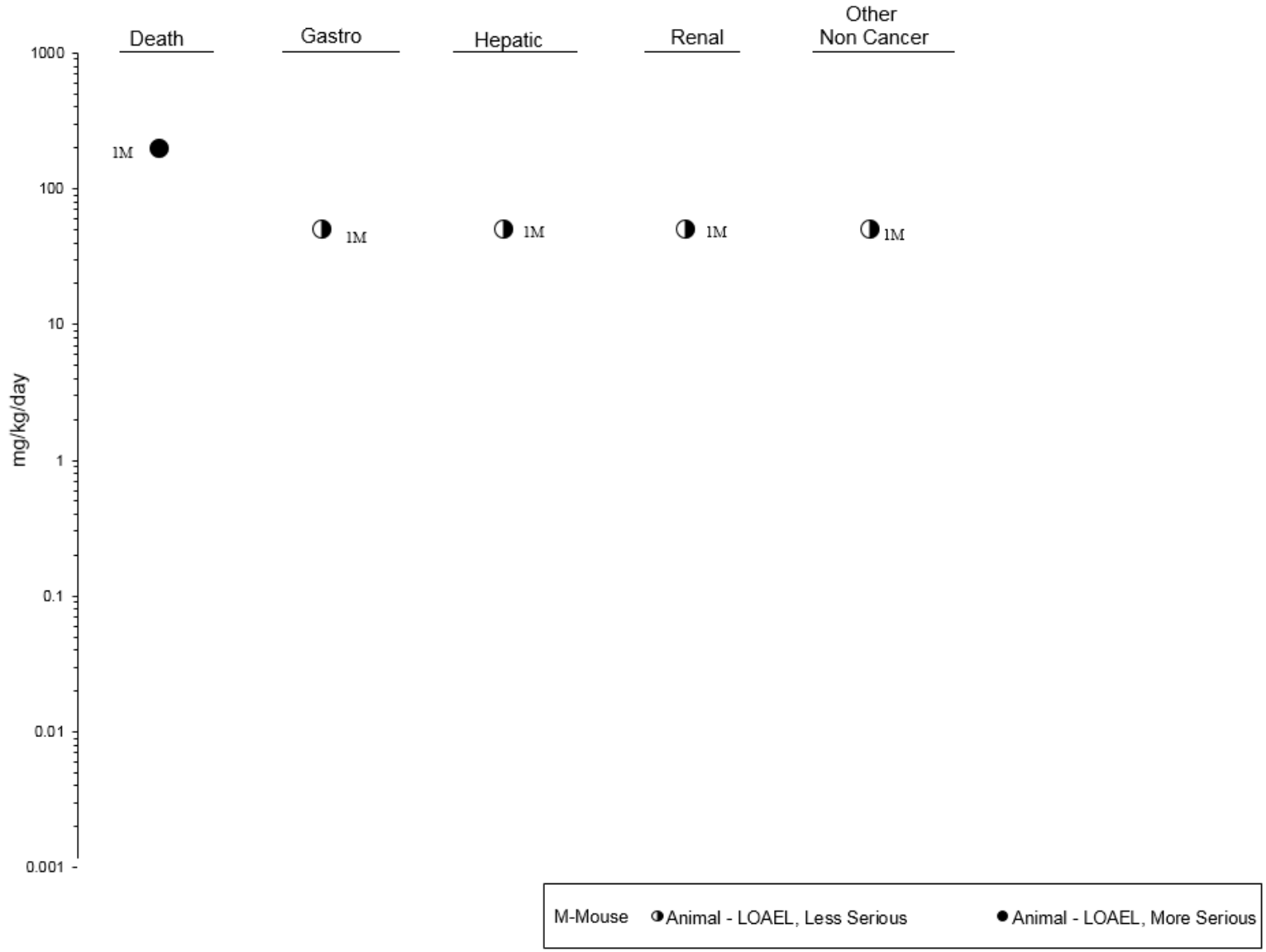


**Figure 2-2. Levels of Significant Exposure to MBOCA – Oral**
Intermediate (15-364 days)

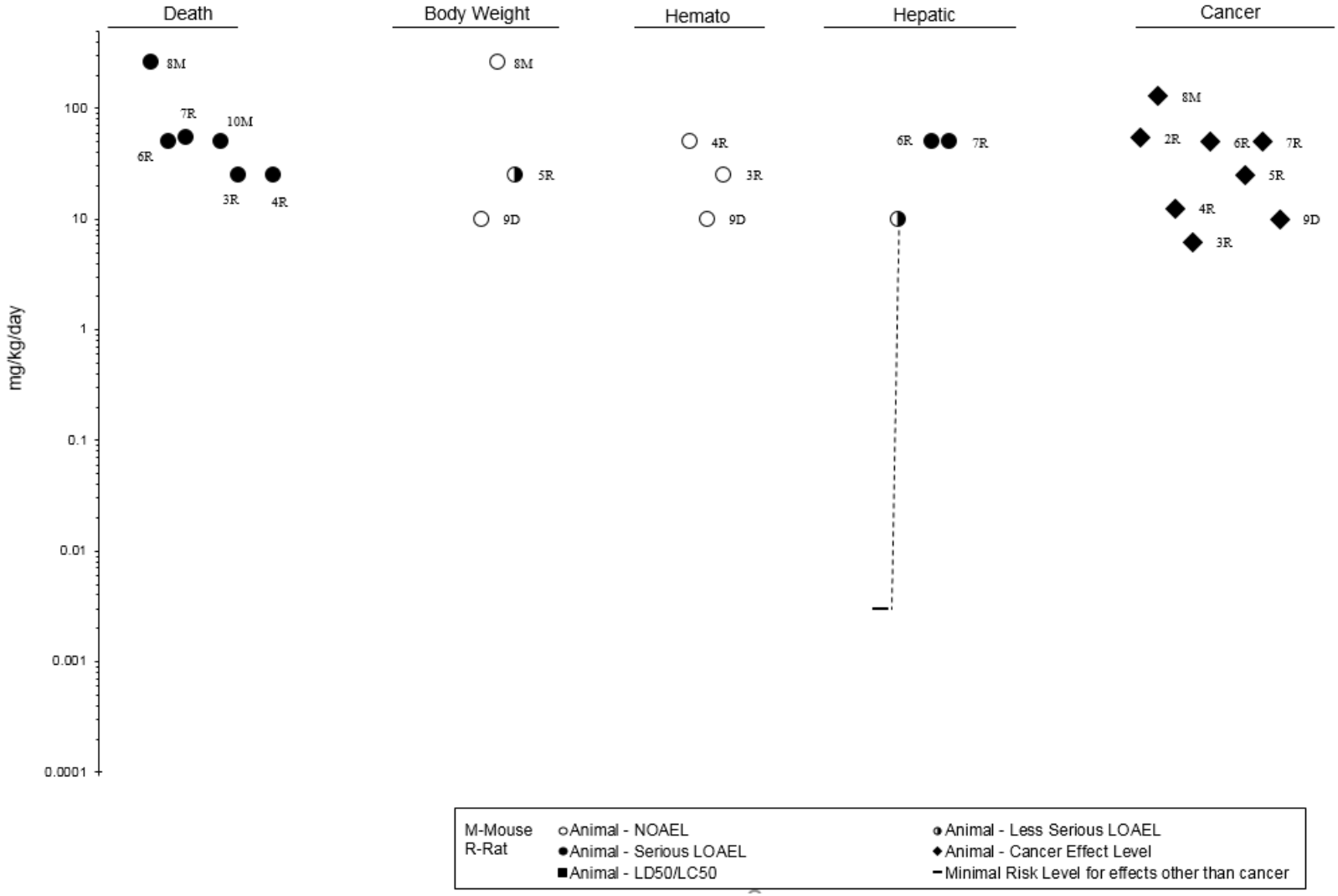


**Table 2-3. Levels of Significant Exposure to MBOCA – Dermal[a]**

| Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effect |
|---|---|---|---|---|---|---|---|---|
| **ACUTE EXPOSURE** | | | | | | | | |
| Mouse (SENCAR) 40 M, 40 F | Once | 0, 0.1, 10, 100, 200 mg | HP | Cancer | | | | MBOCA was not active as a tumor initiator for TPA-induced skin tumors |
| **Nesnow et al. 1985** | | | | | | | | |
| **INTERMEDIATE EXPOSURE** | | | | | | | | |
| Mouse (ICR) 6–10 M | 3 months 1 time/day | 0, 100, 200 mg/kg/day | BI, HP, LE | Gastro | | 100 | | Degeneration and/or dysplasia of the stomach and intestine |
| | | | | Hepatic | | 100 | | Liver degeneration and/or dysplasia |
| | | | | Renal | | 100 | | Renal degeneration and/or dysplasia |
| | | | | Other noncancer | | 100 | | Urinary bladder degeneration and/or dysplasia |
| **Chen et al. 2014** (Note: Histological exam conducted 6 months after final exposure.) | | | | | | | | |

[a]Available dermal studies did not indicate if steps were taken to prevent oral exposure to the compound after dermal application.

BI = biochemical analysis; F = female(s); HP = histopathology; LE = lethality; LOAEL = lowest-observed-adverse=effect level; M = male(s); MBOCA = 4,4'-methylenebis(2-chloroaniline); NOAEL = no-observed-adverse-effect level; TPA = 12-O-tetradecanoylphorbol-13-acetate

## 2.2 DEATH

No studies were located regarding death in humans after exposure to MBOCA.

Decreased lifespan has been noted in rats after chronic oral exposure to MBOCA. Decreased survival was observed in Sprague-Dawley rats at dietary doses ≥25 mg/kg/day using either a standard diet (with 27% protein) or a protein-deficient diet (with 8% protein) containing MBOCA for 18–24 months; lifespans were comparable to control at doses ≤12.5 mg/kg/day (Kommineni et al. 1979; Stula et al. 1975). A similar decrease in lifespan was observed in Wistar rats fed an average dose of 54 mg/kg/day of MBOCA for 500 days in a low-protein diet (Grundmann and Steinhoff 1970). The mean lifespans were 535 days in exposed females and 565 days in males, compared with mean survival time in controls of 730 days (sex not specified). The first death in exposed females occurred on day 200 of treatment and in exposed males on day 390 of treatment. However, no exposure-related changes in survival were noted in Sprague-Dawley rats exposed to dietary levels of MBOCA up to 50 mg/kg/day in a standard diet for up to 2 years (Russfield et al. 1975).

In CD mice, decreased survival was observed in females, but not males, exposed to MBOCA at dietary levels of 260 mg/kg/day in a standard diet for 18 months; no changes in survival were observed in either sex at 130 mg/kg/day (Russfield et al. 1975). However, in a 3-month study with a subsequent 6-month observation period, exposure to 0, 50, 100, or 200 mg/kg/day resulted in 0/6, 1/10, 2/9, and 8/8 deaths, respectively, within 4 months of the final exposure in male ICR mice (females not evaluated) (Chen et al. 2014). In the companion dermal study, topical application of 0, 100, or 200 mg/kg/day for 3 months, followed by a 6-month observation period, only resulted in the death of 1/10 high-dose mice (Chen et al. 2014).

In dogs, one of six female beagle dogs died after 3.4 years of oral administration of an average dose of 10 mg/kg/day of MBOCA (Stula et al. 1977). However, the report concludes that the death was not MBOCA-related, because the dog died from pyelonephritis. The report did not discuss any possible connection between MBOCA administration and pyelonephritis. No additional deaths were reported for the five remaining dogs that were part of the same 9-year study (Stula et al. 1977).

Intraperitoneal injections of 100 or 200 mg/kg/day for 10 days in ICR mice resulted in 100% mortality within 6–18 days (Chen et al. 2014). Dead mice showed visceral organ necrosis. No signs of toxicity or

mortality were observed in 10 Wistar rats following a single subcutaneous injection of 5,000 mg/kg (Grundmann and Steinhoff 1970).

## 2.3 BODY WEIGHT

No studies were located regarding body weight effects in humans after exposure to MBOCA.

In rats fed 25 or 50 mg/kg/day of MBOCA-hydrochloride for 18 months, the average body weight was reportedly 50 g and 100 g lower, respectively, than the body weight of controls at the end of the treatment period (Russfield et al. 1975). The study does not provide the body weights for experimental animals at either dose level; however, average control body weight was reported as 780 g. This indicates a 6–13% decrease in body weight in exposed animals. During the first 20–25 weeks of the experiment, there was no difference in food consumption between MBOCA-treated animals and control animals (Russfield et al. 1975). In the companion mouse study, no exposure-related body weight effects were noted at dietary doses up to 260 mg/kg/day for 18 months (Russfield et al. 1975).

No exposure-related body weight effects were noted in female beagle dogs after 9 years of exposure to MBOCA. Dogs were fed MBOCA at a concentration of 10 mg/kg/day, 3 days/week, for the first 6 weeks of the study and then an average of 10 mg/kg/day, 5 days/week, for the remaining 9 years (Stula et al. 1977).

## 2.4 RESPIRATORY

In an occupational health survey of 31 MBOCA-exposed workers and 31 unexposed referents, the disability rate attributed to respiratory system dysfunction was comparable between exposed and referent groups (Linch et al. 1971). MBOCA workers were exposed to air levels ranging from 0.002 to 002 ppm; dermal exposure was also expected in these workers, but no dermal exposure estimates were not reported. Linch et al. (1971) also did not find an increased incidence of respiratory system dysfunction in a retrospective analysis of 172 former MBOCA-exposed workers compared with all former employees (number not reported; exposure levels not estimated) (Linch et al. 1971).

In ICR mice, exposure to MBOCA via drinking water or daily topical application of doses up to 200 mg/kg/day for 3 months did not result in histopathological lesions in the lungs when mice were evaluated 6 months later (Chen et al. 2014). However, none of the mice were evaluated immediately after

exposure. Due to long recovery period prior to evaluation, this endpoint was not included in the LSE table.

## 2.5 CARDIOVASCULAR

In an occupational health survey, there were no increases in the disability rate attributed to cardiovascular disease in 31 current or 172 former employees exposed to MBOCA via dermal and inhalation exposure (current air levels of 0.002–0.02 ppm), compared with never-exposed referents (Linch et al. 1971).

No studies were located regarding nonneoplastic cardiovascular effects in animals after exposure to MBOCA.

## 2.6 GASTROINTESTINAL

In a case report of accidental occupational exposure, a worker complained of feeling ill in the stomach shortly after ingesting some MBOCA after being accidentally sprayed in the face with molten MBOCA (Hosein and Van Roosmalen 1978). No increases in gastrointestinal system dysfunction were observed in 31 current or 172 former employees exposed to MBOCA via dermal and inhalation, compared with never-exposed referents (Linch et al. 1971). Measured air exposure for current workers ranged from 0.002 to 0.02 ppm; dermal exposure estimates were not reported.

Gastrointestinal effects were reported in male ICR mice following exposure to MBOCA via drinking water or daily dermal application for 3 months (Chen et al. 2014). In the oral study, mice that died following exposure to 50, 100, or 200 mg/kg/day presented with swelling and distention of the intestines. Of the surviving animals exposed orally to 50 or 100 mg/kg/day, degeneration and/or dysplasia was observed in the stomach of 12/16 mice and intestines of 10/16 mice 6 months postexposure (incidence per dose group not reported; n=9 for 50 mg/kg/day, n=7 for 100 mg/kg/day). Similarly, of the surviving animals dermally exposed to 100 or 200 mg/kg/day, degeneration and/or dysplasia was observed in the stomach of 12/18 mice and intestines of 11/18 mice 6 months postexposure (incidence per dose group not reported; n=9 per group). These lesions were not observed in control mice in either study. The study authors did not indicate if steps were taken to prevent oral exposure to the compound after application of MBOCA to the dorsal skin; therefore, oral exposure may have contributed to observed effects in the dermal study.

## 2.7 HEMATOLOGICAL

An occupational health survey found no evidence of increased hematological system dysfunction in 31 current or 172 former employees exposed to MBOCA, compared with unexposed referents (Linch et al. 1971). Expected routes of exposure included inhalation (current air levels of 0.002–0.02 ppm) and dermal (exposure levels not quantified).

Marked methemoglobinemia has been observed in dogs after a single oral dose of MBOCA (no additional data available) (Barnes 1964). In a chronic dog study, no changes in hemoglobin, hematocrit, erythrocyte count, or mononuclear leukocyte count were noted in female beagle dogs after 9 years of exposure to MBOCA. Dogs were fed MBOCA at a concentration of 10 mg/kg/day, 3 days/week, for the first 6 weeks of the study and then an average of 10 mg/kg/day, 5 days/week, for the remaining 9 years (Stula et al. 1977). In rats, mean hemoglobin and hematocrit levels were within normal ranges following exposure to dietary doses up to 50 mg/kg/day in standard or low-protein diets for 18 months (Kommineni et al. 1979).

## 2.8 MUSCULOSKELETAL

Musculoskeletal system disabilities were not associated with MBOCA exposure in an occupational health survey conducted by Linch et al. (1971). This study included 31 current and 172 former employees exposed to MBOCA and never-exposed current and former employee referents. Occupational exposure was via inhalation exposure (current air levels of 0.002–0.02 ppm) and dermal exposure (exposure levels not quantified).

No studies were located regarding musculoskeletal effects in animals after exposure to MBOCA.

## 2.9 HEPATIC

No studies were located regarding hepatic effects in humans after exposure to MBOCA.

Hepatic effects have been reported in male ICR mice following intermediate-duration oral or dermal exposure to MBOCA. Chen et al. (2014) examined liver histology in male ICR mice 6 months after a 3-month exposure to MBOCA via drinking water or dermal application. In the oral study, liver degeneration and/or dysplasia was observed in 13/16 surviving mice exposed to 50 or 100 mg/kg/day (incidence per dose group not reported; n=9 for 50 mg/kg/day, n=7 for 100 mg/kg/day). In the dermal study, liver degeneration and/or dysplasia was observed in 14/18 surviving mice exposed topically to

100 or 200 mg/kg/day (incidence per dose group not reported; n=9 per group). These lesions were not observed in control mice in either study. The study authors did not indicate if steps were taken to prevent oral exposure to the compound after application of MBOCA to the dorsal skin; therefore, oral exposure may have contributed to observed effects in the dermal study.

Evidence of adverse hepatic effects was seen in rats and dogs after chronic oral exposure to MBOCA. Sprague-Dawley rats exposed to MBOCA at dietary levels of 50 mg/kg/day in either standard or low-protein feed showed several nonneoplastic changes in the liver, including hepatomegaly, fatty change, necrosis, fibrosis, and bile duct proliferation (Stula et al. 1975). Similar changes were seen in dogs fed 10 mg/kg/day of MBOCA 3 days/week for the first 6 weeks and then an average of 10 mg/kg/day, 5 days/week, for 9 years (Stula et al. 1977). Histopathology revealed nodular hepatic hyperplasia and disruption of liver architecture in three of six MBOCA-treated dogs but not in controls. Another indication of liver damage was a statistically significant increase in serum alanine aminotransferase (ALT) in MBOCA-treated dogs. The highest levels of ALT occurred during the first 2 years and after 7.5–8 years of treatment (Stula et al. 1977).

## 2.10 RENAL

Evidence for adverse renal effects of MBOCA exposure in humans is limited to a single case study of accidental occupational exposure. Five hours after a worker was accidentally sprayed in the face with molten MBOCA, his urine contained 220 mg/L of protein, indicating damage to the renal tubules (Hosein and Van Roosmalen 1978). However, 11 hours after the accident, there was only a trace of protein in the urine. Two urine specimens collected within 24 hours after the accident had low specific gravities, indicating possible transient damage to the renal tubules and an inability to concentrate urine. The level of exposure was not reported.

Renal effects have been reported in male ICR mice following intermediate-duration oral or dermal exposure to MBOCA. Chen et al. (2014) examined kidney histology in male ICR mice 6 months after a 3-month exposure to MBOCA via drinking water or dermal application. In the oral study, kidney degeneration and/or dysplasia was observed in 9/16 surviving mice exposed to 50 or 100 mg/kg/day (incidence per dose group not reported; n=9 for 50 mg/kg/day, n=7 for 100 mg/kg/day). In the dermal study, kidney degeneration and/or dysplasia was observed in 10/18 surviving mice exposed topically to 100 or 200 mg/kg/day (incidence per dose group not reported; n=9 per group). These lesions were not observed in control mice in either study. The study authors did not indicate if steps were taken to prevent

oral exposure to the compound after application of MBOCA to the dorsal skin; therefore, oral exposure may have contributed to observed effects in the dermal study.

## 2.11 DERMAL

Information on the dermal effects of MBOCA exposure in humans is limited to two case studies reporting a burning sensation after accidental occupational exposure to molten MBOCA (Hosein and Van Roosmalen 1978; NIOSH 1986a; Osorio et al. 1990). It is unclear if the burning sensation was a thermal or chemical effect of exposure to molten MBOCA.

No studies were located regarding dermal effects in animals after exposure to MBOCA.

