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)

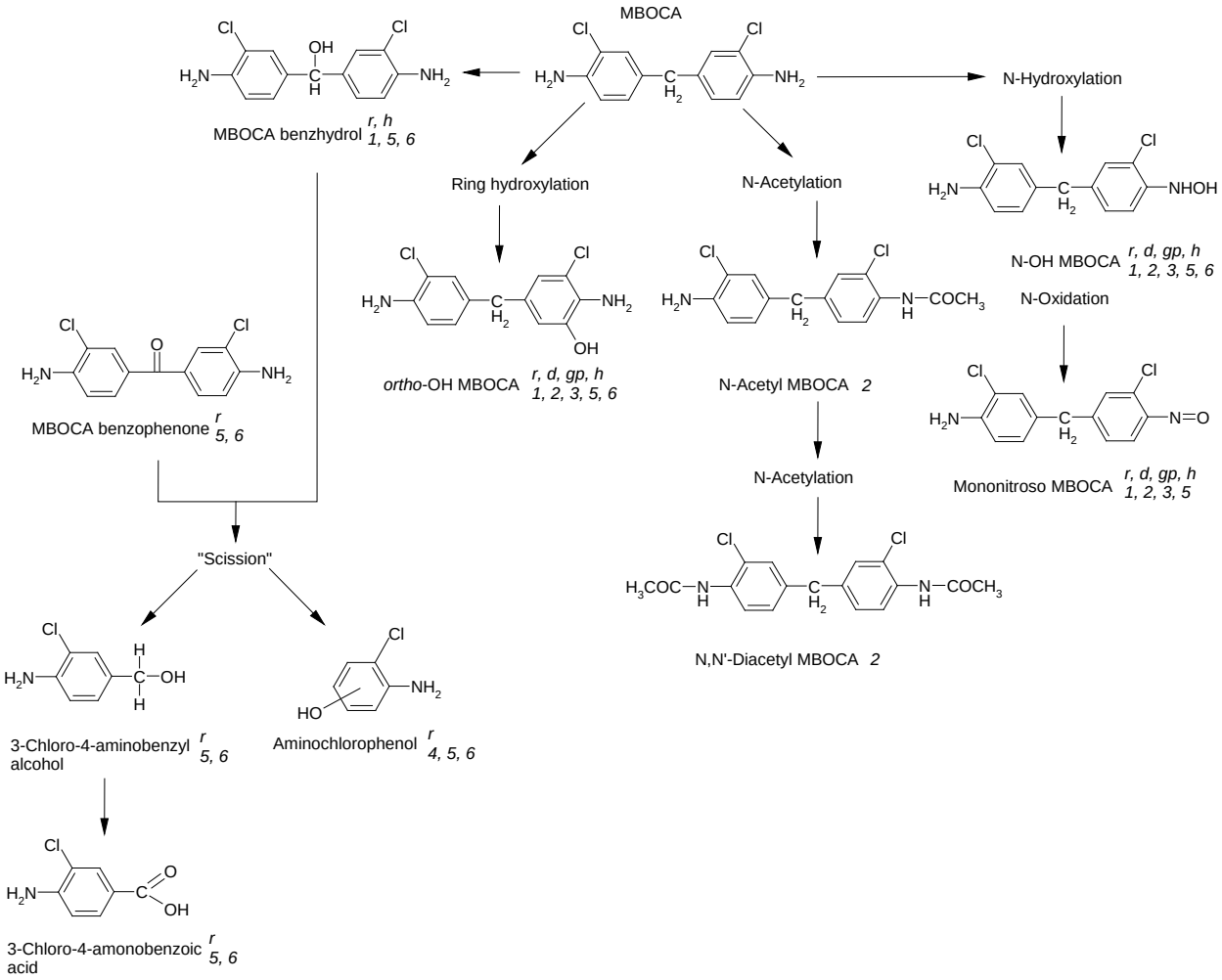
Distribution of radiolabeled MBOCA (and its metabolites) following a single dermal exposure in dogs was rapid, with no measurable radioactivity in blood or plasma up to 24 hours later (Manis et al. 1984). The highest concentration of radioactivity was found in the bile. Detectable concentrations of radioactivity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