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.

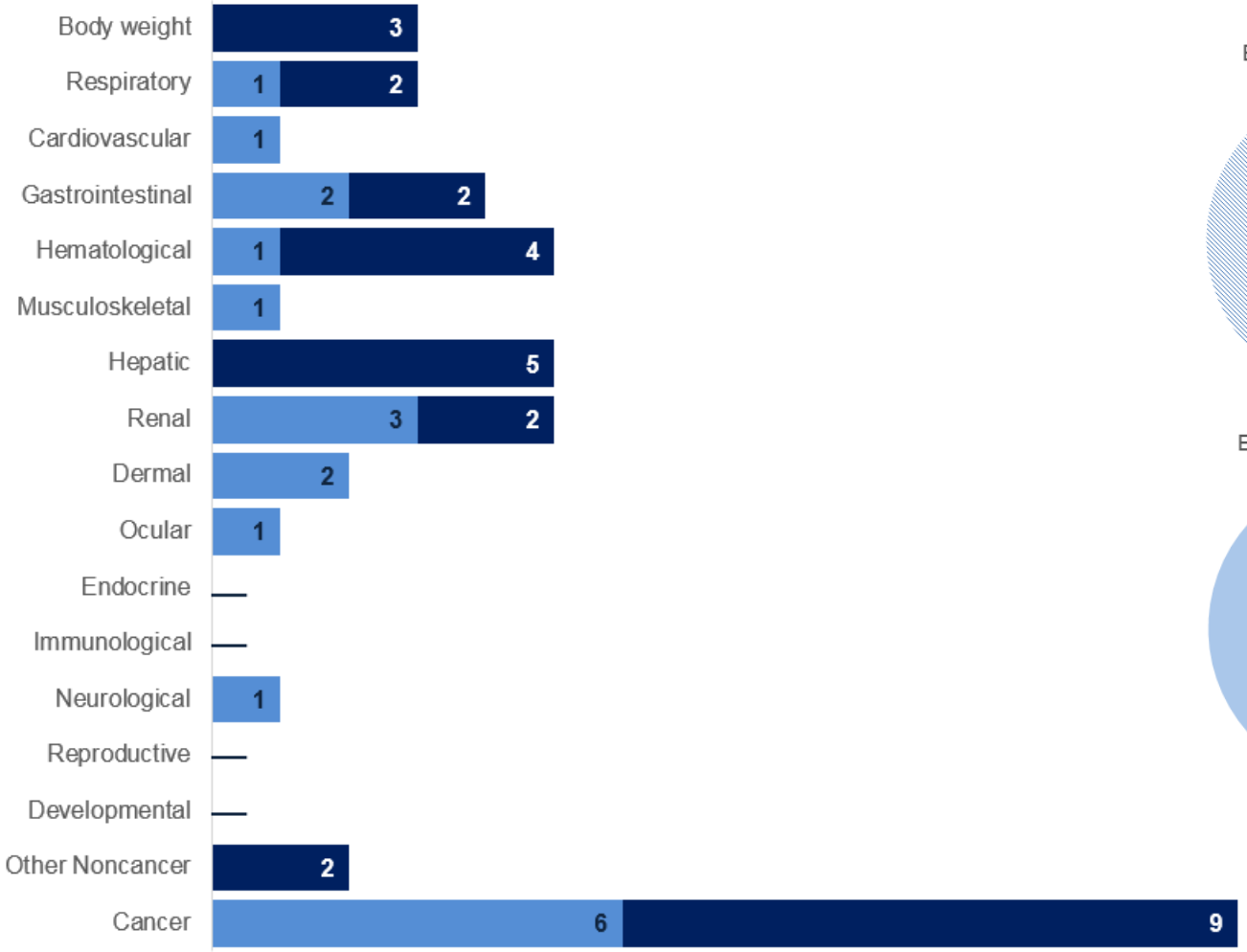
- **Body Weight Endpoint:** Mild decreases in body weight were observed in rats following chronic oral exposure, but not mice or dogs.