## 2.12 OCULAR

Information on the ocular effects of MBOCA exposure in humans is limited to a single case study of a worker complaining of burning eyes after direct exposure to molten MBOCA in an occupational accident (Hosein and Van Roosmalen 1978). It is unclear if this was a thermal or chemical effect of exposure to molten MBOCA.

No studies were located regarding ocular effects in animals after exposure to MBOCA.

## 2.13 ENDOCRINE

No studies were located regarding endocrine effects in humans after exposure to MBOCA.

Cystic hyperplasia of the pars intermedia of the anterior pituitary gland was found in one of five female beagle dogs after 8.3 years of treatment with an average dose of 10 mg/kg/day of MBOCA (Stula et al. 1977). This change was not present in other treated dogs or in controls and was not considered to be treatment related.

## 2.14 IMMUNOLOGICAL

No studies were located regarding immunological effects in humans or animals after exposure to MBOCA.

## 2.15 NEUROLOGICAL

In an occupational health survey, there were no increases in the disability rate attributed to central nervous system dysfunction in 31 current or 172 former employees exposed to MBOCA via dermal and inhalation exposure (current air levels of 0.002–0.02 ppm), compared with never-exposed referents (Linch et al. 1971).

No studies were located regarding neurological effects in animals after exposure to MBOCA.

## 2.16 REPRODUCTIVE

No studies were located regarding reproductive effects in humans or animals after exposure to MBOCA.

## 2.17 DEVELOPMENTAL

No studies were located regarding developmental effects in humans or animals after exposure to MBOCA.

## 2.18 OTHER NONCANCER

Histopathological changes in the urinary bladder have been reported in male ICR mice following intermediate-duration oral or dermal exposure to MBOCA. Chen et al. (2014) examined bladder histology in male ICR mice 6 months after a 3-month exposure to MBOCA via drinking water or dermal application. In the oral study, bladder degeneration and/or dysplasia was observed in 9/16 surviving mice exposed to 50 or 100 mg/kg/day (incidence per dose group not reported; n=9 for 50 mg/kg/day, n=7 for 100 mg/kg/day). In the dermal study, bladder degeneration and/or dysplasia was observed in 10/18 surviving mice exposed topically to 100 or 200 mg/kg/day (incidence per dose group not reported; n=9 per group). These lesions were not observed in control mice in either study. The study authors did not indicate if steps were taken to prevent oral exposure to the compound after application of MBOCA to the dorsal skin; therefore, oral exposure may have contributed to observed effects in the dermal study.

## 2.19 CANCER

***Human Studies.*** Bladder cancer has been reported in some epidemiological studies of occupational exposure to MBOCA (see Table 2-1). An occupational cohort study of MBOCA workers reported atypical cytology in 21/385 workers (a potential biomarker for bladder cancer); however, none of the

cytology readings were classified as "suspicious" or suggestive of bladder cancer (Ward et al. 1990). Bladder tumors were found in 3/200 workers previously exposed to MBOCA at unknown levels over a short duration (mean employment 3.2 months); exposure is expected to be via dermal and inhalation routes (Ward et al. 1990). The average lag-time prior to study initiation was 11.5 years. This incidence was not compared with a referent group and potential co-exposures included 4,4'-methylenedianiline, 4-chloro-ortho-toluidine, aniline, and *ortho*-toluidine (Hogan 1993; Ward 1993). Dost et al. (2009) also reported bladder cancer in 2/308 workers previously exposed to MBOCA at unknown levels for at least 1 year; again, the exposure is expected to be via dermal and inhalation routes. The observed incidence rate was 3.3-fold higher than expected based on rates in the general population; however, the increase was nonsignificant. Other cancer incidences in MBOCA-exposed workers were at or below expected incidences (Dost et al. 2009). No evidence of abnormal urine sediment cytology suggestive of urinary tract pathology or increased risk of any type of cancer was reported in 203 current or former workers exposed to MBOCA via dermal and inhalation exposure (current air levels of 0.002–0.02 ppm), compared with never-exposed referents (Linch et al. 1971).

Additional case reports of bladder cancer have been attributed to occupational MBOCA exposure (see Table 2-1), including a grade 1 papillary urothelial neoplasm in the bladder of a 29-year-old man exposed for 9 months (Ward et al. 1988), a grade 1–2 noninvasive papillary transitional cell tumor in the bladder of a 28-year-old man exposed for 1 year (Ward et al. 1988), and a grade 3 invasive transitional cell carcinoma of the bladder in a 52-year-old man exposed for 14 years (Liu et al. 2005). Exposure levels and routes were not available for these case studies; however, the primary routes of exposure are expected to be inhalation and dermal.

***Animal Studies.*** Various tumor types have been associated with chronic oral exposure to MBOCA in laboratory animals, including lung, liver, blood, bladder, and mammary gland tumors.

Dose-dependent increases in lung adenocarcinoma tumors were observed in Sprague-Dawley rats exposed to dietary concentrations ≥12.5 mg/kg/day for 18 months (Kommineni et al. 1979). Stula et al. (1975) also reported significant increases in lung adenocarcinomas, as well as lung adenomatosis (pre-neoplastic or early neoplastic lesion), in Sprague-Dawley rats fed 50 mg/kg/day of MBOCA for 2 years (only dose tested). In a study in CD-1 rats with low animal numbers (<25/dose), lung ademato sis was increased in a dose-related manner after dietary exposure to doses ≥25 mg/kg/day for 18 months, but exposure-related increases in lung adenocarcinoma were not observed (Russfield et al. 1975). All of these studies were done with animals fed standard protein diets. Lung tumors were still observed when

rats were exposed to MBOCA in a protein-deficient diet, but incidence was generally reduced by approximately 50% (Grundmann and Steinhoff 1970; Kommineni et al. 1979; Stula et al. 1975). MBOCA incidence of lung adematosis was comparable between standard and low-protein diet (Stula et al. 1975). These results indicate that, in general, rats given MBOCA in a low-protein diet have a decreased incidence of lung adenocarcinomas when compared to rats given MBOCA in a standard-protein diet. Some exceptions to this generalization occur. Species, strain, and gender may also play a role.

Most chronic rodent studies report liver tumors following dietary exposure to MBOCA. In rat studies with standard diets, a significant increase in hepatic carcinomas was reported in Sprague-Dawley rats exposed to 50 mg/kg/day for 18 months (Kommineni et al. 1979), but not 2 years (Stula et al. 1975), and a nonsignificant increase in hepatomas was reported in Charles River CD rats 6 months after an 18-month exposure to 50 mg/kg/day (Russfield et al. 1975). Use of a low-protein diet increased the incidence of hepatocellular carcinoma in male Sprague-Dawley rats in the 24-month study by Stula et al. (1975) but did not significantly alter incidences in the 18-month study by Kommineni et al. (1979). Another study reported high incidences of liver cancer in Wistar rats exposed to 54 mg/kg/day for 500 days in a low-protein diet; a companion standard diet experiment was not performed in this study (Grundmann and Steinhoff 1970). In mice, hepatomas were significantly increased in random-bred female albino mice 6 months after an 18-month exposure to ≥130 mg/kg/day, but not in male mice up to 260 mg/kg/day (Russfield et al. 1975). Collectively, these results indicate that species, strain, sex, and diet may play a role. Furthermore, the possible contrasting effects of a protein-deficient diet on MBOCA-induced lung and liver tumors suggests different induction mechanisms for the formation of these two tumors by MBOCA (Kommineni et al. 1979).

Another target organ for MBOCA carcinogenesis is the urinary bladder. Six female beagle dogs were fed an average of 10 mg/kg/day of MBOCA for 9 years. Of the five surviving dogs at scheduled sacrifice, three developed papillary transitional cell carcinomas of the urinary bladder, and one dog had a combined urethral adenocarcinoma and transitional cell carcinoma (Stula et al. 1977). Consistent with these findings, exposed dogs showed abnormal cytology in urine sediment after 8–9 years of treatment, which is suggestive of urinary tract pathology. Despite the small number of animals used, this study demonstrates that ingestion of MBOCA over 9 years was associated with the appearance of carcinomas of the urinary bladder and urethra in dogs.

Other tumor types were also found less consistently after chronic oral administration of MBOCA. Malignant mammary tumors were significantly increased in female Sprague-Dawley rats fed 50 mg/kg/day of MBOCA in a low-protein diet for 2 years, but not a standard diet (Stula et al. 1975). Zymbal's gland carcinomas were increased in male Sprague-Dawley rats exposed to ≥12.5 mg/kg/day for 18 months in either a standard or low-protein diet (Kommineni et al. 1979). Low incidences of mammary tumors and hemangiosarcomas were also observed in these rats at ≥25 mg/kg/day (Kommineni et al. 1979). In another study, vascular tumors (generally subcutaneous hemangiomas and hemangiosarcomas) were observed in randomly bred albino mice 6 months after an 18-month exposure to dietary concentrations ≥130 mg/kg/day in males and 260 mg/kg/day in females (Russfield et al. 1975).

No skin papillomas were observed in SENCAR mice dermally exposed once to MBOCA at a dose up to 200 mg following by biweekly exposures to 2 µg of the tumor promotor 12-o-tetradecanoylphorbol-13-acetate (TPA) for 26 weeks (Nesnow et al. 1985). Several methods were inadequately reported in this study, including whether or not TPA was administered to control animals, if MBOCA was applied to shaved skin, or if was the area was protected after treatment.

***Mechanisms of Carcinogenicity.*** MBOCA is was initially suspected of being a human carcinogen because its chemical structure is similar to that of a known human bladder carcinogen, benzidine, and to that of a potent animal carcinogen, 3,3'-dichloro-benzidine (Osorio et al. 1990). The precise mechanism of action of MBOCA is not completely understood. However, strong evidence of genotoxicity (see Section 2.20 Genotoxicity) suggests that the carcinogenicity of MBOCA is mediated via a genotoxic MOA similar to other well-known cancer-causing aromatic amines (IARC 2012).

In support of a genotoxic MOA, MBOCA has been shown to be electrophilically reactive, forming adducts with hemoglobin (Cheever et al. 1988, 1990, 1991; Chen et al. 1991; Sabbioni and Neumann 1990), tissue DNA (Cheever et al. 1990; Segerback and Kadlubar 1992; Silk et al. 1989), and globin and serum albumin (Cheever et al. 1991). These findings are consisted with the proposed mechanism for chemical carcinogenesis involving the formation of chemical adducts in DNA through covalent binding. In support, MBOCA produces stable DNA adducts in rat liver at levels characteristic of genotoxic carcinogens (Cheever et al. 1990; Kugler-Steigmeier et al. 1989; Segerback and Kadlubar 1992; Silk et al. 1989), and the liver is one of the primary cancer targets in rats exposed to MBOCA (Kommineni et al. 1979; Russfield et al. 1973).

The N-hydroxylation/N-oxidation metabolic pathway for MBOCA, is considered to be an activation step related to adduct formation (Morton et al. 1988). Studies in isolated rat hemoglobin confirmed that two MBOCA metabolites, N-hydroxy MBOCA and mononitroso-MBOCA, formed measurable amounts of adducts with both human and rat hemoglobin, while the amount of adducts formed by the parent compound itself was very small (Chen et al. 1991). Adducts identified in rat liver and kidney DNA following intraperitoneal or oral exposure include N-(deoxyadenosine-8-yl)-4-amino-3-chlorobenzyl alcohol and N-(deoxyadenosine-S-yl)-4-amino-3-chlorotoluene (Segerback and Kadlubar 1992; Silk et al. 1989). The precise mechanism of single-ring MBOCA DNA adduct formation is still not completely understood because of the indication that there is an unstable intermediate formed prior to the formation of the two major identified DNA adducts and that single-ring MBOCA adducts were not readily detectable (Segerback and Kadlubar 1992).

Limited evidence for metabolic formation of N,N'-diacetyl MBOCA in workers exposed to MBOCA (Ducos and Gaudin 1983) suggests that N-hydoxy-N,N'-diacetyl MBOCA can be formed, using benzidine metabolism as a model. N-Hydoxy-N,N'-diacetyl MBOCA is important because it can directly bind to nucleic acids. If the existence of N-hydoxy-N,N'-diacetyl MBOCA is confirmed, it would provide a plausible biochemical basis for adduct formation following MBOCA exposure.

There is some evidence that MBOCA itself may be a tumor promoter. MBOCA caused a statistically significant dose-dependent inhibition of gap-junctional cell communication (GJC) at noncytotoxic doses (Kuslikis et al. 1991). Since GJC is important in controlling cell proliferation, and many known tumor promoters inhibit GJC, GJC inhibition assays have been proposed as short-term screens for promoters.

## 2.20 GENOTOXICITY

***Overview.*** The genotoxicity of MBOCA has been extensively evaluated in *in vivo* and *in vitro* systems; see Tables 2-4 and 2-5, respectively. Evidence indicates that MBOCA and/or its metabolites are mutagenic, directly interact with DNA to form adducts and cause DNA damage, and induce cell transformation. There is limited evidence that it is also clastogenic.

**Table 2-4. Genotoxicity of MBOCA *In Vitro***

| Species (test system) | Endpoint | Results | | Reference |
|---|---|---|---|---|
| | | Activation | | |
| | | With | Without | |
| **Prokaryotic organisms** | | | | |
| *Salmonella typhimurium* strains TA98, TA100 | Gene mutation | + | – | Baker and Bonin 1981 |
| *S. typhimurium* strains TA98, TA100 | Gene mutation | + | – | Brooks and Dean 1981 |
| *S. typhimurium* strain TA100[a] | Gene mutation | + | ND | Cocker et al. 1986 |
| *S. typhimurium* strain TA100[a] | Gene mutation | + | – | Cocker et al. 1985 |
| *S. typhimurium* strain TA98, TA100 | Gene mutation | + | – | Dunkel et al. 1984 |
| *S. typhimurium* strain TA98, TA100 | Gene mutation | + | – | Garner et al. 1981 |
| *S. typhimurium* strain TA1538 | Gene mutation | + | – | Gatehouse 1981 |
| *S. typhimurium* strain TA100 | Gene mutation | + | ND | Hesbert et al. 1985 |
| *S. typhimurium* strain TA100 | Gene mutation | + | – | Hubbard et al. 1981 |
| *S. typhimurium* strain TA100 | Gene mutation | + | ND | Ichinotsubo et al. 1981a |
| *S. typhimurium* strain TA100 | Gene mutation | + | ND | Kugler-Steigmeir et al. 1989 |
| *S. typhimurium* strains TA98, TA100 | Gene mutation | + | – | MacDonald et al. 1981 |
| *S. typhimurium* strains TA98, TA100 | Gene mutation | + | – | Martire et al. 1981 |
| *S. typhimurium* strain TA100 | Gene mutation | + | – | McCann et al. 1975 |
| *S. typhimurium* strain TA100 | Gene mutation | + | – | Messerly et al. 1987 |
| *S. typhimurium* strains TA98, TA100 | Gene mutation | + | – | Nagao and Takahashi 1981 |
| *S. typhimurium* strain TA98 | Gene mutation | + | ND | Rao et al. 1982 |
| *S. typhimurium* strain TA100 | Gene mutation | + | – | Rowland and Severn 1981 |
| *S. typhimurium* strains TA98, TA100 | Gene mutation | + | – | Simmon and Shepherd 1981 |
| *S. typhimurium* strain TM677 (contains pKM101; 8-azaguanine$^{r}$) | Gene mutation | + | ND | Skopek et al. 1981 |
| *S. typhimurium* strains TA1535, TA98, TA100 | Gene mutation | + | ND | Trueman 1981 |
| *S. typhimurium* strains TA98, TA100 | Gene mutation | + | – | Venitt and Crofton-Sleigh 1981 |

**Table 2-4. Genotoxicity of MBOCA *In Vitro***

| Species (test system) | Endpoint | Results: Activation With | Results: Activation Without | Reference |
|---|---|---|---|---|
| *Escherichia coli* strain WP2 uvrA | Gene mutation | – | – | Gatehouse 1981 |
| *E. coli* strain WP2 uvrA/pKM101 | Gene mutation | + | – | Matsushima et al. 1981 |
| *E. coli* strains WP2/pkM1010 and WP2 uvrA/pKM101 | Gene mutation | + | – | Venitt and Crofton-Sleigh 1981 |
| *E. coli* strain 58-161 envA (lambda lysogen) | DNA repair (SOS induction) | + | ND | Thomson 1981 |
| *E. coli* strain JC2921 (recA$^-$), JC5519 (recBC$^-$) | DNA repair (rec$^-$ assay) | + | ND | Ichinotsubo et al. 1981b |
| *E. coli* strain P3478 (polA$^-$) /W3110 (polA$^+$) | DNA repair (differential killing in deficient strains) | – | + | Rosenkranz et al. 1981 |
| *E. coli* strain WP67, CN871 | DNA repair (differential killing in deficient strains) | – | + | Tweats 1981 |
| *Bacillus subtilis* rec$^-$ | DNA repair (rec$^-$ assay) | + | + | Kada 1981 |
| **Eukaryotic organisms, non-mammalian** | | | | |
| *Saccharomyces cerevisiae* XV185-14C | Gene mutation | – | – | Mehta and Von Borstel 1981 |
| *S. cerevisiae* D4 | Mitotic gene conversion | – | – | Jagannath et al. 1981 |
| *S. cerevisiae* JD1 | Mitotic gene conversion | + | + | Sharp and Parry 1981 |
| *S. cerevisiae* XII | Mitotic recombination | – | – | Kassinova et al. 1981 |
| *S. cerevisiae* D6 | Mitotic aneuploidy | + | + | Parry and Sharp 1981 |
| **Mammalian cells** | | | | |
| Mouse lymphoma (L5178Y TK +/–) cells | Forward gene mutation | + | – | Caspary et al. 1988 |
| Mouse lymphoma (L5178Y TK +/–) cells | Forward gene mutation | + | – | Myhr and Caspary 1988 |
| CHO cells | Chromosomal aberrations | – | – | Galloway et al. 1985 |
| CHO cells | Sister chromatid exchange | +/– | +/– | Galloway et al. 1985 |
| CHO cells | Sister chromatid exchange | – | – | Perry and Thomson 1981 |
| Human HeLa cells | Unscheduled DNA synthesis | + | – | Martin and McDermid 1981 |
| Rat primary hepatocytes | Unscheduled DNA synthesis | ND | + | McQueen et al. 1981 |

**Table 2-4. Genotoxicity of MBOCA *In Vitro***

| Species (test system) | Endpoint | Results | | Reference |
|---|---|---|---|---|
| | | Activation | | |
| | | With | Without | |
| Rat primary hepatocytes | Unscheduled DNA synthesis | ND | + | Mori et al. 1988 |
| Rat primary hepatocytes | Unscheduled DNA synthesis | ND | + | Williams et al. 1982 |
| Mouse primary hepatocytes | Unscheduled DNA synthesis | ND | + | McQueen et al. 1981 |
| Hamster primary hepatocytes | Unscheduled DNA synthesis | ND | + | McQueen et al. 1981 |
| Rabbit primary hepatocytes | Unscheduled DNA synthesis | ND | + | McQueen et al. 1983 |
| Human embryonic lung cells | Single strand DNA breaks | ND | + | Casto 1983 |
| Human primary lung cells | Single strand DNA breaks | ND | + | Robbiano et al. 2006 |
| Rat primary lung cells | Single strand DNA breaks | ND | + | Robbiano et al. 2006 |
| Primary hamster embryo cells | Single strand DNA breaks | ND | + | Casto 1983 |
| Human uroepithelial cells | DNA adduct formation | ND | + | DeBord et al. 1996 |
| Human bladder explant culture | DNA adduct formation | ND | + | Stoner et al. 1988 |
| Dog bladder explant culture | DNA adduct formation | ND | + | Stoner et al. 1988 |
| RLV-infected rat embryo ($2FR_4$50) | Cellular transformation | ND | + | Dunkel et al. 1981 |
| RLV-infected rat embryo ($2FR_4$50) | Cellular transformation | ND | + | Traul et al. 1981 |
| Balb/3T3 mouse cells | Cellular transformation | ND | + | Dunkel et al. 1981 |
| Baby hamster kidney (BHL21 V13) cells | Cellular transformation | + | + | Daniel and Dehnel 1981 |
| Baby hamster kidney (BHL21) cells | Cellular transformation | + | ND | Styles 1981 |

[a]Strains listed are those in which there was a positive response; not all strains were tested in each assay.

