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 of the toxicology of 4,4'-methylene-bis(2-chloroaniline) (MBOCA). It contains descriptions and evaluations of toxicological and epidemiological investigations and provides conclusions, where possible, on the relevance of MBOCA toxicity to public health.

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

MBOCA is an aromatic diamine that is used as a curing agent for polyurethane and epoxy resins. It has some of the general toxicity characteristics of aromatic amines. However, methemoglobinemia and the resulting cyanosis, hallmark symptoms of exposure to aromatic amines, are reported with much less frequency after exposure to MBOCA (Linch et al. 1971).

The majority of studies on MBOCA were undertaken to investigate its potential to cause cancer in different animal species because MBOCA was suspected of being a human carcinogen. A large number of studies focused on tumor incidence and did not investigate other toxic effects of MBOCA. Another limitation of the animal studies is the information on acute toxicity. An acute effect of MBOCA in the dog was marked methemoglobinemia after a single oral dose (Barnes 1964). Information on intermediate and chronic exposure, however, indicates that MBOCA is carcinogenic in animals.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure - inhalation, oral, and dermal; and then by health effect -death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods -acute (14 days or less), intermediate (1.5-364 days), and chronic (36.5 days or more).

Levels of significant exposure (LSE) for each route and duration are presented in Table 2-1 and illustrated in Figure (2-l). The points in the figure showing no-observable-adverse-effect levels (NOAELs) or lowest-observable-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of a significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points, which can be found in Tables 2-1, 2-2, 2-3, and 2-4. 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" and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

Levels of MRLs) may be of interest to health professionals and citizens alike.
Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of MBOCA are reported in Table 2-1 and Figure 2-1. Because cancer effects could occur at lower exposure levels, Figure 2-1 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 ($10m^{-4}$ to 10^{-7}), as developed by EPA.

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

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1989a), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

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

2.2.1 Inhalation Exposure

The studies presented in the section on inhalation exposure address occupational or accidental exposures. In occupational settings, dermal, inhalation and oral exposures often occur simultaneously. Therefore, many of the effects described in this section may be partially due to dermal or oral exposures to MBOCA. Exact levels of occupational and accidental exposures are not known. For this reason, the results in this section are not presented in table or figure form.

2.2.1.1 Death

No studies were located regarding death in humans or animals after inhalation exposure to MBOCA. A literature search did not reveal any human or animal studies that reported death due to MBOCA inhalation.

2.2.1.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, hematological, musculoskeletal, or hepatic effects in humans or animals after inhalation exposure to MBOCA. Other systemic effects observed following inhalation exposure are discussed below.

Gastrointestinal Effects. Information on the gastrointestinal effects of MBOCA following inhalation exposure is extremely limited. In a case study of accidental occupational exposure, a worker complained of feeling ill in the stomach after he was sprayed in the face with molten MBOCA (Hosein and Van Roosmalen 1978). Some of the MBOCA entered his mouth. He was wearing overalls, gloves, and safety glasses, but no respirator or face shield. He washed his face and eyes immediately. no follow-up information was given about his condition. There was no information on the dose to which he was exposed.

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

Renal Effects. 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, suggesting that damage to the renal tubules was transitory. The level of exposure was not reported.

In a retrospective bladder cancer incidence study conducted with 532 workers exposed to MBOCA from 1968 to 1979 and 20 workers who were first employed in 1980 and 1981, 385 participated in a urine screening test (Ward et al. 1990). Exfoliative cytology revealed that the morning urine samples obtained from 21 workers contained atypical cells. no cytology readings were positive for cancer. Urine samples obtained from 16 workers were positive for heme (Ward et al. 1990). Almost 90% of the exposed workers were males, and the median duration of exposure was greater than 3 months. This study gathered information on a relatively large group of workers exposed solely to MBOCA (and not to benzidine and P-naphthylamine, which can confound exposures in other occupational settings) and because it alerted medical professionals to the limitations of follow-up procedures generally used after MBOCA exposure. In the course of screening, one participant who was negative for cytology and heme impairment was diagnosed with bladder cancer. Cystoscopy was introduced as a part of the follow-up procedure, and two additional low-grade papillary tumors of the bladder were detected. Data on MBOCA in urine from workers in companies belonging to the Polyurethane Manufacturers Association (PMA) confirmed the importance of biological monitoring (Lowry and Clapp 1992). The percentage of urine samples with <25 μg MBOCA/L increased from 77% in 1985 to 86% in 1990. The percentage of urine samples with >5 μg MBOCA/L decreased from 12% in 1985 to 8% in 1990.