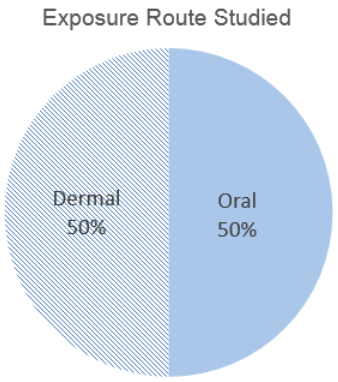
- **Cancer:** A small number of retrospective occupational cohort studies and case reports report bladder cancer in humans following occupational exposure to MBOCA. Chronic oral studies in animals indicate that MBOCA is a multi-site carcinogen in rats, mice, and dogs. Observed tumors include lung, liver,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

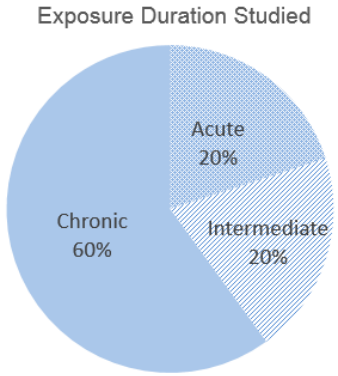
Figure 2-1. Overview of the Number of Studies Examining MBOCA Health Effects

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 Exposure groups: 31 current workers exposed to MBOCA (6 months–16 years); 172 workers formerly exposed to MBOCA Referent groups: 31 never-exposed referents (current employees); all former workers (number not reported)	Current maximum exposure levels: Personal air monitoring level: 0.02 mg/m^3 (0.002 ppm) Air levels in close proximity to manufacturing equipment: 0.25 mg/m^3 (0.02 ppm) 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. No abnormal class IV or V cells were identified in the urine of current or former workers using the Papanicolaou technique[a]. No increase in incidence of cancer (any kind).
Dost et al. 2009 Exposure group: 308 MBOCA-exposed workers (minimum employment of 1 year between 1973 and 2000) 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:** Observed: 1/308 Expected: 0.18/308 SMR (95% CI): 560 (14–3,122) **Bladder cancer registrations:** Observed: 2/308 Expected: 0.61/308 SMR (95% CI): 328 (40–1,184) Cases were employed for 6–10 years; one case was a smoker (smoking was not controlled for in analysis). Other cancer incidences were at or below expected incidences.
Ward et al. 1990 Exposure group: 385 MBOCA-exposed workers (mean employment 3.2 months) Referent group: none	No exposure levels reported; most likely exposed via dermal and inhalation routes. Interval between time of first exposure and study initiation was an average of 11.5 years. Additional co-exposures may have included 4,4'-methylenedianiline, 4-chloro-*ortho*-toluidine, aniline, and *ortho*-toluidine (Hogan 1993; Ward 1993).	**Urinalysis:** Suspicious or positive cytology: 0/385 Evidence of atypical cells: 21/385 Heme: 60/385 **Bladder cystoscopy:** Low-grade papillary tumors: 2/200 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 Case report of occupational accident; one male worker	Routes of exposure: dermal, oral, and inhalation; exposure level high judged by urinary biomarkers. 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). Note: unclear if the burning sensations were a thermal or chemical effect of exposure to molten MBOCA.
Liu et al. 2005 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 Case report of occupational accident; one male worker	Routes of exposure: dermal and inhalation; exposure level high judged by urinary biomarkers. Worker was sprayed over chest, abdomen, and extremities with molten MBOCA.	Worker reported burning sensation of the skin. Note: unclear if the burning sensation was a thermal or chemical effect of exposure to molten MBOCA.
Ward et al. 1988 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 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)