– = negative result; + = positive result; +/– = inconclusive results; 8-azaguanine$^r$ = 8-azaguanine resistance; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; MLV = Moloney mouse sarcoma-leukemia virus; ND = no data; RLV = Rauscher leukemia virus; SOS induction = induction of an error-prone repair system; TK = thymidine kinase

**Table 2-5. Genotoxicity of MBOCA *In Vivo***

| Species (exposure route) | Endpoint | Results | Reference |
|---|---|---|---|
| **Mammals** | | | |
| Human (occupational, multiple) | Sister chromatid exchange in peripheral lymphocytes | + | Edwards and Priestly 1992 |
| Rat (Wistar; i.p.) | Sister chromatid exchange in peripheral lymphocytes | + | Edwards and Priestly 1992 |
| Human (occupational, multiple) | Micronuclei in exfoliated urothelial cells | + | Murray and Edwards 1999, 2005 |
| Human (occupational, multiple) | Micronuclei in peripheral lymphocytes | + | Murray and Edwards 1999, 2005 |
| Human (occupational, multiple) | Micronuclei in peripheral lymphocytes | + | Wang et al. 2017 |
| Rat (Sprague-Dawley, i.p.) | Micronuclei in bone marrow | – | Wakata et al. 1998 |
| Rat (Sprague-Dawley, i.p.) | Micronuclei in peripheral lymphocytes | – | Wakata et al. 1998 |
| Mouse ($B6C3F_1$/BR; i.p.) | Micronuclei in bone marrow | + | Katz et al. 1981 |
| Mouse (CD-1; i.p.) | Micronuclei in erythrocytes | - | Tsuchimoto and Matter 1981 |
| Mouse ($B6C3F_1$; i.p.) | Micronuclei in bone marrow | + | Salamone et al. 1981 |
| Human (occupational, multiple) | Oxidative DNA damage (plasma 8-OHdG levels) | – | Chen et al. 2007 |
| Human (occupational, multiple) | Oxidative DNA damage (plasma 8-OHdG levels) | – | Lin et al. 2013 |
| Rat (Sprague-Dawley; oral) | Single strand DNA breaks in lung and liver | + | Robbiano et al. 2006 |
| Rat (Sprague-Dawley; oral) | Single strand DNA breaks in kidney | – | Robbiano et al. 2006 |
| Human (occupational, multiple) | DNA adduct formation in exfoliated urothelial cells | + | Kaderlik et al. 1993 |
| Rat (Sprague-Dawley; oral) | DNA adduct formation in liver, bladder, and lymphocytes | + | Cheever et al. 1990 |
| Rat (Sprague-Dawley; oral) | DNA adduct formation in liver | + | DeBord et al. 1996 |
| Rat (Sprague-Dawley; oral) | DNA adduct formation in liver and lung | + | Kugler-Steigmeir et al. 1989 |
| Rat (Sprague-Dawley; oral) | DNA adduct formation in liver, lung, and kidney | + | Segerback and Kadlubar 1992 |
| Rat (Sprague-Dawley; dermal) | DNA adduct formation in liver, bladder, and lymphocytes | + | Cheever et al. 1990 |
| Rat (Sprague-Dawley; i.p.) | DNA adduct formation in liver | + | DeBord et al. 1996 |
| Rat (Wistar; i.p.) | DNA adduct formation in liver | + | Silk et al. 1989 |

**Table 2-5. Genotoxicity of MBOCA *In Vivo***

| Species (exposure route) | Endpoint | Results | Reference |
|---|---|---|---|
| **Nonmammalian organisms** | | | |
| *Drosophila melanogaster* (oral, dermal) | Wing-spot test (somatic mutation and recombination) | + | Kugler-Steigmeier et al. 1989 |
| *D. melanogaster* (inhalation) | Sex-linked recessive lethal mutation | + | Donner et al. 1983 |
| *D. melanogaster* (oral, dermal) | Sex-linked recessive lethal mutation | + | Vogel et al. 1981 |

+ = positive results; – = negative results; DNA = deoxyribonucleic acid; i.p. = intraperitoneal

***Mutagenicity.*** *In vitro* testing has provided clear and convincing evidence that MBOCA is mutagenic in the *Salmonella typhimurium* mutagenesis assay, and that the mutagenic effect requires exogenous metabolic activation (Baker and Bonin 1981; Cocker et al. 1985; Dunkel et al. 1984; Garner et al. 1981; Gatehouse 1981; Hesbert et al. 1985; Hubbard et al. 1981; Ichinotsubo et al. 1981a; Kugler-Steigmeier et al. 1989; MacDonald et al. 1981; Martire et al. 1981; McCann et al. 1975; Messerly et al. 1987; Nagao and Takahashi 1981; Rao et al. 1982; Rowland and Severn 1981; Simmon and Shepherd 1981; Skopek et al. 1981; Trueman 1981; Venitt and Crofton-Sleigh 1981). Although not all investigators used each tester strain, the general result is that MBOCA is mutagenic only in strains TA98, TA100, and TM677 at 250 μg/plate, with some inconsistency regarding strain TA98. MBOCA and its metabolites are not mutagenic in *S. typhimurium* strains TA1535, TA1537, or TA1538. This suggests that the mutagenic effect of MBOCA metabolites in some bacteria is dependent on the plasmid pKM101; strains TA98, TA100, and TM677 contain this plasmid, but strains TA1535, TA1537, and TA1538 do not (Ames et al. 1975; Skopek et al. 1981). This hypothesis is supported by the finding that S9-activated MBOCA is mutagenic in *Escherichia coli* strain WP2uvrA only in the presence of the plasmid pKM101 (Gatehouse 1981; Matsushima et al. 1981;Venitt and Crofton-Sleigh 1981). The plasmid carries genes involved in an "error-prone" DNA repair system that introduces mutations as it removes DNA damage (Walker 1984). Gene mutations observed in mouse lymphoma cells cultured with MBOCA also required exogenous metabolic activation (Caspary et al. 1988; Myhr and Caspary 1988). Gene mutations were not induced in *Saccharomyces cerevisiae* with or without metabolic activation (Mehta and Von Borstel 1981).

*In vivo* animal studies also provide direct and indirect evidence that MBOCA is a mutagen; MBOCA metabolites were bound to DNA following oral (Cheever et al. 1990; Kugler-Steigmeier et al. 1989) or dermal (Cheever et al. 1990) exposure in rats. Additionally, small increases in the sex-linked recessive lethal mutations were observed in *Drosophila melanogaster* adults following inhalation, oral, or dermal

exposure to MBOCA (Donner et al. 1983; Vogel et al. 1981). MBOCA also induced somatic mutations and recombination in the *D. melanogaster* wing spot assay (Kugler-Steigmeier et al. 1989).

Most of MBOCA's mutagenic activity appears to be due to the N-hydroxy metabolite, which caused dose-dependent increases in mutations of *S. typhimurium* strains TA100 and TA98 in a pre-incubation assay using nonactivated doses ≥5 μg/plate (Kuslikis et al. 1991) and HPRT gene mutations in human AHH-1 lymphoblastoid cells (Reid et al. 1998). This metabolite is produced by several species, including dogs and humans (Butler et al. 1989; Chen et al. 1989; Morton et al. 1988). The mononitroso derivative appears to be direct-acting mutagen, but is much less potent, causing a statistically significant revertant increase in the pre-incubation assay at the highest tested nontoxic dose (50 μg/plate). Neither the o-hydroxy nor the dinitroso derivatives were direct-acting mutagens at up to 50 or 500 μg/plate, respectively; neither chemical was tested to cytotoxic levels (Kuslikis et al. 1991). N-Acetylation is considered a deactivating step, and neither n-acetyl nor N,N-diacetyl derivatives were mutagenic in *S. typhimurium* in the absence of activation (Hesbert et al. 1985). In the presence of metabolic activation, the mutagenic activity of the acetylated derivatives is less than that of the parent compound (Cocker et al. 1986; Hesbert et al. 1985).

***Clastogenicity.*** There is limited evidence of clastogenicity in mammalian cells following *in vitro* exposure to MBOCA with or without metabolic activation. MBOCA did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cells and only weakly induced sister chromatid exchanges (SCE) in CHO cells in 1/2 assays (Galloway et al. 1985; Perry and Thomson 1981). Findings in rodents following *in vivo* exposure are also inconsistent, with micronuclei induction in mouse bone marrow following intraperitoneal injections of MBOCA, but not in mouse erythrocytes or rat bone marrow or lymphocytes (Katz et al. 1981; Tsuchimoto and Matter 1981; Salamone et al. 1981; Wakata et al. 1998). In contrast, increased SCE and micronuclei have been reported in peripheral lymphocytes and exfoliated urothelial cells of humans occupationally exposed to MBOCA (Edwards and Preistly 1992; Murray and Edwards 1999, 2005; Wang et al. 2017). These effects are expected to be mediated via metabolites, as increased micronuclei were observed in workers with *CYP3A4* polymorphisms A/A and A/G (increased activity), compared with *CYP3A4* G/G (decreased activity); CYP3A4 is implicated in the N-hydroxylation and N-oxidation of MBOCA (Wang et al. 2017).

In *Saccharomyces cerevisiae,* MBOCA induced mitotic gene conversion in the JD1 strain, but not the D4 strain, both with and without metabolic activation (Jagannath et al. 1981; Sharp and Parry 1981). It did

not induce mitotic recombination in *S. cerevisiae* XII (Kassinova et al. 1981), but mitotic aneuploidy was observed both with and without metabolic activity in *S. cerevisiae* D6 (Parry and Sharp 1981).

***Interaction with DNA.*** There is strong and consistent evidence that MBOCA metabolites bind directly to DNA, forming adducts. DNA adducts have been detected in exfoliated urothelial cells of MBOCA workers (Kaderlik et al. 1993) as well as human uroepithelial cells and bladder explant cultures exposed to MBOCA *in vitro* (DeBord et al. 1996; Stoner et al. 1988). DNA adducts in bladder tissue have also been observed in rats following oral or dermal exposure to MBOCA (Cheever et al. 1990) and in dog bladder explant cultures following *in vitro* exposure (Stoner et al. 1988). DNA adduct formation in human and dog bladder tissue is of particular note, since MBOCA is suspected of causing bladder cancer in humans and has been found to cause bladder tumors in dogs (see Section 2.19 Cancer for more details). In the study by Stoner et al. (1988), the level of binding increased with dose, but the increase was not linear. Considerable individual variation in binding levels, varying over at least a 10-fold range, was found in both dogs and humans. At least six adducts were found in dog bladder epithelium; four adducts were found in human bladder epithelium, three of which appeared to be the same as those found in dogs. DNA adducts have also been observed in the liver, lung, kidney, and lymphocytes of rats following oral, dermal, or intraperitoneal exposure (Cheever et al. 1990; DeBord et al. 1996; Kugler-Stegmeier et al. 1989; Segerback and Kadlubar 1992; Silk et al. 1989). The N-hydroxy and N-acetoxy metabolites have also been shown to form DNA adducts when cultured with isolated rat DNA (Segerback and Kadlubar 1992). The *in vivo* and *in vitro* studies in rats identified two other major MBOCA adducts, N-(deoxyadenosin-8-yl)-4-amino-3-chlorobenzyl alcohol and N-(deoxyadenosin-8-yl)-4-amino-3-chlorotoluene (Segerback and Kadlubar 1992). As with adduct formation in the bladder, DNA adduct formation in the liver and lung are of particular note due to induction of lung and liver tumors in rodents following chronic oral exposure to MBOCA (see Section 2.19 Cancer for more details).

DNA damage has been consistently observed in both *in vitro* and *in vivo* studies. DNA damage (single-strand breaks) were observed in human embryonic and primary lung cells, rat primary lung cells, and hamster embryo cells following exposure to MBOCA (Casto 1983; Robbiano et al. 2006). Unscheduled DNA synthesis was also observed in rat, mouse, hamster, and rabbit primary hepatocytes exposed to MBOCA (McQueen et al. 1981, 1983; Mori et al. 1988; Williams et al. 1982). Sensitivity to MBOCA showed species-specific susceptibility: rat > mouse > hamster > rabbit (McQueen et al. 1981, 1983). Metabolic activation was not used with primary cells because they are metabolically competent. Unscheduled DNA synthesis was also observed in human HeLa cells with metabolic activation (Martin and McDermid 1981). *In vivo*, single-strand DNA breaks were observed in the lung and liver, but not

kidney, of Sprague-Dawley rats following a single oral exposure to MBOCA of 570 mg/kg (Robbiano et al. 2006). DNA repair mechanisms have also been induced both with and without metabolic activation in *E. coli* and *Bacillus subtilis* assays (Ichniotsubo et al. 1981b; Kada et al. 1981; Rosenkranz et al. 1981; Thomson 1981; Tweats 1981). Observed DNA damage appears to be due to direct interaction with DNA, and there is no evidence for oxidative DNA damage in MBOCA workers (based on plasma 8-OHdG levels) (Chen et al. 2007; Lin et al. 2013).

***Cell Transformation.*** MBOCA induced cell transformation in RLV-infected rat embryos, Balb/3T3 mouse cells, and baby hamster kidney cells without metabolic activation and baby hamster kidney cells with metabolic activation (other cells not tested with metabolic activation) (Daniel and Dehnel 1981; Dunkel et al. 1981; Styles 1981; Traul et al. 1981).

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

## 3.1 TOXICOKINETICS

Toxicokinetic data are available from both human and animal studies. These data are summarized below.

- Evidence indicates that MBOCA can be absorbed following inhalation, oral, or dermal exposure. However, the extent and rate of absorption have not been determined.
- MBOCA appears to be widely distributed throughout the body following oral or dermal exposure, with the highest concentration in the liver. No data are available following inhalation exposure.
- MBOCA metabolism can proceed via several pathways: N-acetylation, N-hydroxylation, which may be followed by n-oxidation, and ring hydroxylation. Some of these processes may be followed by conjugation.
- MBOCA is excreted in the urine in humans after occupational exposure (assumed to be dermal and inhalation exposure); however, data on the kinetics of excretion are conflicting. It is unknown if other excretion routes exist in humans (no data). In rats, 60% of an oral dose was excreted in the feces.

### 3.1.1 Absorption

No studies were located that directly assessed absorption of MBOCA following exposure in humans via any exposure route. However, absorption was indirectly estimated in five male factory workers by measuring urinary MBOCA levels over a 5-day period (Ichikawa et al. 1990). Personal air exposure levels were obtained by continuously monitoring the breathing zone of each worker for 6–7 hours every other day. The air MBOCA levels ranged from 0.0002 to 0.0089 mg/m$^3$. The amount of MBOCA measured in the urine was much higher than the amounts of inhaled MBOCA as estimated from personal exposure measurements. This observation suggests that a certain amount of MBOCA exposure occurs in the workplace by some additional route(s), potentially via dermal absorption. Detectible MBOCA levels in the urine have also been reported in other studies of workers following acute or long-term exposure (Clapp et al. 1991; Cocker et al. 1988; Ducos and Gaudin 1983; Edwards and Priestly 1992; Hosein and Van Roosmalen 1978; Keen et al. 2012; NIOSH 1986a, 1986b; Osorio et al. 1990; Robert et al. 1999b; Thomas and Wilson 1984), supporting absorption of MBOCA following occupational exposure.

No studies were located regarding absorption in animals after inhalation exposure to MBOCA.

Several studies in animals following oral MBOCA exposure detected MBOCA or its metabolites in body tissues, urine, and feces (Cheever et al. 1988; Farmer et al. 1981; Groth et al. 1984). When rats were given a single oral dose of $^{14}$C-MBOCA by gavage, 16.5% of the dose was excreted in the urine within 72 hours, while 13.7% was retained in the tissues (Groth et al. 1984). These data indicate that some MBOCA is absorbed after oral exposure in animals. Approximately 60% remained unabsorbed in the feces (Groth et al. 1984).

Differential absorption rates of $^{14}$C-MBOCA were investigated following dermal and intravenous exposures in beagle dogs (Manis et al. 1984). By comparing the levels of MBOCA excreted via the urinary and biliary systems, following percutaneous and intravenous (100% absorption) administration, the investigators calculated that, after 24 hours, only 2.4% of the applied MBOCA was absorbed through the skin. In another study in rats, 11.5–21.9% of MBOCA applied to the skin was calculated to have been absorbed within 72 hours of application (Groth et al. 1984).

Chin et al. (1983) indicate that MBOCA is rapidly absorbed through human skin *in vitro*. Using radiolabeled MBOCA and fresh human neonatal foreskin organ cultures, the absorption and penetration of MBOCA through a 7x7 mm area was evaluated over 4 hours. One hour after application, 46% of the radiolabeled MBOCA was detected on the skin and 0.5% was detected on the underlying membrane (the remaining radioactivity was unabsorbed and stayed on the coverglass). Four hours later, 61% was detected in the skin, 26% had passed through and was on the underlying membrane filter, and 12% remained unabsorbed. The absorption process was optimal at 37°C and decreased sharply at 0°C. These findings indicate that MBOCA penetrates the neonatal foreskin readily without being metabolized, and that the absorption process is temperature dependent (Chin et al. 1983). However, using full-thickness human and rat skin sections, Hotchkiss et al. (1993) showed poor absorption of MBOCA. In human breast skin preparations, only 2.4 and 5.9% of an applied dose was absorbed within 72 hours under unoccluded and occluded conditions, respectively. In rat skin samples, only 1.3 and 1.8% of an applied dose was absorbed within 72 hours under unoccluded and occluded conditions, respectively (Hotchkiss et al. 1993).

### 3.1.2 Distribution

No studies were located regarding distribution in humans after exposure to MBOCA via any route. No studies were located regarding distribution in animals after inhalation exposure to MBOCA.

In oral studies, the liver showed the highest level of radioactivity in rats following acute exposure to radiolabeled MBOCA (Cheever et al. 1991; Farmer et al. 1981; Sabbioni and Neumann 1990). The next highest levels were observed in the kidney, lung, gastrointestinal tract, white fat, and blood, followed by lower levels in the testes, brain, lymphocytes, spleen, and uterus. The remaining radioactivity was recovered in urine and feces. Following repeated exposure to radiolabeled MBOCA for 28 days, the radioactivity accumulated more rapidly in the liver than in the blood (1,455 femtomoles/mg versus 122 femtomoles/mg tissue, respectively) (Cheever et al. 1991). Similar distribution patterns were observed in rats and dogs following acute intravenous or intraperitoneal exposure to radiolabeled MBOCA, with the liver as the primary site of accumulation (Cheever et al. 1991; Farmer et al. 1981; Manis et al. 1984; Tobes et al. 1983)

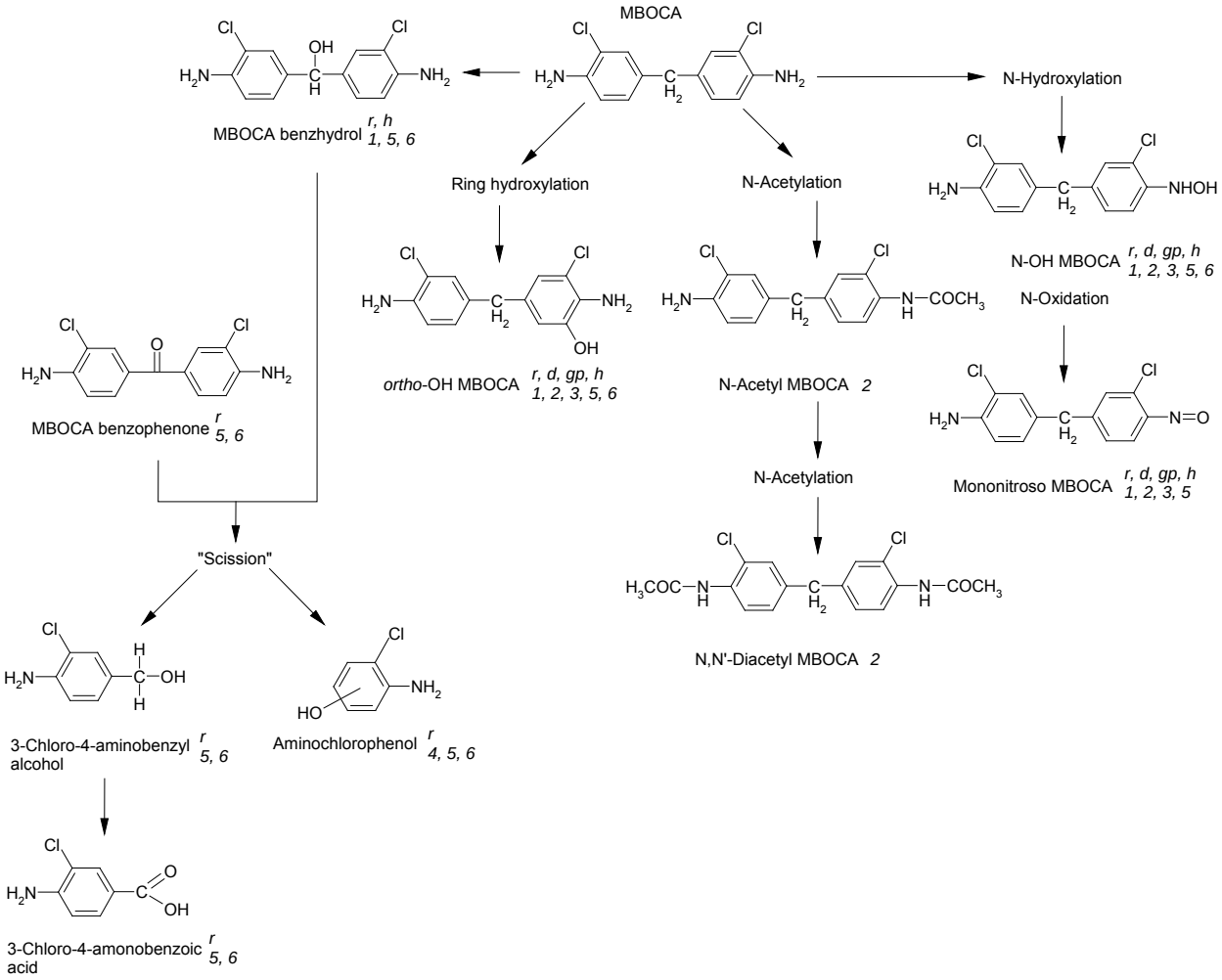
Distribution of radiolabeled MBOCA (and its metabolites) following a single dermal exposure in dogs was rapid, with no measurable radioactivity in blood or plasma up to 24 hours later (Manis et al. 1984). The highest concentration of radioactivity was found in the bile. Detectable concentrations of radioactivity were found in the liver, kidney, fat, lung, and urine.