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

Dermal/Ocular Effects. Very limited information is available regarding dermal/ocular effects after inhalation exposure to MBOCA. When a worker was accidentally sprayed with molten MBOCA, he experienced burning of the face and eyes shortly thereafter (Hosein and Van Roosmalen 1978). The worker was wearing gloves and safety glasses but no respirator or face shield, so the exposure may have occurred by ingestion or dermal absorption as well. The limitations of this study are: (1) no further follow-up information was provided on the worker's condition; (2) the dose of MBOCA was not known; (3) exposure involved mixed routes; and (4) only one exposed individual was described.

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

No studies were located regarding the following health effects in humans or animals after inhalation exposure to MBOCA:

2.2.1.3 Immunological Effects

2.2.1.4 Neurological Effects

2.2.1.5 Reproductive Effects

2.2.1.6 Developmental Effects

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after inhalation exposure to MBOCA. The sex-linked recessive lethal (SLRL) test in *Drosophiki melu*n*oguster* was used to monitor the air in the vulcanizing, weighing, calendaring, and molding areas of a rubber plant (Donner et al. 1983). Male flies that were exposed in the molding area exhibited a small but significant increase ($p<0.05$) in SLRL genotoxicity. Exposure data were not provided, but high ambient exposures were expected to occur in the tested areas. Acute lethality to fruit flies in the molding area was 50%; sterility was not reported. Concomitant exposures to other chemicals may also have occurred.

Other genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

MBOCA is suspected of being a human carcinogen because its chemical structure is similar to a known human bladder carcinogen, benzidine, and to that of a potent animal carcinogen, 3,3'-dichlorobenzidine (Osorio et al. 1990; Ward et al. 1990). A limited number of epidemiological studies were found that examined the incidence of cancer in workers exposed to MBOCA. Workers who were exposed to MBOCA for a median duration of employment of 3.2 months (between 1968 and 1981) were examined for bladder cancer (Ward et al. 1990). Of the 200 workers examined, 3 men were diagnosed with bladder tumors by cystoscopy: 1 with a papillary tumor, and 2 with low-grade papillary transitional cell tumors. Two of the men were less than 30 years old. The interval between the time of first exposure and diagnosis was 11.5 years; the latency period for most bladder carcinogens is about 20 years (Ward et al. 1990). Since bladder carcinoma in young men is very uncommon, this finding increases the concern that MBOCA may cause bladder tumors in humans. A limitation of this study is that there were no controls (there were no data on the incidence of bladder tumors diagnosed by cystoscopy in an unexposed population). The study did not provide statistical evidence for an association between bladder tumor incidence and MBOCA exposure in workers. The statistical significance of three cases of bladder tumors could not be evaluated. There is no information on the daily amount of MBOCA to which workers were exposed, and a possibility exists of coexposure to other chemicals such as 4,4'-methylenedianiline, 4-chloro ortho-toluidine, aniline, and otiho-toluidine (Hogan 1993; Ward 1993). MBOCA may not be solely responsible for the observed carcinogenic effects.

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

2.2.2 Oral Exposure

Very little information on acute oral toxicity of MBOCA in humans or animals is available. Information on the long-term health effects of MBOCA in humans is also limited. However, several chronic oral exposure studies in mice (Russfield et al. 1975), rats (Kommineni et al. 1979; Stula et al. 1975) and dogs (Stula et al. 1977) clearly demonstrate the carcinogenic potential of MBOCA in these species. These studies are discussed in Section 2.2.2.8.