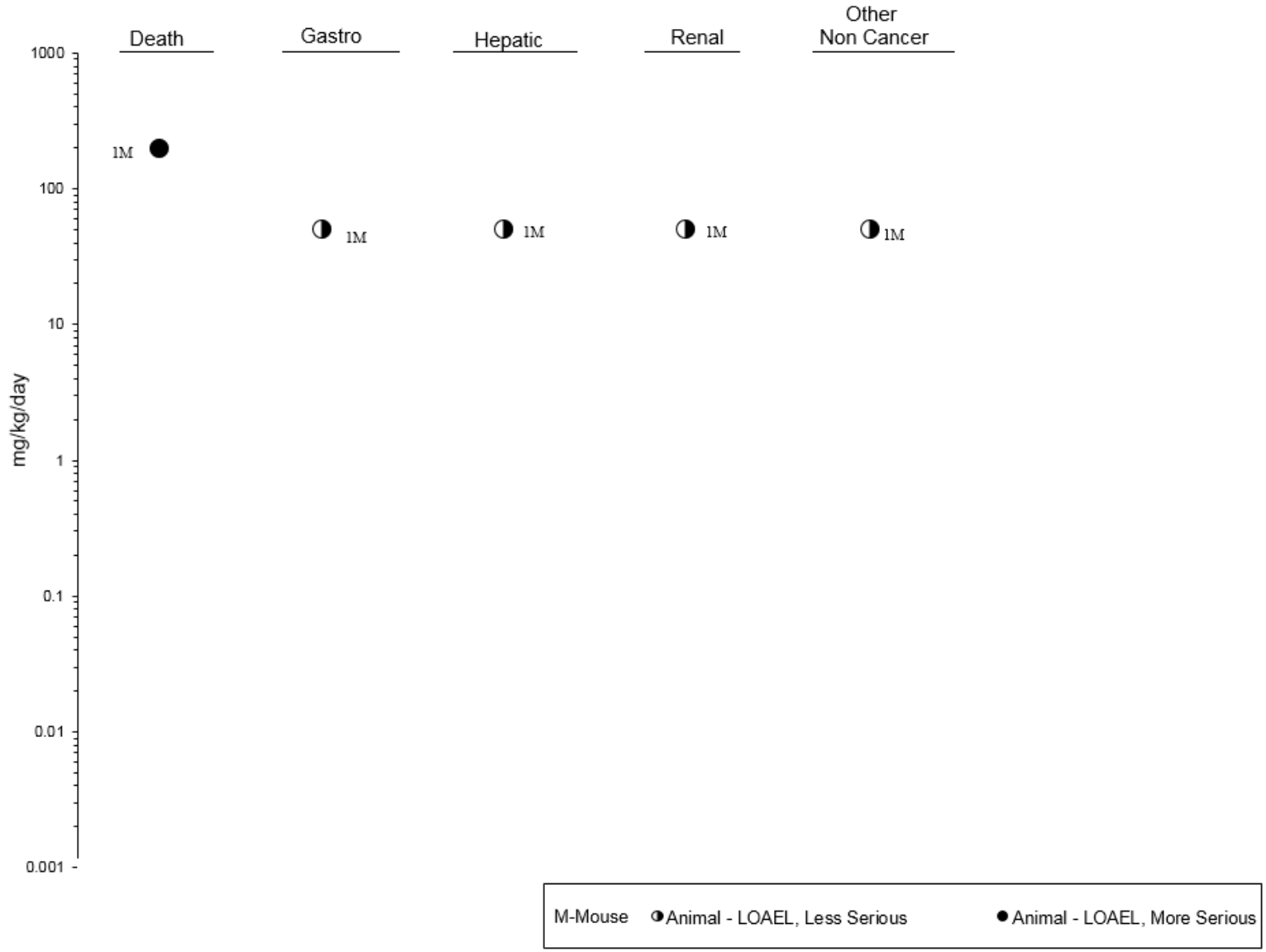


Figure 2-2. Levels of Significant Exposure to MBOCA – Oral
Intermediate (15-364 days)