### 3.1.3 Metabolism

MBOCA is extensively metabolized in experimental animals (Morton et al. 1988), and its metabolism can follow several pathways: N-acetylation, N-hydroxylation/N-oxidation, and ring hydroxylation. The proposed scheme of MBOCA metabolism is presented in Figure 3-1.

The majority of metabolic information has been determined in *in vitro* systems. Morton et al. (1988) quantified the formation of metabolites using appropriate chemically synthesized standards in human and rat liver microsomes with $^{14}$C-MBOCA. The rate of N-hydroxylation of MBOCA, an obligatory step in metabolic activation of aromatic amines, was higher in rat than in human microsomes (Morton et al. 1988). Rat liver microsomes were also found to be more efficient in O-hydroxy-MBOCA formation when compared with human microsomes (see Figure 3-1). The same *in vitro* microsomal system was used to elucidate the role of hepatic cytochrome P-450 monooxygenases in metabolic oxidation and detoxification of MBOCA (Butler et al. 1989). The analysis of 22 human liver microsome preparations showed that there was variation in N-oxidation of MBOCA by different preparations, and analysis of metabolism catalyzed by different rat isozymes showed that the process was catalyzed by phenobarbital-inducible cytochrome P-450 species. Since MBOCA is considered a potential human carcinogen, this

## Figure 3-1. Proposed Metabolic Pathway of 4,4'-Methylenebis(2-chloroaniline) (MBOCA)




*d* = dog; *gp* = guinea pig; *h* = human; *r* = rat

Sources: Butler et al. 1989 (1); Cheever et al. 1991 (2); Chen et al. 1989 (3); Farmer et al. 1961 (4); Kuslikis et al. 1991 (5); Morton et al. 1988 (6)

result indicates that individual profiles of cytochromes P-450 may be important determinants of an individual's susceptibility to MBOCA carcinogenesis (Butler et al. 1989). This is supported by evidence of increased frequency of lymphocyte micronuclei in MBOCA workers with *CYP3A4* A/A or A/G phenotypes (increased CYP activity) compared with MBOCA workers with *CYP3A* G/G phenotype (Wang et al. 2017). Hydroxylation by liver microsomes results in two major metabolites, N-hydroxy- and O-hydroxy-MBOCA (Chen et al. 1989; Morton et al. 1988) (see Figure 3-1). The formation of these two metabolites was evaluated using canine, guinea pig, and rat liver microsomes (Chen et al. 1989). These results indicate that there are species differences in the oxidation of MBOCA. The major metabolite in the guinea pig liver microsome system was N-hydroxy MBOCA, while dog microsomes oxidized MBOCA to the O-hydroxylated metabolite with significant amounts of hydroxylamine. In the rat liver microsome system, other polar metabolites were predominant, while there were fewer N- and O-hydroxylated MBOCA derivatives (Chen et al. 1989).

MBOCA metabolites have been investigated in workers following occupational exposure to MBOCA. Of 23 urine samples, small amounts of N-acetyl MBOCA were present in only 10 samples, even after heat treatment, while MBOCA was present in all of the samples (Cocker et al. 1988). A similar observation was made by Ducos et al. (1985) who found that the level of N-acetyl MBOCA in urine was <10% of the level of MBOCA recovered in urine of exposed workers. Skin absorption of MBOCA was considered an important factor in both of these studies. In a further attempt to identify the heat-labile MBOCA urine metabolites, Cocker et al. (1990) compared them to the chemically synthesized glucuronide. The results indicate that the major heat-labile conjugate of MBOCA in the urine of exposed workers is probably the β-N-glucuronide of the unmetabolized compound. MBOCA glucuronide spontaneously decomposes at 37°C within 24 hours to yield the unmetabolized MBOCA (Cocker et al. 1990). In 122 workers from 19 different plants, the predominant metabolite in urine was monoacetylated MBOCA (Ducos and Gaudin 1983). Another metabolite, N,N'-diacetyl MBOCA, was also identified in one of the workers. In another study of occupational exposure, 35% of the MBOCA metabolites were excreted as conjugates in the urine (Osorio et al. 1990).

Studies in dogs (Manis et al. 1984) and rats (Groth et al. 1984) indicate that MBOCA is rapidly and extensively metabolized following dermal exposure to $^{14}$C-MBOCA and that urinary levels of unmetabolized MBOCA represent only a small fraction of the total (MBOCA plus metabolites) excreted in urine. Twenty-four hours after exposure, 1.3% of the dose applied to the skin of dogs was excreted in the urine as total radioactivity, of which only 0.005% represented unmetabolized MBOCA. Similarly, in

rats after 72 hours, 2.54% of the amount applied to the skin was excreted in the urine as total radioactivity, while only 0.008% represented unmetabolized MBOCA.

After acute intragastric exposure of male rats to radiolabeled MBOCA, the levels of MBOCA and its metabolites were determined in the urine. The level of MBOCA was higher when urine was treated with exogenous glucuronidase or sulfatase, indicating the presence of conjugates (Morton et al. 1988). The conjugates, which are highly polar, can be digested with a sulphatase-glucuronidase mixture leading to 30–50% of deconjugation. When glucuronidase alone was tested, it had a small effect on conjugates (Farmer et al. 1981). These findings suggest that a large proportion of the conjugates consists of sulfates (Farmer et al. 1981).

### 3.1.4 Excretion

Occupational biomonitoring studies and case reports of accidental occupational exposure have reported urinary excretion of MBOCA and its metabolites, and urinary levels correlated with the level of exposure or expected level of exposure based on job description (Clapp et al. 1991; Cocker et al. 1988; Ducos and Gaudin 1983; Edwards and Priestly 1992; Hosein and Van Roosmalen 1978; Keen et al. 2012; NIOSH 1986a, 1986b; Osorio et al. 1990; Robert et al. 1999b; Thomas and Wilson 1984). It was unclear from these studies whether urine was the primary route of MBOCA excretion because other potential routes of excretion were not quantified.

Estimated urinary clearance rates in humans varied between studies. Based on urine samples taken from a worker exposed to 11.34 L of molten MBOCA containing 1,700 ppb of MBOCA, the biological half-life of MBOCA in the urine was estimated to be approximately 23 hours (NIOSH 1986a; Osorio et al. 1990). Hosein and Van Roosmalen (1978) also reported rapid excretion of MBOCA in the urine during the first 18 hours in a man accidently sprayed with molten MBOCA. Five hours after the spill, the level of MBOCA in the urine was 3.6 mg/L (3,600 ppb), 24 hours after exposure, the level of MBOCA in the urine was down to 0.03 mg/L (30 ppb), and 3 weeks later, it was below that level (Hosein and Van Roosmalen 1978). Similarly, MBOCA was only detectable in the urine from 1/13 MBOCA workers who had been absent from work for 48 hours, while all current workers had detectable MBOCA levels in their urine (Clapp et al. 1991; NIOSH 1986b). However, in another study on five male workers exposed to MBOCA for 3–27 years, MBOCA levels in preshift (before starting work) and postshift (after work) urine samples were not significantly different (Ichikawa et al. 1990). Also, the MBOCA urine levels 48 hours after exposure were similar to preshift levels. These study authors concluded that the biological

half-time of urinary MBOCA is relatively long. The reasons for the different clearance rates observed in the studies described (Clapp et al. 1991; Ichikawa et al. 1990; NIOSH 1986b) are not clear. Since relatively small numbers of workers were observed, the results may merely reflect individual differences.

Feces and urine were major excretion routes in rats after oral exposure to radiolabeled MBOCA, although rate and pattern of excretion differed somewhat depending upon exposure conditions. Excretion was rapid in CD rats following gavage exposure to 44–58 mg/kg of radiolabeled MBOCA, with 64–87% of radioactivity recovered in the urine and feces (Morton et al. 1988). When a single oral dose of 10 mg/kg of radiolabeled MBOCA was administered to female LAC:Porton rats, the majority of the radioactivity was excreted 48 hours later in the feces (60%) (Farmer et al. 1981). Similar observations were made in Sprague-Dawley rats given 12 mg/kg of radiolabeled MBOCA (Groth et al. 1984). However, when male Sprague-Dawley rats were exposed to a single oral dose of 50 mg/kg/day of MBOCA, urinary MBOCA excretion is maximal on the 1st day and steadily decreases thereafter (Ducos and Gaudin 1983). The quantity of free urinary MBOCA is very small, approximately 0.5 parts per 1,000. The most abundant MBOCA metabolite in urine was identified as a monoacetylated compound in comparison to the N-acetyl-4,4-methylenebis(2-chloroaniline) and N,N-diacetyl-4,4-methylenebis(2-chloroaniline) forms (Ducos and Gaudin 1983). The biological half-lives of MBOCA were 4.4 and 16.7 days in rat liver and blood, respectively, after a single oral exposure to 75 mg/kg of $^{14}$C-MBOCA (Cheever et al. 1988). The biological half-lives of MBOCA in adducts with globin and DNA were 14.3 and 11.1 days, respectively (Cheever et al. 1988). After chronic exposure to MBOCA (in either standard-protein or low-protein diets), urine of tumor-bearing rats contained a significantly higher level of MBOCA than urine from animals without tumors (Kommineni et al. 1979).

Feces and urine were major excretion routes in rats after intravenous or intraperitoneal exposure to radiolabeled MBOCA, although rate and pattern of excretion differed somewhat depending upon species and exposure conditions. In rats, 73% of the total cumulative dose of radiolabeled MBOCA was found in the feces 48 hours after a single intravenous dose of 0.51 mg/kg (Tobes et al. 1983). However, studies with intravenous administration of MBOCA in the dog found that the major route of excretion was through the urine, with 46% of the dose excreted in urine and 32% of the dose in the bile 24 hours after intravenous injection of $^{14}$C-MBOCA (Manis et al. 1984). After a single intraperitoneal injection of 1, 13, or 100 mg/kg of MBOCA, the compound was excreted most rapidly in the urine within the first 24 hours. Rats receiving the highest concentration of MBOCA produced 3 times more urine during the first 24 hours than did controls (Farmer et al. 1981).

Male Sprague-Dawley rats were treated with a single dermal dose of 2.5 mg MBOCA or $^{14}$C-MBOCA; within 72 hours, 2.54% of the administered radioactive MBOCA was excreted as $^{14}$C, while only 0.008% was excreted as the parent compound (Groth et al. 1984). Similar results were obtained in dogs. Twenty-four hours after a single dermal application of 0.4 mg/kg of $^{14}$C-MBOCA to beagle dogs, the highest concentration of radioactivity was found in the bile (Manis et al. 1984); no unmetabolized MBOCA was present in the bile. The results support the hypothesis that dermal absorption is a viable mode of entry and that MBOCA is rapidly metabolized and excreted after it enters the body regardless of the route of entry. Urinary excretion of unmetabolized MBOCA was a small but consistent fraction, comprising 0.4–0.5% of the total urinary excretion of radioactivity (Manis et al. 1984).

### 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.

No chemical-specific PBPK/PBPD models have been developed for MBOCA.

### 3.1.6 Animal-to-Human Extrapolations

No studies were identified that could evaluate potential differences in the toxicity or toxicokinetics of MBOCA between humans and animals. Studies using rats (Farmer et al. 1981; Groth et al. 1984; Morton et al. 1988; Tobes et al. 1983) and dogs (Manis et al. 1984b) indicate that the kinetics of MBOCA do not differ significantly across animal species and that the differences are primarily quantitative; however, elimination patterns appear to be different between species. Available mechanistic data are inadequate to evaluate potential species differences.

## 3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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 MBOCA are discussed in Section 5.7, Populations with Potentially High Exposures.

Differences in species sensitivity to MBOCA have been explored in *in vitro* and *in vivo* studies. In one study examining the DNA adduct formation in human bladder explant cultures, there was a distinct difference in the sensitivity to MBOCA (Stoner et al. 1988). Results showed that some cultures had much higher levels of MBOCA binding to bladder epithelium than did others. These findings suggest that some individuals are likely to develop more damage from MBOCA exposure than others. This could be an important consideration in cases of occupational exposure to MBOCA. Another observation concerns the cytochrome P-450 family of enzymes. It is known that profiles of cytochromes P-450, enzymes that play an essential role in detoxification of MBOCA, vary from one individual to another (Butler et al. 1989). Consequently, the rates of MBOCA metabolism may vary significantly among the population because of these differences in individual profiles of cytochrome P-450. This is supported by evidence of increased frequency of lymphocyte micronuclei in MBOCA workers with *CYP3A4* A/A or A/G phenotypes (increased CYP activity) compared with MBOCA workers with *CYP3A* G/G phenotype (Wang et al. 2017). The ability of a person to acetylate may also be of importance since it is well established for benzidine that this ability is directly related to the genotoxicity and carcinogenicity of the compound. However, Lin et al. (2013) found that *N*-acetylation status has minimal effect on the degree of oxidative damage following occupational exposure to MBOCA. It should be noted, however, that no significant increase in oxidative damage was observed in MBOCA-exposed workers compared with unexposed

referents. Therefore, this study did not use a sensitive measure of toxicity to evaluate the potential impact *N*-acetylation status on MBOCA toxicity.

Formation of adducts is a potential MOA of carcinogenesis following MBOCA exposure (see Section 2.19 Cancer). Gluthatione (GSH) has also been shown to protect against hemoglobin adduct formation with oxidized MBOCA metabolites (Chen et al. 1991); therefore, individuals with lowered GSH levels brought about by genetic variations (e.g., G6PD deficiency), oxidative stress, or excessive exposure to GSH depleting xenobiotics (such as acetaminophen) could potentially be at increased risk following MBOCA exposure.

Factors such as these may partially influence individual susceptibilities to MBOCA-induced carcinogenicity. Other populations that may show increased sensitivity include very young children who have an immature hepatic detoxification system and individuals with impaired liver or kidney function.

## 3.3 BIOMARKERS OF EXPOSURE AND EFFECT

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).

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 MBOCA are discussed in Section 3.3.1. 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 MBOCA from this report are discussed in Section 5.6, General Population Exposure.

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 MBOCA 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.

### 3.3.1 Biomarkers of Exposure

Occupational biomonitoring for MBOCA exposure has commonly been determined by measuring levels of MBOCA or its metabolites in urine (Clapp et al. 1991; Cocker et al. 1988; Ducos and Gaudin 1983; Hosein and Van Roosmalen 1978; Keen et al. 2012; NIOSH 1986b; Osorio et al. 1990; Thomas and Wilson 1984). Methods for detection of "total" and "free" MBOCA levels have been developed, with a detection limit of 1 μg/L for a 20-μL injection volume (Robert et al. 1999a, 1999b). Since MBOCA is rapidly metabolized, its presence in urine indicates recent exposure. MBOCA is rapidly biodegraded and converted to its glucuronide conjugates. Since varying levels of β-glucuronidase are present in urine, it may be difficult to determine accurate levels of MBOCA in the urine. Following administration of radiolabeled MBOCA to rats (Farmer et al., 191; Groth et al., 1984) and dogs (Manis et al. 1984b), less than 2 and l%, respectively, of the radioactivity recovered in urine was unmetabolized MBOCA. The biologic half-life of MBOCA in blood following intravenous exposure was estimated to be 0.70 hours; the levels of MBOCA found in various organs 24 hours after dermal exposure were relatively small (Manis et al. 1984). Measuring the metabolites of MBOCA, therefore, would be useful surrogate exposure biomarkers.

Edwards and Priestly (1992) examined whether urinary thioethers would be valid biomarkers for MBOCA exposure since thioethers are metabolic end products of the pathway involving mercapturic acid. The potential biomarker was evaluated in both human and rats. In the human study, pre- and post-work urine samples from 11 employees of a polyurethane manufacturing plant and 10 control subjects were evaluated for MBOCA and thioether levels. Results indicated that the urinary thioether levels were similar in pre- and post-work samples ($p>0.05$) and did not correlate with urinary MBOCA levels. No evaluation of potential correlation between urinary thioether or MBOCA levels and air levels of MBOCA

was conducted. In the rat study, male Wistar rats were exposed intraperitoneally to 125 or 250 mg/kg/day of MBOCA in peanut oil (vehicle) daily for 5 days, while the control received peanut oil only (Edwards and Priestly 1992). Urine was collected for 24 hours prior to and following exposure. Urinary MBOCA levels were significantly higher in the treated groups compared to controls, but the urinary thioether levels were not affected by MBOCA treatment. These findings indicate that urinary thioether levels are not useful for estimating occupational MBOCA exposure.

Male and female CD rats treated with 44 or 58 mg/kg of radiolabeled MBOCA by gavage had o-glucuronide and o-sulfate MBOCA conjugates in urine; the mono-N-glucuronide was the major biliary metabolite 24 hours after treatment (Morton et al. 1988). After a single oral dose of 75 mg/kg, MBOCA formed adducts with globin and liver DNA in Sprague-Dawley rats (Cheever et al. 1988). The half-lives for rat globin and liver DNA were estimated to be 14 and 11 days, respectively. An o-hydroxysulfate, identified as 5-hydroxy-3,3'-dichloro-4,4'-diamino-diphenylmethane-5-sulfate, was the major metabolite found in dog urine. This metabolite also formed adducts with DNA *in vitro* in a time-dependent manner (Manis and Braselton 1984). Studies in rats and guinea pigs have demonstrated MBOCA adducts to hemoglobin (Chen et al. 1991; Sabbioni and Neumann 1990), with a disappearance rate that approximates the life of a red blood cell. These study results suggest that MBOCA adducts may be useful biomarkers to monitor MBOCA exposure.

The relative sensitivity of urinary and hemoglobin adduct biomarkers was evaluated by Vaughan and Kenyon (1996). Evaluations indicated that while urinary MBOCA levels are useful an accurate for acute exposures, the rapid half-life in the urine does not allow for monitoring of long-term exposure. In contrast, hemoglobin adducts can estimate MBOCA exposure over a 3-month period. Therefore, hemoglobin adduct may be more appropriate biomarkers for occupational exposure.

The levels of MBOCA have not been determined in chronic animal studies. It is unknown if chronic sequestering and low-level release of MBOCA, resulting in steady state levels, occur.

The ability of MBOCA to covalently bind to body proteins has been less frequently used to estimate exposure to MBOCA. MBOCA is known to bind to globin (Cheever et al. 1991). Therefore, measurement of MBOCA-globin adducts could be used to monitor MBOCA exposure since the half-life of MBOCA-globin adducts is greater than the half-life of MBOCA.

### 3.3.2 Biomarkers of Effect

The available information suggests that the bladder is a potential target organ for MBOCA-induced carcinogenesis (Dost et al. 2009; Liu et al. 2005; Ward et al. 1988, 1990). Medical surveillance of occupationally exposed workers may help to ascertain the incidence of bladder cancer; however, available techniques are for general identification of bladder lesions and are not specific to MBOCA. Ward et al. (1990) demonstrated that evaluation of urine sediment cytology using the Papanicolaou technique is insensitive for detecting lesions in the urinary tract. Data from that study indicate that cytoscopy is a better biomarker of effect for bladder lesions.

## 3.4 INTERACTIONS WITH OTHER CHEMICALS

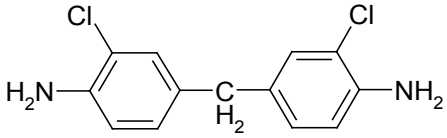
Limited information was located on the interactive effects of other chemicals on MBOCA toxicity. MBOCA metabolism is influenced by phenobarbital (Chen et al. 1991; Morton et al. 1988). *In vivo* treatment with phenobarbital induced cytochrome P-450 enzymes, which resulted in a slight increase in MBOCA hydroxylation (Chen et al. 1991). Rats treated with phenobarbital had a 4–8-fold increased metabolic rate for MBOCA (Morton et al. 1988). Phenobarbital did not, however, affect adduct formation (Chen et al. 1991). *In vivo* treatment with p-naphthoflavone did increase the rate of MBOCA-hemoglobin adduct formation in rats treated subcutaneously with either 100 or 500 mg/kg/day (Chen et al. 1991). MBOCA metabolism is NADPH-dependent. Moreover, MBOCA hydroxylation is inhibited by 2,4-dichloro-6-phenylphenoxyethylamine, an inhibitor of microsomal mixed function oxidases (Chen et al. 1989). Cysteine and glutathione inhibit *in vivo* hemoglobin adduct formation by N-hydroxy-MBOCA and mononitroso-MBOCA (Chen et al. 1991). The most recent findings suggest that binding of N-hydroxy-MBOCA to DNA in rat tissues can be inhibited by ascorbic acid, glutathione, nitrosobenzene, and methyl viologen but not by nitromethane, *p*-nitrobenzylpyridine, or methionine (Segerback and Kadlubar 1992).

# CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

## 4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of MBOCA is located in Table 4-1.

**Table 4-1. Chemical Identity of 4,4'-Methylenebis(2-chloroaniline) (MBOCA)**

| Characteristic | Information | Reference |
|---|---|---|
| Chemical name | 4,4'-Methylenebis(2-chloroaniline) | |
| Synonym(s) and registered trade name(s)[a] | MBOCA, 3,3'-dichloro-4,4'-diaminodiphenylmethane; 4,4'-methylene(bis)-chloroaniline; 4,4'-methylenebis(o-chloroaniline); 4,4'-methylene-bis[2-chlorobenzenamine]; bis(3-chloro-4-aminopropyl) methane; aniline, 4,4'methylene-bis[2-chloro-; bis-(4-amino-3-chlorophenyl) methane; di(4-amino-3-chlorophenyl) methane; bis amine; MCA, CL-MDA; DACPM; Cuamin-M; Activator-M; CA-800; DAC; Bis-Amine A; Curene 442; MOCA; and others | CIS 1992; HSDB 1991; NRC 1981; OHM/TADS 1985; Smith and Woodward 1983 |
| Chemical formula | $C_{13}H_{12}Cl_2N_2$ | IARC 1974 |
| Chemical structure |  | NRC 1981 |
| CAS Registry Number | 101-14-4 | HSDB 1991 |

[a]MBOCA trade names that are not in use: Curalin M, Curalon M, Cyanaset, and $LD_{813}$.

CAS = Chemical Abstracts Service

## 4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of MBOCA is located in Table 4-2.

## Table 4-2. Physical and Chemical Properties of 4,4'-Methylenebis(2-chloroaniline) (MBOCA)

| Property | Information | Reference |
|---|---|---|
| Molecular weight | 267 | IARC 1974 |
| Color | | |
| Pure form | Colorless crystals | IARC 1974 |
| Technical form | Yellow, tan, or brown pellets | Smith and Woodward 1983; NRC 1981 |
| Physical state | Solid | HSDB 1991 |
| Melting point | 110 °C | HSDB 1991 |
| Boiling point | No data | |
| Density at 20 °C | 1.44 g/mL | NRC 1981; Sax and Lewis 1987 |
| Odor | Nearly odorless | NRC 1981 |
| Odor threshold: | No data | |
| Solubility: | | |
| Water at 24 °C | 13.9 mg/L | Voorman and Penner 1986a |
| Organic solvents | Soluble in hot methyl ethyl ketone, alcohol, acetones, trichloroethylene, toluene, ether, esters, and lipids | HSDB 1991; OHM/TADS 1985; Smith and Woodward 1983 |
| Partition coefficients: | | |
| Log $K_{ow}$ | 3.94[a] | HSDB 1991 |
| Log $K_{oc}$ | 4810 | HSDB 1991 |
| Vapor pressure | | |
| at 25 °C | $1.0 \times 10^{-5}$ mmHg | Smith and Woodward 1983 |
| at 60 °C | $1.3 \times 10^{-5}$ mmHg | NRC 1981 |
| at 100 °C | $3.5 \times 10^{-5}$ mmHg | Smith and Woodward 1983 |
| at 120 °C | $5.4 \times 10^{-5}$ mmHg | NRC 1981 |
| Henry's law constant at 25 °C | $4 \times 10^{-11}$ atm $m^3$/mole | |
| Autoignition temperature | No data | |
| Flashpoint | No data | |
| Flammability limits | No data | |
| Conversion factors | No data | |
| Explosive limits | No data | |

[a]Estimated value.

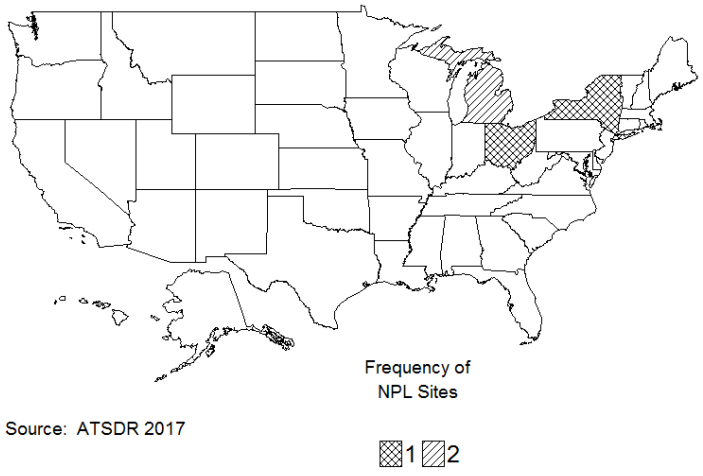
# CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

## 5.1 OVERVIEW

MBOCA has been identified in at least 4 of the 1,854 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2017). However, the number of sites in which MBOCA has been evaluated is not known. The number of sites in each state is shown in Figure 5-1. All four these sites are located within the United States.

MBOCA may be discharged to the environment as a result of an uncontained, open cycle, manufacturing process. Such a discharge could constitute a release to the atmosphere as a fugitive dust or as a spill of MBOCA pellets or heated liquid MBOCA. Otherwise nonhazardous solid wastes may become contaminated by MBOCA in the manufacturing process, thus making such wastes hazardous. The dust can settle to soil or surface waters where it will be strongly adsorbed to the organic matter in the soil or water column; therefore, it is unlikely to contaminate groundwater. Microbial degradation is a potentially significant degradation process and may be quite rapid if appropriate organisms are present in the soil or water. In air or surface waters, MBOCA may undergo photooxidation by alkoxyradicals. Members of the general population are unlikely to be exposed to MBOCA unless they live in an area that has been contaminated. Workers in plants that manufacture or use MBOCA have the potential to be highly exposed by inhalation or dermal contact.

**Figure 5-1. Number of NPL Sites with 4,4'-Methylenebis(2-chloroaniline) (MBOCA) Contamination**




Source: ATSDR 2017

- The general population is not expected to be exposed to MBOCA. However, ingestion of plants grown in contaminated soil could lead to oral exposure.

- MBOCA is primarily used as a curing agent for polymers. Occupational exposure is expected to be primarily via inhalation and/or dermal contact during use or production of MBOCA.

- MBOCA is not expected to volatize from water or soil. It is not expected to transport through soil due to rapid and tight adsorption to organic matter. MBOCA is bioaccumulated by food plants grown in contaminated soil.

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

### 5.2.1 Production

MBOCA is a man-made chemical and has not been found in nature (IARC 1974). It is produced commercially by reacting formaldehyde with *o*-chloroaniline (HSDB 1991; IARC 1974). Pure MBOCA is a colorless crystalline solid (Smith and Woodward 1983). The technical grade of MBOCA that is available in the United States comes mainly from Japan in the form of tan/yellow fused prills or pastilles. The diamine purity is 99.8%, typically with 0.2% free *o*-chloroaniline (monomer). Isomers are produced as side reactions such as trimers and tetramers-diamines with three- and four-ring structures joined by methylene groups. Isomers constitute up to 8–10% of MBOCA. The dimer makes up to 90–92% of the MBOCA produced today for coatings and cast polyurethanes. There is no commercial use for pure dimer MBOCA other than for laboratory work.

MBOCA has been produced commercially in the United States for some time. The first reported production was in 1956 (IARC 1974). U.S. production of MBOCA was estimated to be 3.3–5.5 million pounds in 1970 and 7.7 million pounds in 1972 (IARC 1974). In 1982, production of MBOCA in the United States was reported to have ceased (HSDB 1991).

MBOCA has been manufactured in the United States by two companies: E.I. Du Pont de Nemours and Company (Deepwater, New Jersey) and Anderson Development Company (Adrian, Michigan). However, E.I. Du Pont de Nemours and Company ceased MBOCA production in 1978, and Anderson Development Company ceased production in 1979. Presently, all MBOCA used in the United States is imported. As of 1985, there were at least four production sites in the United States that use imported MBOCA: Polyester Corporation (Southampton, new York), American Cyanamid Company (Bound Brook, new Jersey), E.I. Du Pont de Nemours and Company (Deepwater, New Jersey), and Anderson Development Company (Adrian, Michigan) (OHM/TADS 1985). However, in 1992, Allchem Industries,

Inc. (Gainesville, Florida), Maypro Industries, Inc. (Harrison, New York), and Miki Sangyo (USA), Inc. (New York, New York), were also reported to produce MBOCA for commercial sale (Van et al. 1992).

Twenty-four industrial sites (Table 5-1) were listed in the 2016 Toxics Release Inventory (TRI) as producers and/or users of MBOCA (TRI16 2017). However, since not all producers of MBOCA are required to report to TRI, the companies listed on the inventory cannot be considered the exclusive producers of MBOCA in the United States. This is not an exhaustive list.

**Table 5-1. Facilities that Produce, Process, or Use MBOCA**

| State[a] | Number of facilities | Minimum amount on site in pounds[b] | Maximum amount on site in pounds[b] | Activities and uses[c] |
|---|---|---|---|---|
| DE | 2 | 1,000 | 9,999 | 6 |
| FL | 1 | 100,000 | 999,999 | 7 |
| IA | 1 | 1,000 | 9,999 | 6 |
| IL | 3 | 1,000 | 9,999 | 12 |
| IN | 1 | 1,000 | 9,999 | 6 |
| MI | 1 | No data | No data | No data |
| NE | 1 | 10,000 | 99,999 | 12 |
| NJ | 1 | No data | No data | No data |
| NY | 1 | 10,000 | 99,999 | 6 |
| OH | 3 | 100 | 99,999 | 6, 12 |
| PA | 1 | No data | No data | No data |
| TN | 1 | 1,000 | 9,999 | 10, 11 |
| TX | 4 | 1,000 | 99,999 | 6, 7 |
| WI | 3 | 1,000 | 99,999 | 6 |
| WV | 1 | No data | No data | No data |

[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)

### 5.2.2 Import/Export

In 1978, approximately 0.4 million pounds of MBOCA were imported into the United States (HSDB 1991). The amount of MBOCA imported into the United States increased in 1983 to 1.51 million pounds. In 1991, approximately 2.0 million pounds of MBOCA were imported into the United States. The MBOCA was manufactured by two Japanese producers and a Taiwanese producer.

### 5.2.3 Use

The majority of MBOCA consumed in the United States has been used as a curing agent for isocyanate-containing polymers, and only about 1% is used in epoxy/epoxy-urethane resin blends (IARC 1974). These cured polymers have many commercial and military uses. MBOCA was reported to be the most widely used agent for curing castable liquid polyurethane elastomers (HSDB 1991; IARC 1974; Sax and Lewis 1987). Commercially, these MBOCA-cured polyurethanes have been used to produce shoe soles, rolls for postage stamp machines, cutting bars in plywood manufacturing, rolls and belt drives in cameras, computers, and reproducing equipment, and wheels and pulleys for escalators and elevators (NRC 1981). MBOCA has also been reported to be formulated with other aromatic diamines and sold under trade names as a curing agent (IARC 1974). MBOCA has also been used in the manufacture of gun mounts, jet engine turbine blades, radar systems, and components in home appliances (HSDB 1991), and as a wiring patting and curing agent (Cowles 1978). Military applications of MBOCA-cured polyurethanes include ball seals on nuclear submarines, positioning strips in Poseidon missiles, and encapsulation of electric components (NRC 1981).

### 5.2.4 Disposal

Because MBOCA is defined as a "hazardous waste," companies that generate wastes containing ≥100 kg MBOCA are required to conform with EPA regulations (EPA 1989; HSDB 1991). For more information on the regulations and guidelines that apply to MBOCA, see Chapter 7.

No universal method exists for the disposal of carcinogenic compounds such as MBOCA (HSDB 1991). Product residues and sorbent media containing MBOCA have been packaged in epoxy-lined drums and disposed of at EPA-approved sites (OHM/TADS 1985). Destruction via chemical reaction is another method that has been used to dispose of small amounts of MBOCA (HSDB 1991). This method, in which MBOCA is oxidized with potassium permanganate, is generally used for laboratory wastes containing small amounts of MBOCA.

Incineration technologies have been investigated for the disposal of MBOCA. MBOCA has been considered a good candidate for rotary kiln incineration at a temperature range of 820–1,600°C, with residence times of seconds for liquids and gases and hours for solids (EPA 1981b; HSDB 1991). MBOCA is also listed as a good candidate for fluidized bed incineration at temperatures ranging from 450 to 980°C and residence times similar to those for rotary kiln incineration (EPA 1981). Disposal of MBOCA contained in waste waters using activated carbon adsorption has been studied (HSDB 1991). Saturated filters used to remove MBOCA from waste water via carbon absorption can subsequently be destroyed by rotary kiln or fluidized bed incineration (EPA 1979). Biodegradation treatment of MBOCA using continuous flow reactors that are designed to remove potential hazardous chemicals from water and waste water may be also useful in clean-up operations. Similarly, activated carbon processes and ozone oxidation provide effective disposal treatment (EPA 1979). There is, however, no information on the availability of MBOCA residues from polyurethanes and other plastics.

## 5.3 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 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).

### 5.3.1 Air

Estimated releases of 1,500 pounds (~0.68 metric tons) of MBOCA to the atmosphere from 25 domestic manufacturing and processing facilities in 2016, accounted for about 54% of the estimated total

environmental releases from facilities required to report to the TRI (TRI16 2017). These releases are summarized in Table 5-2.

**Table 5-2. Releases to the Environment from Facilities that Produce, Process, or Use MBOCA[a]**

| | | Reported amounts released in pounds per year[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Total release | | |
| State[c] | RF[d] | Air[e] | Water[f] | UI[g] | Land[h] | Other[i] | On-site[j] | Off-site[k] | On- and off-site |
| DE | 2 | 0 | 0 | 0 | 0 | 1,213 | 0 | 1,213 | 1,213 |
| FL | 1 | No data | No data | No data | No data | No data | No data | No data | No data |
| IA | 1 | No data | No data | No data | No data | No data | No data | No data | No data |
| IL | 3 | 1 | 0 | 0 | 3 | 0 | 1 | 3 | 4 |
| IN | 1 | No data | No data | No data | No data | No data | No data | No data | No data |
| MI | 1 | No data | No data | No data | No data | No data | No data | No data | No data |
| NE | 1 | 4 | 0 | 0 | 0 | 0 | 4 | 0 | 4 |
| NJ | 1 | No data | No data | No data | No data | No data | No data | No data | No data |
| NY | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| OH | 3 | 6 | 0 | 0 | 65 | 0 | 6 | 65 | 71 |
| PA | 1 | No data | No data | No data | No data | No data | No data | No data | No data |
| TN | 1 | 1,487 | 0 | 0 | 0 | 0 | 1487 | 0 | 1,487 |
| TX | 4 | No data | No data | No data | No data | No data | No data | No data | No data |
| WI | 3 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| WV | 1 | No data | No data | No data | No data | No data | No data | No data | No data |
| Total | 25 | 1,500 | 0 | 0 | 68 | 1,213 | 1,500 | 1,281 | 2,781 |

[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)

### 5.3.2 Water

No MBOCA was released to surface water or publically owned treatment works from 25 domestic manufacturing and processing facilities in 2016 (TRI16 2017). These releases are summarized in Table 5-2.

### 5.3.3 Soil

Estimated releases of 68 pounds (~0.03 metric tons) of MBOCA to soils 25 domestic manufacturing and processing facilities in 2016, accounted for about 2.4% of the estimated total environmental releases from facilities required to report to the TRI (TRI16 2017). No MBOCA was released via underground injection (TRI16 2017). These releases are summarized in Table 5-2.

## 5.4 ENVIRONMENTAL FATE

### 5.4.1 Transport and Partitioning

**Air.** Volatilization of MBOCA from soil or surface waters is unlikely to be a major factor for Environmental fate because of its very low vapor pressure ($1x10^{-5}$ mmHg at 25°C) (Keeslar 1986; NIOSH 1978b) and its strong adsorption to organic matter.

**Water.** MBOCA partitions to soil rather than water as a result of its relatively low solubility in water (13.9 mg/L) and its amine groups, which have an affinity for soil organic matter. This binding is rapid and very tight and results in virtually no movement of MBOCA through soil (Voorman and Penner 1986a).

**Sediment and Soil.** The partitioning of MBOCA in the soil affects the uptake of the compound by plants grown in contaminated soil and its subsequent ingestion by humans. MBOCA is bioaccumulated by food plants (e.g., carrots, orchard grass, beans, cabbage, beet, sorghum, cucumber), but movement of the compound within the plant is extremely limited. MBOCA applied to leaf surfaces resulted in adsorption to the leaf cuticle but no movement beyond the application site. Exposure of roots of bean, sorghum, and carrots to aqueous solutions of 5 mg/L of MBOCA for 8 days resulted in relatively high concentrations on the root surfaces of these plants (37, 2,000, and 20 mg/kg, respectively), demonstrating bioconcentration at that site but limited translocation to plant shoots (1.7 mg/kg, 2–5 mg/kg, and virtually undetectable, respectively). MBOCA applied to soils at a concentration of 5 mg/kg again showed an

uptake in the roots of cucumbers and beans (up to 17 mg/kg MBOCA); the shoots of these plants contained <0.2 mg/kg. This limited translocation may be due to the low water solubility of MBOCA (Voorman and Penner 1986b). This may be of concern in cases of accidents (or even during routine operations) in which MBOCA is released to the air.

### 5.4.2 Transformation and Degradation

**Air.** The photooxidation half-life of MBOCA in air is estimated to be between 0.290 and 2.90 hours based on reactions with hydroxyl radicals (Howard et al. 1991), suggesting that this may be a significant fate process.

**Water.** Studies examining the biodegradation of MBOCA, using activated sludge microorganisms, suggested that MBOCA was readily degraded (from 2.02 to 0.09 mg/L) in a continuous biological reactor within 24 hours, but not during a 7-day static incubation test (EPA 1979; Tabak et al. 1981). Other degradation processes were also effective in reducing the concentrations of MBOCA present in simulated waste water. Ozone oxidation reduced an initial concentration of 1.52 mg/L MBOCA to nondetectable levels within 5 minutes. Between 21 and 35 mg of carbon per liter, depending on the type of carbon, were required to reduce 1.0 mg/L MBOCA to 0.1 mg/L (EPA 1979). MBOCA was not susceptible to oxygen stripping (EPA 1979).

The estimated photooxidation half-life of MBOCA in surface water is between 1.3 and 72 days, while in groundwaters, MBOCA may have a half-life of 8 weeks to 1 year (Howard et al. 1991). The estimated hydrolysis half-life of MBOCA in water at 25°C and pH 7 is >800 years (EPA 1988c). Studies of microbial degradation of MBOCA showed several biodegradation products, including N-monoacetyl MBOCA and N,N'-diacetyl MBOCA (Yoneyama and Matsumura 1984). 4,4'-Diamino-3,3'-dichloro-benzophenone was produced from metabolic conversion of MBOCA by soil microorganisms (Voorman and Penner 1986a).

**Sediment and Soil.** Carbon dioxide production from soil samples treated with MBOCA was <1% of the total applied, suggesting that aromatic rings are resistant to microbial degradation and oxidation (Voorman and Penner 1986a). These investigators did detect a metabolite with the methylene carbon oxidized to a carbonyl. Microbial degradation of MBOCA has been shown to occur using *Bacillus megatetium* and *nocardiopsis* sp. isolated from soil. These microorganisms readily metabolize MBOCA, with 39 and 24%, respectively, of the original concentration remaining after 3 hours of incubation. The

major degradation pathways were: (1) acetylation of MBOCA to N-monoacetyl MBOCA and then to N,N'-diacetyl MBOCA and (2) hydroxylation of N-monoacetyl MBOCA to N-hydroxy-N-acetyl MBOCA with the final metabolite being N-hydroxy-N,N'-diacetyl MBOCA (Yoneyama and Matsumura 1984). Also present was a metabolite with the methylene carbon oxidized to a carbonyl (Voorman and Penner 1986a).

The estimated half-life of MBOCA in soil based on aerobic biodegradation may range between 1 and 6 months (Howard et al. 1991).

## 5.5 LEVELS IN THE ENVIRONMENT

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

Table 5-3 shows the lowest limit of detections that are achieved by analytical analysis in environmental media. An overview summary of the range of concentrations detected in environmental media is presented in Table 5-4.