2.2.2.1 Death

No studies were located regarding increased mortality in humans from any cause following exposure to MBOCA.

Decreased lifespan has been noted in rats after chronic exposure to MBOCA (Kommineni et al. 1979). Male Sprague-Dawley rats were fed a standard diet (with 27% protein) containing 0, 12.5, 25, or 50 mg/kg/day of MBOCA or a protein-deficient diet (with 8% protein) containing 0, 6.25, 12.5, or 25 mg/kg/day of MBOCA for 18 months. Decreased lifespan was observed in the rats fed 25 mg/kg/day of MBOCA in either the standard or protein-deficient diet. Rats fed the standard diet containing the highest concentration of MBOCA, 50 mg/kg/day, also experienced a shortened lifespan. Similar decrease in lifespan was observed in Wistar II 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 first death in females occurred on day 200 of treatment and in males on day 390 of treatment; the mean lifespan in control females was 535 days and in males 565 days.

In another study, 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).

The LOAEL value for death in rats for chronic exposure is reported in Table 2-1 and Figure 2-l.

2.2.2.2 Systemic Effects

No studies were located regarding cardiovascular or musculoskeletal effects in humans or animals after oral exposure to MBOCA. The systemic effects observed after oral exposure are discussed below.

The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species for chronic duration are presented in Table 2-1 and Figure 2-1.

Respiratory Effects. no studies were located regarding respiratory effects in humans after oral exposure to MBOCA.

Lung adenomatosis was found in rats fed 2.5 (low dose) or 50 (high dose) mg/kg/day of MBOCA for 18 months. Three of 22 rats from the low-dose group and 4 of 19 from the high-dose group developed this preneoplastic condition (Russfield et al. 1975). Although this finding was not statistically significant, it is important because exposure to MBOCA induces preneoplastic lesions in the lungs.

Gastrointestinal Effects. Extremely limited information is available regarding gastrointestinal effects of MBOCA after oral exposure. In a case study of an accidental occupational exposure, a worker complained of feeling ill in the stomach after his face was sprayed with molten MBOCA that also entered his mouth (Hosein and Van Roosmalen 1978). He wore overalls, gloves, and safety glasses but no respirator or face shield. He washed his face and eyes immediately after the exposure. no further information was given about his condition. As with other occupational exposure studies, there was no information on the dose to which he was exposed. Another limitation of this study is the possibility that some of the MBOCA was inhaled or dermally absorbed.

No studies were located regarding gastrointestinal effects in animals following oral exposure to MBOCA.

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to MBOCA.

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). The analyses were performed only twice a year, so the reported observations may not be meaningful.

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

While no information is available on acute or intermediate exposure, evidence of adverse hepatic effects was seen in rats and dogs after chronic exposure to MBOCA. Several adaptive changes occurred in the livers of rats fed 50 mg/kg/day of MBOCA for 2 years. These changes included 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 glutamic-pyruvic transaminase (SGPT) in MBOCA-treated dogs. The highest levels of SGPT occurred during the first 2 years and after 7.5-8 years of treatment (Stula et al. 1977).

Based on the occurrence of hepatic effects in dogs exposed to MBOCA, a chronic-duration MRL of 0.003 mg/kg/day has been calculated from the LOAEL of 10 mg/kg/day (Stula et al. 1977) as described in the footnote on Table 2-1.

Renal Effects. There is very limited information regarding renal effects in humans after oral exposure to MBOCA. Five hours after an acute exposure to molten MBOCA, in which MBOCA was accidentally sprayed over the worker's face and some entered his mouth, a urine sample from the exposed worker contained 220 mg/L of protein indicating an immediate, transient effect on tubular reabsorption of low molecular weight proteins (Hosein and Van Roosmalen 1978). However, 11 hours after the accident, there was only a trace of protein in the urine of this worker. The urine specimens collected in the course of 24 hours after the accident had low specific gravity, indicating possible transient damage to the renal tubules and an attendant inability to concentrate urine.