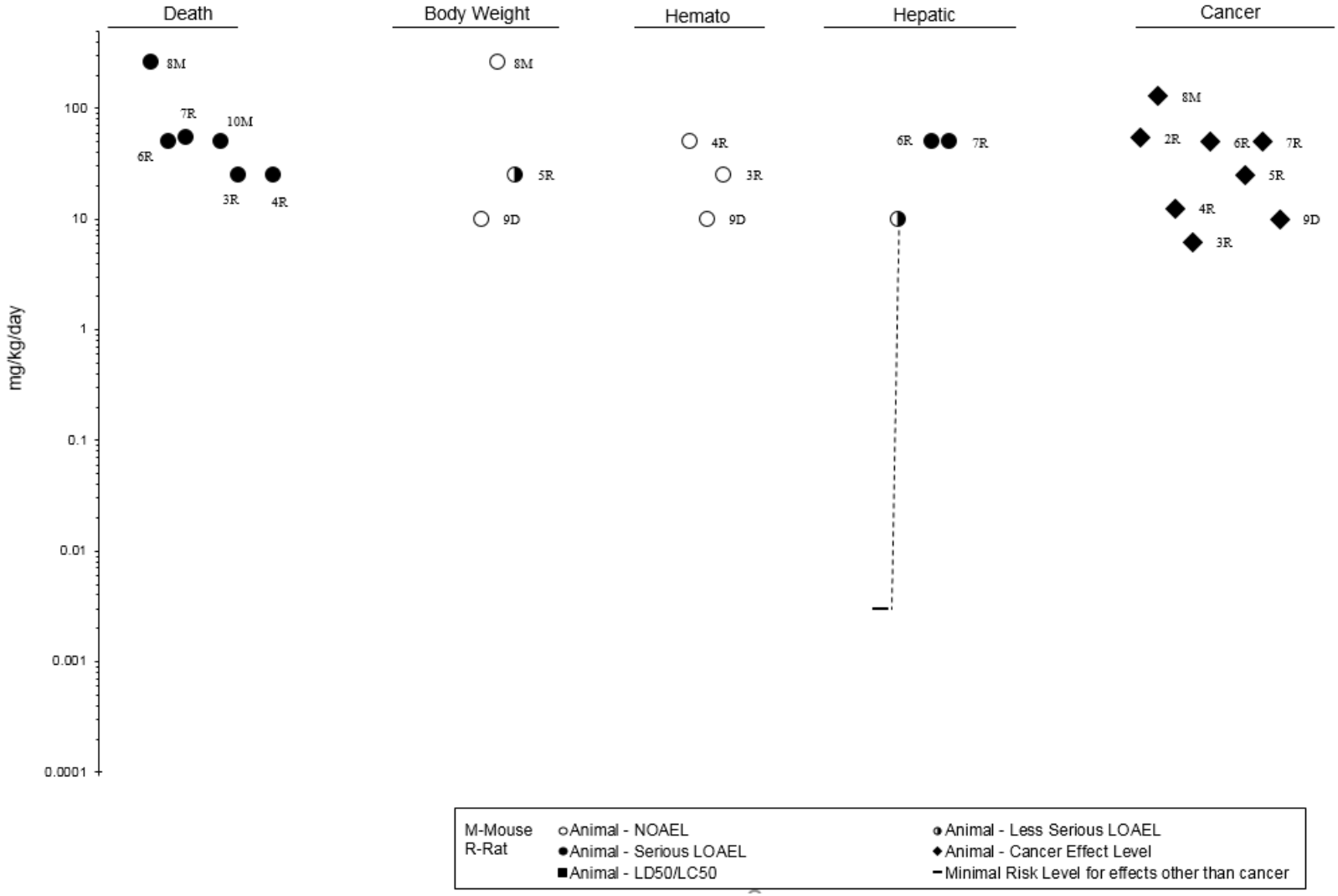


Table 2-3. Levels of Significant Exposure to MBOCA – Dermal[a]

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 ademastosis 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 ($2FR_450$)	Cellular transformation	ND	+	Dunkel et al. 1981
RLV-infected rat embryo ($2FR_450$)	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).