**Table 5-3. Lowest Limit of Detection Based on Standards[a]**

| Media | Detection limit | Reference |
|---|---|---|
| Air | 1 ng/m$^3$ | Skarping et al. 1985 |
| Water | 25 ng/L | Rice and Kissinger 1981, 1982 |
| Soil/sediment | 25 ng/L | Rice and Kissinger 1981, 1982 |
| Whole blood | 25 pg | Sabbion and Neumann 1990 |

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.

**Table 5-4. Summary of Environmental Levels of MBOCA**

| Media | Low | High | For more information |
|---|---|---|---|
| Outdoor air (ppbv) | No monitoring data were identified. | | |
| Indoor air (ppbv) | No monitoring data were identified. | | |
| Surface water (ppb) | 1 | 1 | Section 5.5.2 |
| Ground water (ppb) | No monitoring data were identified. | | |
| Drinking water (ppb) | No monitoring data were identified. | | |
| Food (ppb) | No monitoring data were identified. | | |
| Soil (ppm) | 4.6 | 1146 | Section 5.5.2 |

Detections of MBOCA in air, water, and soil at NPL sites are summarized in Table 5-5.

**Table 5-5. MBOCA 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) | 1.84 | 1.56 | 2.29 | 2 | 1 |
| Soil (ppb) | 28 | 41.6 | 124 | 4 | 1 |

[a]Concentrations found in ATSDR site documents from 1981 to 2017 for 1,854 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.

### 5.5.1 Air

Monitoring of MBOCA dust and vapor in the ambient air of a production facility in 1969 showed that the maximum 8-hour average concentrations were 0.32 and 0.25 mg/m$^3$, respectively. Significant levels were detected only in areas adjacent to the pelletizing unit, although even these levels were only intermittently high. Skin absorption was the major source of exposure and could be effectively controlled with appropriate protective clothing and engineering controls (Linch et al. 1971).

Ambient air and personal air monitoring was conducted at a plastics factory where MBOCA was used in the production of urethane. The results obtained from 10 ambient air samples (6 in the general work area, 4 in the area where MBOCA was melted) indicated that MBOCA was not present in the general area above the level of detection (0.015 µg/filter), and was present in the air near the MBOCA melting pot at levels up to 92 µg/m$^3$. Personal air monitoring indicated that only those employed as mixers and molders were exposed to detectable levels of MBOCA, ranging from 0.06 to 0.70 µg/m$^3$. Wipe samples of

surfaces that workers were most likely to be exposed to contained low levels of MBOCA throughout. Surface wipe samples showed MBOCA contamination ranging from 0.1 μg/100 $cm^2$ near the trimmers work table to 19.1 μg/100 $cm^2$ adjacent to the MBOCA melting pot. Surfaces that were rarely wiped clean, but were not near the melting pot (such as the tops of storage cabinets), contained an average of 4.7 μg/100 $cm^2$ (Clapp et al. 1991). MBOCA air levels were also evaluated in a polyurethane elastomer factory, and the air exposure levels ranged from 0.2 to 8.9 μg/$m^3$ (Ichikawa et al. 1990). MBOCA levels were measured at 0.001–0.042 mg/$m^3$ in the air of a factory producing rubber ski boots (Smith and Woodward 1983).

MBOCA was present at only trace concentrations in air samples of dust taken 1.5 feet above ground level in a residential area known to be contaminated with MBOCA (Keeslar 1986).

### 5.5.2 Water

A specialty chemical manufacturing plant in Adrian, Michigan, that produced >1 million pounds/year of MBOCA during the late 1970s was found to have released significant quantities of it in its waste water discharges. Water sampling surveys found the following concentrations of MBOCA associated with the facility (Parris et al. 1980): >1,600 ppm in industrial lagoon sediment; 250 ppb in industrial lagoon effluent water; 1.5 ppb in industrial site deep well water; 1 ppb in surface runoff water from site; <0.5 ppb in sewage treatment plant, influent water; <0.5 ppb in sewage treatment plant, effluent water <0.5 ppb; 18 ppm (estimated) in sewage treatment plant, activated sludge; and ≤0.1 ppb in Raisin River water.

Samples of well water from a residential area adjacent to the manufacturing plant, however, did not contain detectable levels of MBOCA, suggesting that groundwater contamination had not occurred (Keeslar 1986).

### 5.5.3 Sediment and Soil

Soil samples taken on the site of a manufacturing plant using MBOCA contained levels as high as 1,146 ppm, while concentrations along public roads near the site ranged from 4.6 to 590 ppm (Keeslar 1986). Soil from the yards of residences adjacent to the site (within a 1-km radius) typically had 1.74 mg/kg MBOCA in the top 2 inches of soil and 0.02 mg/kg in the next 4 inches.

### 5.5.4 Other Media

No studies were located on the levels of MBOCA found in other environmental media. However, it has been shown that MBOCA binds to, and penetrates, the roots of plants grown in contaminated soil. Once in the plant, MBOCA stays very close to the root surface and is not distributed throughout the plant (Voorman and Penner 1986b).

## 5.6 GENERAL POPULATION EXPOSURE

The general population is not likely to be exposed to MBOCA. However, members of the general population may be exposed to MBOCA if they consume certain types of plants (e.g., root crops) grown in MBOCA-contaminated soil. MBOCA has been found to adhere to the leaves and roots of plants, and the compound is not removed by rinsing with water (Voorman and Penner 1986b).

## 5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Populations living near areas known to be contaminated with MBOCA can be considered to have high potentials for exposure. Although adults living in a contaminated area of Adrian, Michigan did not have detectable levels of MBOCA in their urine, young children (all under the age of 6 years) from the area had urine concentrations of 0.3–1.0 ppb. The levels in children were believed to be a direct consequence of their frequent contact with contaminated soils. MBOCA was detected in the residences of this area, primarily on floors, carpeting, and vacuum cleaner bags; however, other household surfaces did not have significant concentrations of MBOCA (Keeslar 1986).

Workers using or producing MBOCA have the highest potential for exposure. MBOCA is commercially used as a curing agent for isocyanate polymers by specialty manufacturers of industrial and commercial polyurethane products (e.g., as gears, gaskets, sport boots, and roller skate wheels). The form of MBOCA to which workers could be exposed is likely to be either a liquid emulsion, dust, or solid pellets (NIOSH 1986b; Schulte et al. 1988). Occupational exposures may occur at several stages of polymer production, especially where prepolymers are mixed with molten curing agent before molding (Edwards and Priestly 1992). In most cases, dermal absorption is the most important occupational exposure pathway (Edwards and Priestly 1992; Lowry and Clapp 1992). The National Occupational Health Survey estimated that 2,094 workers were potentially exposed to MBOCA in the workplace in 1980 (Schulte et al. 1988).
A urinary monitoring program can determine aggregate worker exposure to MBOCA (Ward et al. 1986). Workers in a plastics plant that mixed or molded urethane products containing MBOCA were found to

have detectable levels of MBOCA in their urine. Concentrations for the mixers ranged from 5 to >100 µg/L urine (average concentration, 61.9 µg/L), whereas concentrations for the molders were considerably lower, nondetectable to 50 µg/L urine (average concentration, 14.8 µg/L). The greatest exposure route was inferred to be direct skin contact with MBOCA, despite the fact that the mixers wore gloves while transferring dry MBOCA, melting it, or dispensing the molten fluid (Clapp et al. 1991). After 2 days away from work, only 1 of 13 workers in the plastics plant had detectable levels of MBOCA in his urine. This worker also had the highest peak urinary MBOCA levels during the preceding week (Clapp et al. 1991). Another investigation of workers in a polyurethane elastomer factory reported that pre- and postshift urinary levels were not significantly different in all exposed workers and that levels measured 48 hours after cessation of work were not always the lowest (Ichikawa et al. 1990). The difference may partially be explained by the actual levels of MBOCA in the workplace; workers that had MBOCA in the urine after 2 days away from work had the highest levels of the compound when last measured, suggesting that they were exposed to higher MBOCA levels than workers without any MBOCA in urine after the weekend. The reported findings may also reflect differences in metabolic rates between workers, or that different depots of MBOCA are excreted over different time frames from the body.

Workers in a manufacturing plant using MBOCA had urine concentrations of MBOCA ranging from 13 to 458 ppb (mean 145 ppb). Their immediate families were found to have had exposures to MBOCA also; urine levels of MBOCA ranged from 0 to 15 ppb (Keeslar 1986). These findings suggest that direct exposure to MBOCA itself in an occupational setting or at a hazardous waste site may not be necessary for exposure, and that people can also be exposed to MBOCA by contact with an MBOCA-exposed individual. Monitoring of workers at seven facilities in Australia that used MBOCA in the manufacture of polyurethane polymers showed that average MBOCA levels in the urine of the workers dropped from 29.6 to 10.4 mg/L within 8–9 months after the implementation of an exposure prevention program (Wan et al. 1989). Another study of 150 workers in 19 factories with industrial exposure to MBOCA showed that, at the end of the workshift, excretion levels ranged from <0.5 to 1,600 µg/L of MBOCA, with the highest average urine concentrations (600 µg/L) in workers directly involved in MBOCA manufacture or use; urine MBOCA levels dropped after exposure controls were implemented in the plant (Ducos et al. 1985). Similar decreases in urine MBOCA concentrations were observed following improvements in ventilation and with the use of protective clothing by workers exposed to MBOCA (Thomas and Wilson 1984).

Results of a voluntary biological monitoring program implemented by the Polyurethane Manufacturers Association suggest that exposure to MBOCA among users of the compound decreased between 1985 to 1990. Following implementation of a number of engineering controls to limit exposure, including the use of closed transfer systems and the use of a fused, hardened MBOCA pellet, worker urine specimens containing <25 μg MBOCA/L increased from 77 to 86% of the total amount collected. Over this same time period, urine samples containing >50 μg MBOCA/L decreased from 12 to 8% of the total number of samples collected (Lowry and Clapp 1992). Workers in shipyards where MBOCA is used as a potting and molding agent for wiring may also have potentially high exposures to MBOCA (Cowles 1978).

# CHAPTER 6. ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of MBOCA 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 MBOCA.

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.

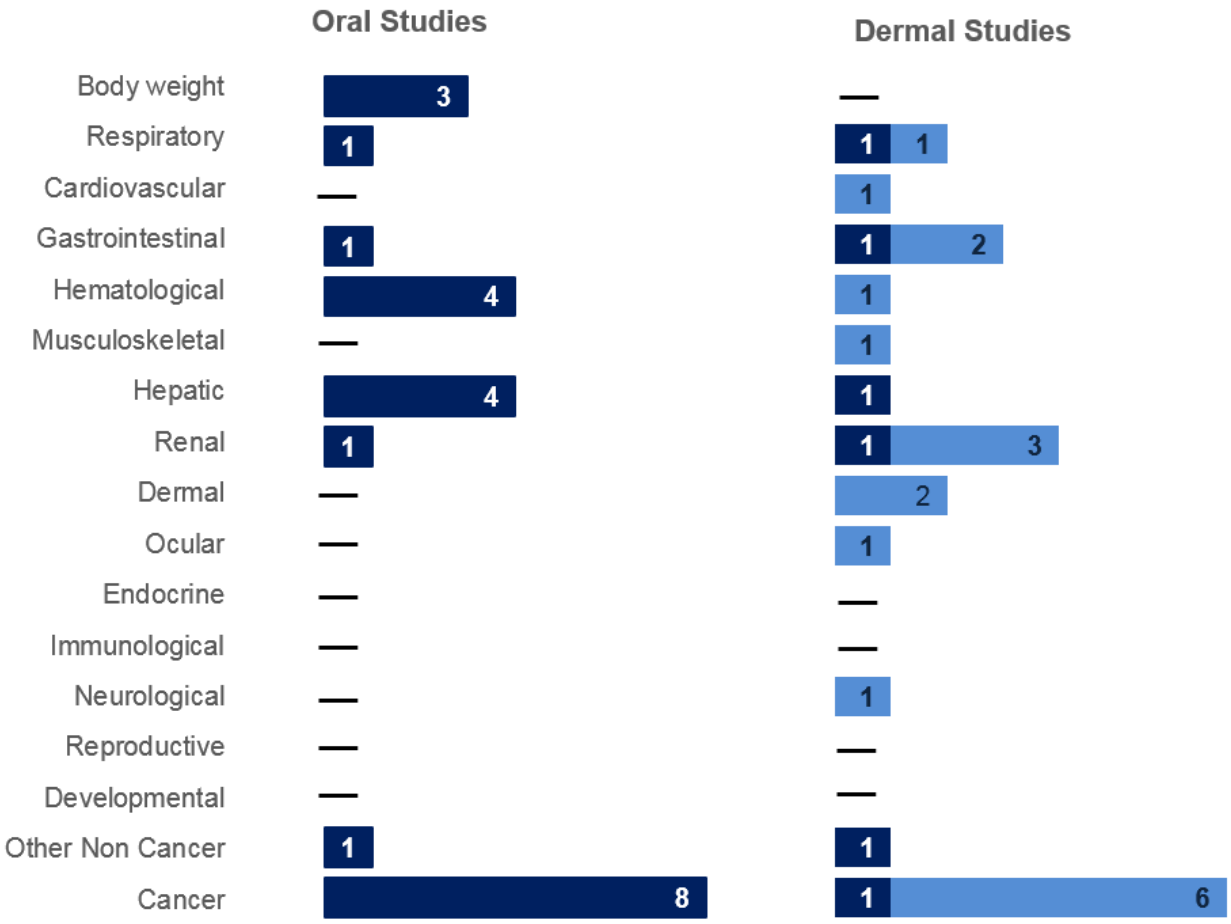
## 6.1 Information on Health Effects

Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to MBOCA 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 MBOCA. 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.

With regard to human health effects of MBOCA, the few available studies were either case reports of acute occupational exposure or involved intermediate or chronic epidemiological studies. Acute exposures to MBOCA were by the inhalation, oral, and/or dermal routes, although in some studies, it was difficult to clearly define the exposure route. Intermediate exposures were by either inhalation and/or dermal contact; no intermediate oral exposure studies were located. Chronic exposure in humans occurred by inhalation and/or dermal contact; no chronic oral studies were located. These studies are included as dermal studies in Figure 6-1 to avoid double counting of the studies; however, it is acknowledged that exposure may have occurred via multiple routes. No information is available regarding body weight, hepatic, endocrine, immunological, reproductive, or developmental effects in humans by any route of exposure. Studies on cancer incidence in humans after inhalation and/or dermal exposure to MBOCA were located.

## Figure 6-1. Summary of Existing Health Effects Studies on MBOCA By Route and Endpoint*

**Potential carcinogenic, liver, and hematological effects were the most studied endpoints**
The majority of the studies examined oral exposure in animals (versus humans)




*Includes studies discussed in Chapter 2; the number of studies include those finding no effect. No inhalation studies in animals were located. Human occupational exposure is expected to be predominately via dermal absorption and/or inhalation. For this figure, all human studies were counted only once under dermal exposure to avoid double counting the same study. However, it is acknowledged that exposure likely occurred via multiple routes.

Virtually all of the data regarding the health effects of MBOCA in animals were obtained from studies in which MBOCA was administered orally. No information is available regarding cardiovascular, musculoskeletal, dermal, ocular, endocrine, neurological, reproductive, or developmental effects in animals by any route of exposure. Extremely limited information is available regarding health effects in animals dermal exposure. No information is available regarding health effects in animals following inhalation exposure

## 6.2 Identification of Data Needs

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.

**Acute-Duration MRLs.** Data were inadequate to derive acute-duration oral or inhalation MRLs. Acute data were limited to a single report of methemoglobinemia in dogs following a single oral dose (Barnes et al. 1964). Acute-duration studies are needed to identify sensitive targets of toxicity and establish dose-response relationships.

**Intermediate-Duration MRLs.** Data were inadequate to derive intermediate-duration oral or inhalation MRLs. Several limitations were identified in the only available intermediate-duration study (Chen et al. 2014), including examination of a limited number of endpoints, inadequate data reporting, and long recovery period (6 months) prior to examination. Additional intermediate-duration oral studies are needed; these studies should include examination of a wide range of potential targets. Intermediate-duration inhalation studies are needed to identify sensitive targets of toxicity and establish dose-response relationships.

**Chronic-Duration MRLs.** Data were inadequate to derive chronic-duration inhalation MRLs. Chronic-duration inhalation studies are needed to identify sensitive targets of toxicity and establish dose-response relationships. Data were considered adequate to derive a chronic-duration oral MRL; however, only a limited number of nonneoplastic endpoints have been evaluated following chronic oral exposure and a NOAEL for the critical effect (liver toxicity) has not been established. Additional chronic-duration

oral studies examining a comprehensive set of nonneoplastic endpoints over a range of doses would decrease the uncertainty in the MRL.

**Health Effects.** As discussed above, oral and inhalation studies of all durations would be helpful to identify the most sensitive nonneoplastic effects. Specifically, no studies were identified evaluating endocrine, reproductive, immunological, neurological, or developmental endpoints. Studies examining effects on these systems by any route of exposure would be useful.

**Epidemiology and Human Dosimetry Studies.** Human studies on MBOCA consist of either case reports of accidental exposure, occupational health surveys, or retrospective cohort analyses of workers previously exposed to MBOCA. Exposures are expected to be mainly inhalation and dermal. A common limitation in these studies is small sample size, lack of control for concurrent exposures, confounding factors (e.g., smoking), lack of exposure monitoring, and/or exposure via multiple routes. A well-designed prospective occupational study controlling for confounders would better evaluate the potential link between occupational MBOCA exposure and bladder cancer (and/or other health effects). In the absence of additional epidemiological studies, studies designed to evaluate potential MOAs, particularly cancer MOAs, would be useful to determine relevance of animal findings.

**Biomarkers of Exposure and Effect.**

***Exposure.*** Sensitive methods for evaluation of MBOCA in urine are available. Since MBOCA can bind to body proteins and DNA, the presence of MBOCA adducts is an indication of exposure. Although information on some MBOCA metabolites and adducts is available, the development of sensitive methods for their determination is needed. Further identification of those two classes of biomarkers in humans would be helpful in assessing MBOCA exposure levels in high-risk populations.

Studies in rats showed that intraperitoneal injection of ring-labeled MBOCA, or ring-labeled n-acetylated MBOCA, resulted in the generation of three DNA adducts (Silk et al. 1989). These adducts were also produced by the *in vitro* reaction of the N-hydroxy derivative of MBOCA with rat liver slices. The major product of this reaction was also formed following incubation of DNA with N-hydroxy-6-amino-3-chlorobenzyl alcohol, the compound resulting from cleavage of the methylene bridge of N-hydroxy MBOCA. While the single ring species appears to be an intermediate in the formation of the DNA adduct, it is not known whether N-hydroxylation or bridge cleavage occurs first in the formation of the reactive species. The DNA adduct was analytically identified as N-(deoxyadenosin-8-yl)-4-amino-

3-chlorobenzyl alcohol. Since MBOCA adducts can be used as biomarkers of exposure, additional information regarding their characteristics such as half-life would be useful in estimating MBOCA exposures.

***Effect.*** The urinary bladder is a potential target organ for MBOCA-induced carcinogenicity (Dost et al. 2009; Liu et al. 2005; Ward et al. 1988, 1990). Ward et al. (1990) demonstrated that evaluation of urine sediment cytology using the Papnicolaou technique (a current biological monitoring practice for occupational hygiene) is insensitive for detecting lesions in the urinary tract. Data from that study indicate that cystoscopy is a better biomarker of effect. Retrospective biological monitoring using cystoscopy would help identify new biomarkers necessary to characterize the preneoplastic state of the urinary bladder.

**Absorption, Distribution, Metabolism, and Excretion.** Quantitative data on the absorption of MBOCA in humans and animals following all routes of exposure are very limited. Human studies indicate that MBOCA is absorbed rapidly and that the amount absorbed is proportional to the dose for the inhalation (Cocker et al. 1988, 1990; Ichikawa et al. 1990; NIOSH 1986b) and/or dermal routes (Chin et al. 1983; NIOSH 1986b). Data on absorption rates for all three routes are needed. Additional quantitative absorption data in animals via all three routes would be useful because they could be used to estimate absorption in humans.

No studies were located regarding distribution in humans following inhalation, oral, or dermal exposures to MBOCA. Animal kinetic studies (following intraperitoneal or intravenous exposure) in rats and dogs indicate that MBOCA is distributed in the blood to liver, bile, kidney, lung, and fat (Cheever et al. 1991; Farmer et al. 1981; Manis et al. 1984; Morton et al. 1988; Sabbioni and Neumann 1990). It is not known if MBOCA reaches a steady state after repeated exposures. Additional inhalation and dermal exposure studies regarding distribution would be useful because of the potential for human exposure via those two routes.

Limited information is available regarding metabolism in humans (Cocker 1988, 1990; Ducos et al. 1985; Osorio et al. 1990) to MBOCA. Metabolism has been partially characterized in animals following oral exposure. *In vitro* studies investigating the capacity of MBOCA to form adducts characterized one of its metabolites, a product of cleavage between the methylene bridge and one of the aromatic nuclei, as a DNA adduct-forming metabolite, N-hydroxy-MBOCA (MBOCA-NHOH) (Silk et al. 1989). Several MBOCA metabolites were identified following N- and o-hydroxylation of MBOCA by the canine, guinea

pig, and rat liver mixed-function oxidase systems (Chen et al. 1989). Because differences in metabolism may occur with differences in the route of exposure, more data on metabolism following inhalation and dermal exposures would be useful. Also needed is information on MBOCA metabolites in terms of their potential carcinogenic capacities.

There is limited information on excretion in humans occupational exposure showing that the metabolites (N-acetyl MBOCA, β-N-glucuronide of MBOCA) and very limited amounts of parent MBOCA are excreted in the urine (Cocker et al. 1988; Ichikawa et al. 1990; NIOSH 1986b). Studies in rats show that after acute oral exposure to radioactive MBOCA, the majority of the label is in the feces (Farmer et al. 1981; Groth et al. 1984). More information is needed on the excretion rate in animals after exposure to MBOCA via all three routes in order to establish which is the major route of excretion.

**Comparative Toxicokinetics.** Studies using rats (Farmer et al. 1981; Groth et al. 1984; Morton et al. 1988; Tobes et al. 1983) and dogs (Manis et al. 1984b) indicate that the kinetics of MBOCA do not differ significantly across species and that the differences are primarily quantitative. Since the kinetic data alone do not allow for the identification of target organs common to humans and animals, additional studies on the distribution and toxicity may allow for identification of similar target organs. Additional studies in dogs would be helpful since they are similar to humans in that they develop bladder cancer following exposure to MBOCA. No animal data on toxicokinetics were located regarding interspecies differences or sex-related differences. The limited amount of animal data, as well as a relative lack of data across different routes of exposure, indicate that it may be difficult to compare the kinetics of MBOCA in animals with that in humans. Additional studies using several species and all three exposure routes are needed in order to determine similarities and differences between humans and animals.

**Children's Susceptibility.** No human or animal data are available regarding children's susceptibility. Children are not likely to be exposed to MBOCA; however, the potential for MBOCA to cause developmental effects in pregnant workers has not been evaluated. Developmental studies would be useful to address this data gap.

**Physical and Chemical Properties.** The physical and chemical properties of MBOCA are sufficiently defined (see Chapter 4) to allow assessments of the environmental fate of the compound to be made; no further information is needed.

**Production, Import/Export, Use, Release, and Disposal.** Presently, conflicting information exists on the number of people who have been or are being exposed to MBOCA in the workplace; the numbers range from 114 (NOES 1992) to 2,094 (Schulte et al. 1988). The general population is not likely to be exposed to MBOCA.

Information is unavailable on current or historical MBOCA production in the United States. MBOCA is only used in the workplace in 24 facilities in the United States (TR15 2017). Information on potential food contamination with MBOCA would also be useful in reducing risks associated with general population exposures. MBOCA may be released to the environment in waste waters or fugitive emissions from plants. Additional information is needed on atmospheric releases of MBOCA from manufacturing facilities to assess the potential for general population exposure.

At the present time, there is no information on the amounts of MBOCA disposed by different methods except for TRI data on the amounts released into different media (see Tables 5-1 and 5-2). Additional information on currently used disposal methods would allow the determination of their efficiency. Also needed is information on the availability of MBOCA residues from polyurethanes and other plastics.

**Environmental Fate.** The fate of MBOCA in soil has been described (EPA 1979; Voorman and Penner 1986a; Yoneyama and Matsumura 1984), and some information is available on the spread and transport of MBOCA in surface waters (Parris et al. 1980). Additional data on the aquatic fate of MBOCA, its residence time in the water column, and its absorption to sediment or organic matter in the water would assist in assessing drinking water contamination. Information on the fate of MBOCA adsorbed to sediment would be useful in assessing uptake by aquatic organisms and re-entry of MBOCA into the water column. Information on the half-life of MBOCA in the environment would also be useful for assessing the risk for human exposure.

**Bioavailability from Environmental Media.** Available pharmacokinetic data suggest that MBOCA is absorbed by humans following dermal and inhalation exposures (Chin et al. 1983; Cocker et al. 1988, 1990; Ichikawa et al. 1990; NIOSH 1986b). MBOCA has been measured in the urine of workers following dermal and/or inhalation exposures, suggesting rapid absorption and excretion. Information on the absorption of MBOCA by humans as a result of ingestion of contaminated water or food has not been found and would be useful in assessing the uptake of MBOCA from contaminated foods. Further information on the uptake of MBOCA by all three exposure routes, particularly the differentiation of

dermal and inhalation exposure in workers, would be helpful in determining potential uptake of MBOCA as a result of exposure to contaminated air, water, or foods, or contact with contaminated surfaces.

**Food Chain Bioaccumulation.** The bioconcentration factor of MBOCA has been estimated to be 5.75 in aquatic organisms (HSDB 1991). In addition, it has been shown that MBOCA binds to and penetrates the roots of plants grown in contaminated soil and is not easily removed by rinsing. However, MBOCA stays very close to the root surface and is not distributed throughout the plant, and the roots bioaccumulate the chemical (Voorman and Penner 1986b). This information suggests that there is a potential for food chain bioaccumulation both from aquatic organisms and the root systems of terrestrial plants. Actual data on the potential for aquatic organisms to bioaccumulate MBOCA would be useful in determining potential food chain concentrations.

**Exposure Levels in Environmental Media.** Some information exists on levels of MBOCA found in the workplace and the environment around facilities that manufacture or use MBOCA (Keeslar 1986; Parris et al. 1980). Further information on atmospheric levels of MBOCA in areas other than the workplace would be helpful for estimating general population exposure.

Reliable monitoring data for the levels of MBOCA at hazardous waste sites are also needed. The information collected on levels of MBOCA in the environment could be combined with the information on body burden to assess the potential risk of adverse health effects in populations living near hazardous waste sites.

**Exposure Levels in Humans.** Certain population groups are known to have a higher risk of exposure to MBOCA than others. The highest exposures are found in workers at manufacturing facilities that use MBOCA in the production of polyurethane plastics (Clapp et al. 1991; Ichikawa et al. 1990; Schulte et al. 1988; Ward et al. 1987). The next highest levels are found in populations that live near facilities where uncontrolled MBOCA releases occur (Keeslar 1986). Specific information on where such releases occur, on the populations living near such facilities, and on the levels to which they may be exposed was not found. This information is needed to assess whether health studies on these populations need to be conducted.

**Exposures of Children.** Children are not likely to be exposed to MBOCA unless they live near facilities where uncontrolled MBOCA releases occur (Keeslar 1986). Specific information on where such

releases occur and on the levels to which children may be exposed was not found. This information is needed to assess whether health studies on children need to be conducted.

**Analytical Methods.** Validated analytical methods exist for determination of MBOCA in urine and hemoglobin adducts (Robert et al. 1999a, 1999b; Vaughan and Kenyon 1996)

## 6.3 Ongoing Studies

No ongoing studies were identified for MBOCA.

# CHAPTER 7. REGULATIONS AND GUIDELINES

Pertinent international and national regulations, advisories, and guidelines regarding MBOCA 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 MBOCA.

**Table 7-1. Regulations and Guidelines Applicable to MBOCA**

| Agency | Description | Information | Reference |
|---|---|---|---|
| | **Air** | | |
| EPA | RfC | No data | IRIS 2017 |
| WHO | Air quality guidelines | No data | WHO 2010 |
| | **Water & Food** | | |
| EPA | Drinking water standards and health advisories | No data | EPA 2012 |
| | National primary drinking water regulations | No data | EPA 2009 |
| | RfD | No data | IRIS 2017 |
| WHO | Drinking water quality guidelines | No data | WHO 2017 |
| FDA | EAFUS | No data[a] | FDA 2013 |
| | **Cancer** | | |
| ACGIH | Carcinogenicity classification | A2[b,c] | ACGIH 2001, 2016 |
| HHS | Carcinogenicity classification | Reasonably anticipated to be a human carcinogen[d] | NTP 2016 |
| EPA | Carcinogenicity classification | No data | IRIS 2017 |
| IARC | Carcinogenicity classification | Group 1[e,f] | IARC 2012, 2017 |
| | **Occupational** | | |
| ACGIH | TLV | 0.01 ppm[g] | ACGIH 2016 |
| OSHA | PEL (8-hour TWA) for general industry, shipyards and construction | No data | OSHA 2016a, 2016b, 2016c |
| NIOSH | REL (up to 10-hour TWA) | 0.003 mg/m$^3$ [g,h] | NIOSH 2016 |

## Table 7-1. Regulations and Guidelines Applicable to MBOCA

| Agency | Description | Information | Reference |
|---|---|---|---|
| | **Emergency Criteria** | | |
| EPA | AEGLs-air | No data | EPA 2016 |
| AIHA | ERPGs | No data | AIHA 2015 |
| DOE | PACs-air | | DOE 2016a |
| | PAC-1[i] | 0.03 ppm | |
| | PAC-2[i] | 0.94 ppm | |
| | PAC-3[i] | 21 ppm | |

[a]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.
[b]A2: Suspected human carcinogen.
[c]Based on clear evidence of lung and liver tumors in rats and mice and bladder tumors in dogs following prolonged exposure.
[d]Based on sufficient evidence of carcinogenicity from studies in experimental animals.
[e]Group 1: Carcinogenic to humans.
[f]Based on sufficient evidence in experimental animals for carcinogenicity and strong mechanistic evidence indicating carcinogenicity.
[g]Skin notation: Potential significant contribution to the overall exposure by the cutaneous route.
[h]Potential occupational carcinogen.
[i]Definitions of PAC terminology are available from U.S. DOE (2016b).

ACGIH = American Conference of Governmental Industrial Hygienists; AEGL = acute exposure guideline levels; AIHA = American Industrial Hygiene Association; DOE = Department of Energy; 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; HHS = Department of Health and Human Services; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; MBOCA = 4,4'-methylenebis(2-chloroaniline); 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

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# APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

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.

APPENDIX A

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.

APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 4,4'-Methylenebis(2-chloroaniline) (MBOCA)
***CAS Numbers:*** 101-14-4
***Date:*** May 1994
March 2017—Updated literature search
***Profile Status:*** Final
***Route:*** Inhalation
***Duration:*** Acute

***MRL Summary:*** There are insufficient data for derivation of an acute inhalation MRL.

***Rationale for Not Deriving an MRL:*** No acute inhalation studies were identified for MBOCA.

***Agency Contact (Chemical Manager):*** Dennis Jones, D.V.M.

APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 4,4'-Methylenebis(2-chloroaniline) (MBOCA)
***CAS Numbers:*** 101-14-4
***Date:*** May 1994
March 2017—Updated literature search
***Profile Status:*** Final
***Route:*** Inhalation
***Duration:*** Intermediate

***MRL Summary:*** There are insufficient data for derivation of an intermediate-duration inhalation MRL.

***Rationale for Not Deriving an MRL:*** No intermediate-duration inhalation studies were identified for MBOCA.

***Agency Contact (Chemical Manager):*** Dennis Jones, D.V.M.

APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 4,4'-Methylenebis(2-chloroaniline) (MBOCA)
***CAS Numbers:*** 101-14-4
***Date:*** May 1994
March 2017—Updated literature search
***Profile Status:*** Final
***Route:*** Inhalation
***Duration:*** Chronic

***MRL Summary:*** There are insufficient data for derivation of a chronic-duration inhalation MRL.

***Rationale for Not Deriving an MRL:*** No chronic-duration inhalation studies were identified for MBOCA.

***Agency Contact (Chemical Manager):*** Dennis Jones, D.V.M.

APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 4,4'-Methylenebis(2-chloroaniline) (MBOCA)
***CAS Numbers:*** 101-14-4
***Date:*** May 1994
March 2017—Updated literature search
***Profile Status:*** Final
***Route:*** Oral
***Duration:*** Acute

***MRL Summary:*** There are insufficient data for derivation of an acute-duration oral MRL.

***Rationale for Not Deriving an MRL:*** Acute oral studies were limited to a single dose study in dogs reporting methemoglobinemia (Barnes, 1964); however, dose information was not available.

***Agency Contact (Chemical Manager):*** Dennis Jones, D.V.M.

APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 4,4'-Methylenebis(2-chloroaniline) (MBOCA)
***CAS Numbers:*** 101-14-4
***Date:*** May 1994
March 2017—Updated literature search
***Profile Status:*** Final
***Route:*** Oral
***Duration:*** Intermediate

***MRL Summary:*** There are insufficient data for derivation of an intermediate-duration oral MRL.

***Rationale for Not Deriving an MRL:*** Only one intermediate-duration oral study was identified (Chen et al. 2014). This study exposed male ICR mice to 0, 50, 100, or 200 mg/kg/day for 3 months via drinking water. Mice were then given standard water for an additional 6 months prior to sacrifice. All high-dose animals died prior to scheduled sacrifice. Effects reported in animals that survived until scheduled sacrifice included degeneration and/or dysplasia in several organs at the lower doses (stomach, intestines, liver, kidney, bladder); however, incidence data for all lesions in each organ were combined for surviving animals from these dose groups. Due to examination of a limited number of endpoints, inadequate data reporting, and long recovery period (6 months) prior to examination, this study is considered inadequate to use as the basis of the intermediate-duration oral MRL.

***Agency Contact (Chemical Manager):*** Dennis Jones, D.V.M.

APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 4,4'-Methylenebis(2-chloroaniline) (MBOCA)
***CAS Numbers:*** 101-14-4
***Date:*** May 1994
March 2017—Updated literature search
***Profile Status:*** Final
***Route:*** Oral
***Duration:*** Chronic
**MRL** 0.003 mg/kg/day
***Critical Effect:*** Hepatic toxicity (increased ALT, nodular hepatic hyperplasia)
***Reference:*** Stula et al. 1977
***Point of Departure:*** LOAEL of 10 mg/kg/day
***Uncertainty Factor:*** 3,000
***LSE Graph Key:*** 9
***Species:*** Dog

***MRL Summary:*** A chronic-duration oral MRL of 0.003 mg/kg/day was derived for MBOCA based on evidence of hepatic toxicity in dogs exposed to concentrations ≥10 mg/kg/day via capsule for 9 years (3–5 days/week), including elevated ALT and nodular hepatic hyperplasia; a NOAEL was not identified for hepatic effects (Stula et al.1977). The MRL is based on the LOAEL of 10 mg/kg/day for hepatic effects and a total uncertainty factor of 3,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, 10 for human variability, and 3 for limitations in the database).

***Selection of the Critical Effect:*** Available chronic oral studies were predominately focused on carcinogenic effects of MBOCA. Based on review of the limited data regarding nonneoplastic results (see Table A-1), the most sensitive effect appears to be liver effects in dogs exposed to 10 mg/kg/day (Stula et al. 1977). Effects noted at higher doses in rats included decreased body weight at 25 mg/kg/day (Russfield et al. 1975) and various hepatic lesions in rats exposed to 50 mg/kg/day (Stula et al. 1975); no NOAELs were identified for these effects.

**Table A-1. Summary of Candidate Critical Effects for Chronic Oral MRL for MBOCA**

| Species | Duration | NOAEL (mg/kg/day) | LOAEL (mg/kg/day) | Effect | Reference |
|---|---|---|---|---|---|
| Hepatic effects[a] | | | | | |
| Dog | 9 years | ND | 10 | Increased ALT, nodular hepatic hyperplasia | Stula et al. 1977 |
| Rat | 2 years | ND | 50 (SLOAEL) | Hepatocytomegaly, fatty change, necrosis, fibrosis, bile duct proliferation | Stula et al. 1975 |
| Body weight effects | | | | | |
| Rat | 18 months | ND | 25 | Decreased body weight | Russfield et al. 1975 |

**[a]Selected critical effect.**

LOAEL = lowest observed adverse effect level; ND = not determined; NOAEL = no-observed-adverse-effect level;

**Table A-1. Summary of Candidate Critical Effects for Chronic Oral MRL for MBOCA**

| Species | Duration | NOAEL (mg/kg/day) | LOAEL (mg/kg/day) | Effect | Reference |
|---|---|---|---|---|---|

SLOAEL = serious LOAEL

***Selection of the Principal Study:*** The study with the lowest identified LOAEL for the critical effect of hepatic toxicity was selected as the principal study (Stula et al. 1977).

***Summary of the Principal Study:***

Stula EF, Barnes JR, Sherman H, et al. 1977. Urinary bladder tumors in dogs from 4,4'-methylenebis(2-chloroaniline) (MOCA). J Environ Pathol Toxicol l(l):3 l-50.

Six 1-year old female Beagle dogs were given a gelatin capsule with 100 mg of MBOCA for 3 days/week for the first 6 weeks, then 5 days/week for up to 9 years. The dose was 8–15 mg/kg/day with an average of 10 mg/kg/day for the 9-year period. Six untreated female dogs served as control. Dogs were weighed weekly. Blood was collected periodically (~2 times/year) for hematology and biochemistry. Urine was collected at the same time for urinalysis and urine sediment cytology. A complete necropsy was performed on all animals with histopathological examination for tumors.

One of six female beagle dogs died after 3.4 years of oral administration; however, the report concludes that the death was not MBOCA-related, because the dog died from pyelonephritis. No evidence of an association between pyelonephritis and MBOCA was identified. No clinical signs of toxicity or body weight effects were noted. No changes in hemoglobin, hematocrit, erythrocyte count, or mononuclear leukocyte count were noted. Statistically significant increase in ALT was observed in MBOCA treated dogs, but not in controls. The highest level of ALT occurred during the first 2 years and after 7–8.5 years of treatment. This change was an indication of liver damage. Histopathology revealed nodular hepatic hyperplasia in 3/5 treated dogs. There was also a disruption of liver architecture; all of these changes were MBOCA-related and were absent in the control animals. Nonmalignant focal liver cell alterations were common in both treated and control dogs. Cystic hyperplasia of the pars intermedia of the anterior pituitary gland was found in one of five female beagle dogs after 8.3 years of treatment. This change was not present in other treated dogs or in controls and was not considered to be treatment related.

Urine sediment cytology revealed abnormalities suggestive of neoplasia of the genitourinary system in dogs after 7 years of MBOCA treatment. The observed changes correspond to grades IV and V of human urinary cells by the Papanicolaou technique. Two of the treated dogs had small amount of blood in the urine observed after 8 years of MBOCA treatment. Three dogs had papillary transitional cell carcinoma of the urinary bladder, tumors infiltrated the lamina propria but not the muscle layer of the bladder. There was no evidence of metastasis to other organs. One dog developed a tumor close to the urethra. Histologically, it was a combined infiltrating transitional cell carcinoma and adenocarcinoma of the urethra. The adenocarcinoma seems to have metastasized to part of the tumor. In spite of the use of a small number of animals, this study shows that the ingestion of MBOCA over 9 years was associated with the incidence of urinary bladder cancer.

***Selection of the Point of Departure for the MRL:*** The LOAEL of 10 mg/kg/day for hepatic toxicity in dogs was selected as the point of departure for derivation of the chronic-duration oral MRL.

.

***Calculations:*** Average dose per dog was calculated by study authors based on average body weight.

***Uncertainty Factor:*** The LOAEL is divided by a total uncertainty factor of 3,000:

- 10 for use of a LOAEL
- 10 for extrapolation from animals to humans
- 10 for human variability
- 3 for database limitations (e.g., limited examination of nonneoplastic endpoints, lack of reproductive/developmental studies)

MRL = The LOAEL ÷ uncertainty factors
10 mg/kg/day ÷ (10 x 10 x 10 x 3) = 0.003 mg/kg/day

***Other Additional Studies or Pertinent Information that Lend Support to this MRL:*** As shown in Table A-1, nonneoplastic effects have been observed in both dogs and rats following chronic oral exposure at the lowest doses evaluated (Stula et al. 1975, 1977). Hepatic degeneration and/or dysplasia have also been observed in mice following subchronic oral or dermal exposure to MBOCA (Chen et al. 2014).

***Agency Contact (Chemical Manager):*** Dennis Jones, D.V.M.

# APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR 4,4'-METHYLENEBIS(2-CHLOROANILINE)

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 4,4'-methylenebis(2-chloroaniline).

## 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, and chemical interactions data for 4,4'-methylenebis(2-chloroaniline). 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 4,4'-methylenebis(2-chloroaniline) 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 4,4'-methylenebis-(2-chloroaniline) 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
    - Neurological effects
    - Reproductive effects

**Table B-1. Inclusion Criteria for the Literature Search and Screen**

| | |
|---|---|
| | Developmental effects |
| | Other noncancer effects |
| | Cancer |
| Toxicokinetics | |
| | Absorption |
| | Distribution |
| | Metabolism |
| | Excretion |
| | PBPK models |
| Biomarkers | |
| | Biomarkers of exposure |
| | Biomarkers of effect |
| Interactions with other chemicals | |

### B.1.1 Literature Search

The current literature search was intended to update the health effects sections of the existing toxicological profile for 4,4'-methylenebis(2-chloroaniline) (ATSDR 1994), thus, the literature search was restricted to studies published between January 1992 to March 2017. The following main databases were searched in March 2017:

- 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, and Medical Subject Headings (MeSH) terms for 4,4'-methylenebis(2-chloroaniline). 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 4,4'-methylene-bis(2-chloroaniline) 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.

APPENDIX B

## Table B-2. Database Query Strings

| Database search date | Query string |
|---|---|
| **PubMed** | |
| 03/2017 | ((101-14-4[rn] OR 3L2W5VTT2A[rn] OR "Methylenebis(chloroaniline)"[MeSH] OR "Methylenebis(chloroaniline)"[nm]) AND (1992/01/01 : 3000[dp] OR 1992/01/01 : 3000[mhda])) OR (("3,3'-Dichlor-4,4'-diaminodiphenylmethan"[tw] OR "3,3'-Dichloro-4,4'-diaminodifenilmetano"[tw] OR "3,3'-Dichloro-4,4'-diaminodiphenylmethane"[tw] OR "3,3'-Dicloro-4,4'-diaminodifenilmetano"[tw] OR "4,4'-Diamino-3,3'-dichlorodiphenylmethane"[tw] OR "4,4'-Methylene(bis)-chloroaniline"[tw] OR "4,4'-Methylenebis(2-chloroaniline)"[tw] OR "4,4'-Methylenebis(o-chloroaniline)"[tw] OR "4,4'-Methylenebis-2-chlorobenzenamine"[tw] OR "4,4-Metilene-bis-o-cloroanilina"[tw] OR "4,4'-methylenebis(2-chloro-Aniline)"[tw] OR "4,4'-methylenebis(2-chloro-Benzenamine)"[tw] OR "Bis amine"[tw] OR "Bis(3-chloro-4-aminophenyl)methane"[tw] OR "Bis(4-amino-3-chlorophenyl)methane"[tw] OR "Bis-amine A"[tw] OR "Bisamine"[tw] OR "Bisamine S"[tw] OR "CL-Mda"[tw] OR "Cuamine M"[tw] OR "Cuamine MT"[tw] OR "Curalin M"[tw] OR "Curene 442"[tw] OR "Cyanaset"[tw] OR "Dacpm"[tw] OR "Di(-4-amino-3-chlorophenyl)methane"[tw] OR "Di-(4-amino-3-clorofenil)metano"[tw] OR "Diamet Kh"[tw] OR "LD 813"[tw] OR "MBOCA"[tw] OR "Methylene 4,4'-bis(o-chloroaniline)"[tw] OR "Methylene-bis-orthochloroaniline"[tw] OR "Methylenebis(3-chloro-4-aminobenzene)"[tw] OR "Methylenebis(chloroaniline)"[tw] OR "Methylene Bis(chloroaniline)"[tw] OR "Millionate M"[tw] OR "p, p'-Methylenebis(alpha-chloroaniline)"[tw] OR "p, p'-Methylenebis(o-chloroaniline)"[tw] OR "p, p'-Methylenebis(ortho-chloroaniline)"[tw] OR "Quodorole"[tw] OR "4,4'-Methylene bis(2-chloroaniline)"[tw]) AND (1992/01/01 : 3000[dp] OR 1992/01/01 : 3000[crdat] OR 1992/01/01 : 3000[edat])) |
| **Toxline** | |
| 03/2017 | ( "3 3'-dichlor-4 4'-diaminodiphenylmethan" OR "3 3'-dichloro-4 4'-diaminodifenilmetano" OR "3 3'-dichloro-4 4'-diaminodiphenylmethane" OR "3 3'-dicloro-4 4'-diaminodifenilmetano" OR "4 4'-diamino-3 3'-dichlorodiphenylmethane" OR "4 4'-methylene ( bis ) -chloroaniline" OR "4 4'-methylenebis ( 2-chloroaniline ) " OR "4 4'-methylenebis ( o-chloroaniline ) " OR "4 4'-methylenebis-2-chlorobenzenamine" OR "4 4-metilene-bis-o-cloroanilina" OR "4 4'-methylenebis ( 2-chloro-aniline ) " OR "4 4'-methylenebis ( 2-chloro-benzenamine ) " OR "bis amine" OR "bis ( 3-chloro-4-aminophenyl ) methane" OR "bis ( 4-amino-3-chlorophenyl ) methane" OR "bis-amine a" OR "bisamine" OR "bisamine s" OR "cl-mda" OR "cuamine m" OR "cuamine mt" OR "curalin m" OR "curene 442" OR "cyanaset" OR "dacpm" OR "di ( -4-amino-3-chlorophenyl ) methane" OR "di- ( 4-amino-3-clorofenil ) metano" OR "diamet kh" OR "ld 813" OR "mboca" OR "methylene 4 4'-bis ( o-chloroaniline ) " OR "methylene-bis-orthochloroaniline" OR "methylenebis ( 3-chloro-4-aminobenzene ) " OR "methylenebis ( chloroaniline ) " OR "methylene bis ( chloroaniline ) " OR "millionate m" OR "p p'-methylenebis ( alpha-chloroaniline ) " OR "p p'-methylenebis ( o-chloroaniline ) " OR "p p'-methylenebis ( ortho-chloroaniline ) " OR "quodorole" OR "4 4'-methylene bis ( 2-chloroaniline ) " OR 101-14-4 [rn] ) AND 1992:2017 [yr] AND ( ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org] ) AND NOT PubMed [org] AND NOT pubdart [org] |
| **Toxcenter** | |
| 03/2017 | FILE 'TOXCENTER' ENTERED AT 10:51:42 ON 21 MAR 2017<br>L1 867 SEA 101-14-4<br>L2 859 SEA L1 NOT TSCATS/FS<br>L3 766 SEA L2 NOT PATENT/DT<br>L4 312 SEA L3 AND PY>=1992 |

## Table B-2. Database Query Strings

| Database search date | Query string |
|---|---|
| | ACTIVATE TOXQUERY/Q |
| | --- |
| | L5 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) |
| | L6 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) |
| | L7 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) |
| | L8 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT |
| | L9 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) |
| | L10 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) |
| | L11 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?) |
| | L12 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE)) |
| | L13 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?) |
| | L14 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?) |
| | L15 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?) |
| | L16 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?) |
| | L17 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?) |
| | L18 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?) |
| | L19 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?) |
| | L20 QUE (ENDOCRIN? AND DISRUPT?) |
| | L21 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?) |
| | L22 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?) |
| | L23 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?) |
| | L24 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?) |
| | L25 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?) |
| | L26 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?) |
| | L27 QUE (NEPHROTOX? OR HEPATOTOX?) |
| | L28 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?) |
| | L29 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?) |
| | L30 QUE L5 OR L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR |

## Table B-2. Database Query Strings

| Database search date | Query string |
|---|---|
| | L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29 |
| | L31 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE |
| | OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE |
| | OR PORCINE OR MONKEY? OR MACAQUE?) |
| | L32 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA |
| | OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE) |
| | L33 QUE L30 OR L31 OR L32 |
| | L34 QUE (NONHUMAN MAMMALS)/ORGN |
| | L35 QUE L33 OR L34 |
| | L36 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR |
| | PRIMATES OR PRIMATE?) |
| | L37 QUE L35 OR L36 |
| | --- |
| | L38 269 SEA L4 AND L37 |
| | L39 42 SEA L38 AND MEDLINE/FS |
| | L40 26 SEA L38 AND BIOSIS/FS |
| | L41 176 SEA L38 AND CAPLUS/FS |
| | L42 25 SEA L38 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS) |
| | L43 207 DUP REM L39 L40 L42 L41 (62 DUPLICATES REMOVED) |
| | L*** DEL 42 S L38 AND MEDLINE/FS |
| | L*** DEL 42 S L38 AND MEDLINE/FS |
| | L44 42 SEA L43 |
| | L*** DEL 26 S L38 AND BIOSIS/FS |
| | L*** DEL 26 S L38 AND BIOSIS/FS |
| | L45 15 SEA L43 |
| | L*** DEL 176 S L38 AND CAPLUS/FS |
| | L*** DEL 176 S L38 AND CAPLUS/FS |
| | L46 135 SEA L43 |
| | L*** DEL 25 S L38 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS) |
| | L*** DEL 25 S L38 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS) |
| | L47 15 SEA L43 |
| | L48 165 SEA (L44 OR L45 OR L46 OR L47) NOT MEDLINE/FS |
| | D SCAN L48 |

## Table B-3. Strategies to Augment the Literature Search

| Source | Query and number screened when available |
|---|---|
| **TSCATS[a]** | |
| 03/2017 | Compound searched: 101-14-4 |
| **NTP** | |
| 03/2017 | 101-14-4<br>3,3'-Dichloro-4,4'-diaminodifenilmetano<br>3,3'-Dichloro-4,4'-diaminodiphenylmethane<br>4,4'-Diamino-3,3'-dichlorodiphenylmethane |

## Table B-3. Strategies to Augment the Literature Search

| Source | Query and number screened when available |
|---|---|
| | 4,4'-Methylene(bis)-chloroaniline<br>4,4'-Methylenebis(2-chloroaniline)<br>4,4'-Methylenebis(o-chloroaniline)<br>4,4'-Methylenebis-2-chlorobenzenamine<br>4,4-Metilene-bis-o-cloroanilina<br>4,4'-methylenebis(2-chloro-Aniline)<br>4,4'-methylenebis(2-chloro-Benzenamine)<br>Bis amine<br>Bis(3-chloro-4-aminophenyl)methane<br>Bis(4-amino-3-chlorophenyl)methane<br>Di(-4-amino-3-chlorophenyl)methane<br>Di-(4-amino-3-clorofenil)metano<br>MBOCA<br>Methylene 4,4'-bis(o-chloroaniline)<br>Methylene-bis-orthochloroaniline<br>Methylenebis(3-chloro-4-aminobenzene)<br>Methylenebis(chloroaniline)<br>Methylene Bis(chloroaniline)<br>p,p'-Methylenebis(alpha-chloroaniline)<br>p,p'-Methylenebis(o-chloroaniline)<br>p,p'-Methylenebis(ortho-chloroaniline)<br>4,4'-Methylene bis(2-chloroaniline) |
| **NIH RePORTER** | |
| 06/2017 | Text Search: "101-14-4" OR "3,3'-Dichlor-4,4'-diaminodiphenylmethan" OR "3,3'-Dichloro-4,4'-diaminodifenilmetano" OR "3,3'-Dichloro-4,4'-diaminodiphenylmethane" OR "3,3'-Dicloro-4,4'-diaminodifenilmetano" OR "4,4'-Diamino-3,3'-dichlorodiphenylmethane" OR "4,4'-Methylene(bis)-chloroaniline" OR "4,4'-Methylenebis(2-chloroaniline)" OR "4,4'-Methylenebis(o-chloroaniline)" OR "4,4'-Methylenebis-2-chlorobenzenamine" OR "4,4-Metilene-bis-o-cloroanilina" OR "4,4'-methylenebis(2-chloro-Aniline)" OR "4,4'-methylenebis(2-chloro-Benzenamine)" OR "Bis amine" OR "Bis(3-chloro-4-aminophenyl)methane" OR "Bis(4-amino-3-chlorophenyl)methane" OR "Bis-amine A" OR "Bisamine" OR "Bisamine S" OR "CL-Mda" OR "Cuamine M" OR "Cuamine MT" OR "Curalin M" OR "Curene 442" OR "Cyanaset" OR "Dacpm" OR "Di(-4-amino-3-chlorophenyl)methane" OR "Di-(4-amino-3-clorofenil)metano" OR "Diamet Kh" OR "LD 813" OR "MBOCA" OR "Methylene 4,4'-bis(o-chloroaniline)" OR "Methylene-bis-orthochloroaniline" OR "Methylenebis(3-chloro-4-aminobenzene)" OR "Methylenebis(chloroaniline)" OR "Methylene Bis(chloroaniline)" OR "Millionate M" OR "p,p'-Methylenebis(alpha-chloroaniline)" OR "p,p'-Methylenebis(o-chloroaniline)" (Advanced), Search in: Projects Admin IC: All, Fiscal Year: Active Projects |
| **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 2017 results were:

- Number of records identified from PubMed, TOXLINE, and TOXCENTER (after duplicate removal): 361
- Number of records identified from other strategies: 26
- Total number of records to undergo literature screening: 387

### B.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on 4,4'-methylenebis(2-chloroaniline):

- 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.

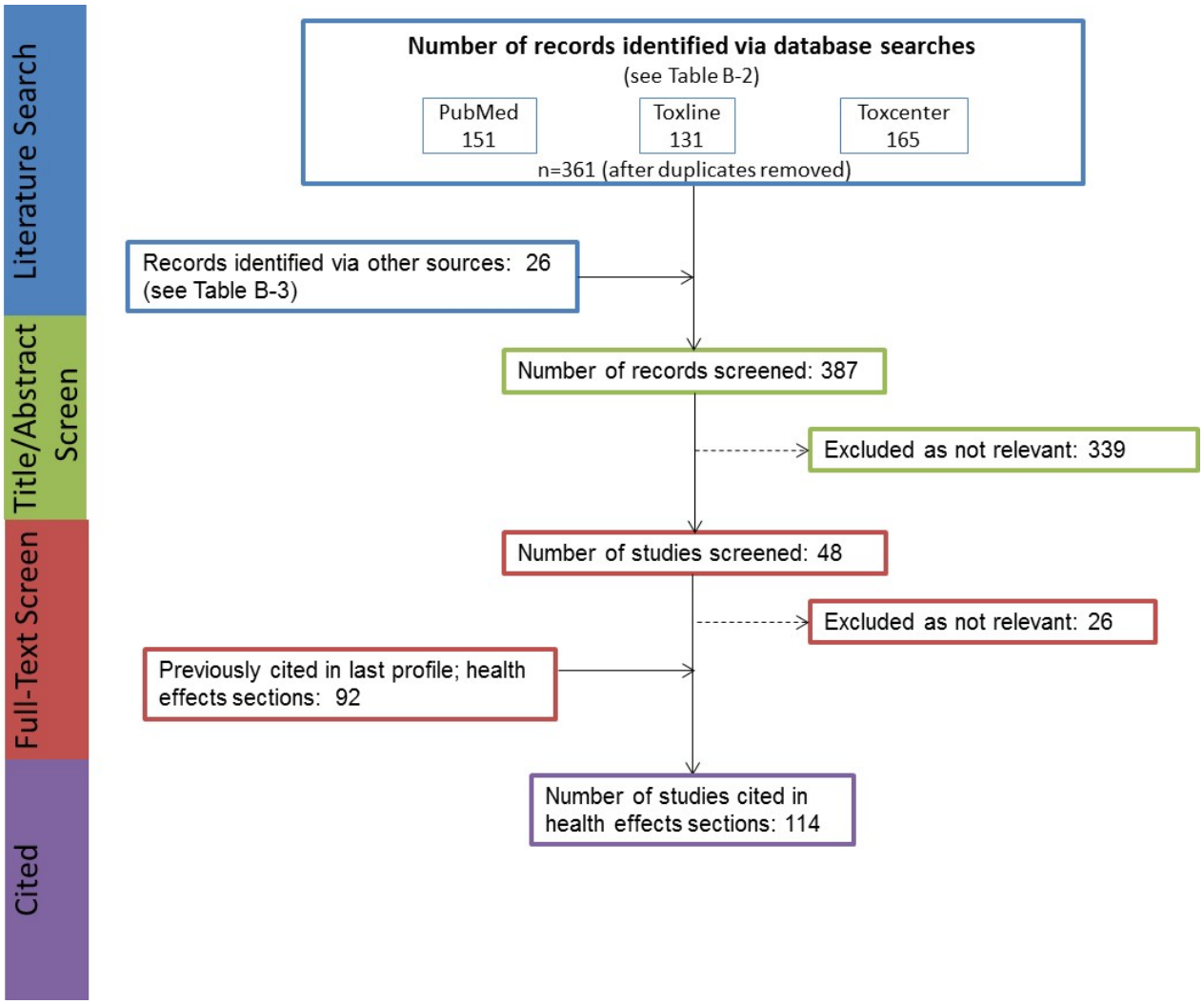
- Number of titles and abstracts screened: 387
- Number of studies considered relevant and moved to the next step: 48

***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: 48
- Number of studies cited in the health effects sections of the existing toxicological profile (May, 1994): 92
- Total number of studies cited in the health effects sections of the updated profile: 114

A summary of the results of the literature search and screening is presented in Figure B-1.

**Figure B-1. March 2017 Literature Search Results and Screen for 4,4'-Methylenebis(2-chloroaniline)**



# APPENDIX C. USER'S GUIDE

## 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-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 C-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.

APPENDIX C

**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** | | | | | | | | | |

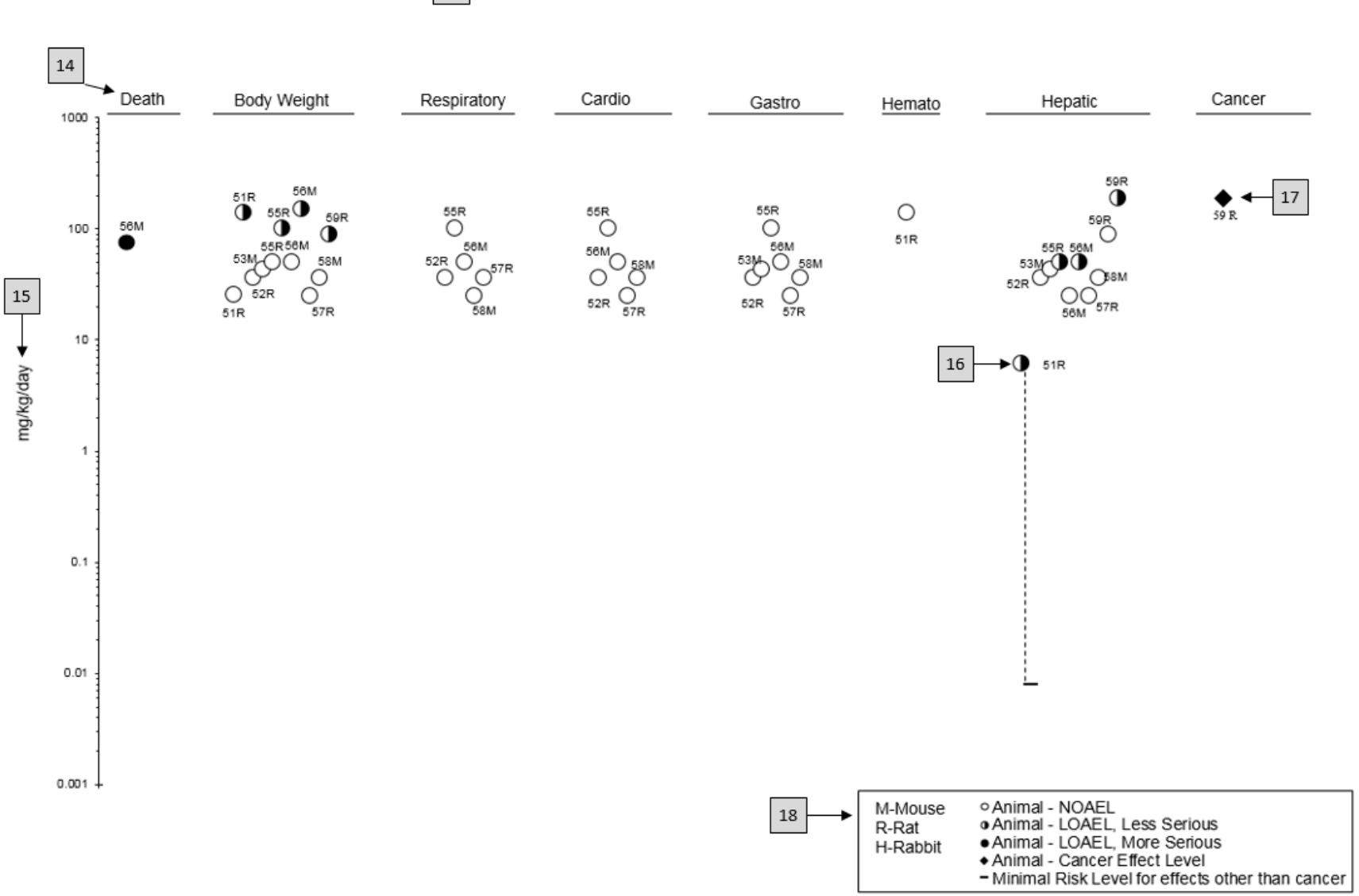
[a]The number corresponds to entries in Figure 2-x.

(11) [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).

[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 C

**Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral**



# APPENDIX D. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances 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 E. GLOSSARY

**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 F. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

| | |
|---|---|
| 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 |