Dermal/Ocular Effects. There is very little information on dermal/ocular effects after oral exposure to MBOCA. In one occupational exposure study, a worker was accidentally sprayed with molten MBOCA, and some of the compound entered his mouth. The worker complained of burning face and eyes shortly after exposure (Hosein and Van Roosmalen 1978). At the time of the accident the worker was wearing gloves and safety glasses, but he had no respirator or face shield. It is therefore likely that exposure also occurred by inhalation and dermal absorption. This study is limited because only one exposed individual was described, and no further information was given regarding the worker's condition, the dose of MBOCA, or the precise exposure route.

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

Other Systemic Effects. No studies were located regarding other systemic effects in humans after oral exposure to MBOCA.

In rats fed 25 or 50 mg/kg/day of MBOCA-hydrochloride for 18 months, the average body weight was 50 g and 100 g lower, respectively, than the body weight of controls at the end of the treatment period. The study does not provide the body weights for experimental animals at either dose level. During the first 20-25 weeks of the experiment, however, there was no difference in food consumption between MBOCA-treated animals and control animals (Russfield et al. 1975). The major limitation of this study is that data on weight changes are incomplete, which makes the interpretation difficult.

2.2.2.3 Immunological Effects

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

2.2.2.4 neurological Effects

No studies were located regarding neurological effects in humans after oral 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. The dog also had a papillary transitional cell carcinoma of the urinary bladder. The limitations of this study are the lack of correlation between pituitary hyperplasia and transitional cell bladder carcinoma and the small number of animals used.

2.2.2.5 Reproductive Effects

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

2.2.2.6 Developmental Effects

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

2.2.2.7 Genotoxic Effects

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

D. melanogaster larvae, heterozygous for visible wing mutations, were fed a medium made with a 5-millimolar aqueous solution of MBOCA for 48 hours (Kugler-Steigmeier et al. 1989) to determine whether MBOCA exposure could induce recombinations and somatic mutations in fruit flies. The resulting adults exhibited a small but statistically significant increase in small single wing spots. Higher concentrations could not be tested because of MBOCA's extremely limited solubility. Because the number of large single spots was not increased, the authors concluded that the somatic mutations occurred late in development. Similarly, the authors concluded, because of the absence of induced twin spots, that MBOCA did not exhibit strong recombinogenic activity. In an SLRL test, adult *D. melanogaster* were fed diets containing MBOCA at a concentration of 3.75 or 7.5millimoles for 3 days (Vogel et al. 1981). A statistically significant increase in SLRL test values was observed at the 7.5-millimolar concentration in one of two tests. The experiment was incomplete because mating was attempted only once after exposure; therefore, germ cells were sampled at only one stage of development. The method of feeding makes concomitant dermal exposure possible in both experiments. However, MBOCA is expected to be too non-polar to penetrate through the larval integument.

Radioactive deoxyribonucleic acid (DNA) adducts were identified in rats administered 75 mg/kg of MBOCA labeled with ^{14}C at the methylene carbon by gavage (Cheever et al. 1990). The highest level of radioactive adducts was found in the liver (49.11 µmol/mg DNA at 24 hours). Covalent binding to bladder and lymphocyte DNA was also observed (about 7 and 0.9 pmol/mg DNA, respectively, at 24 hours). In a separate experiment, in which rats were exposed to 1.4-30 mg/kg [^{14}C-methylene]-MBOCA by gavage. Significant quantities of DNA adducts were found in the liver and lung, marginal amounts were found in the kidney, and no adducts were detected in the bladder (Kugler-Steigmeier et al. 1989). Enzymatic hydrolysis of the DNA showed that two DNA adducts were generated in the liver and two different adducts were found in the lung. Kugler-Steigmeier et al. (1989) did not observe significant binding to kidney or bladder DNA because the quantity of DNA isolated from these tissues was too small to measure the amount of covalently bound MBOCA to DNA in these tissues. The research results suggest that oral exposures to MBOCA induce vascular and liver neoplasms in mice (Russfield et al. 1975) lung and liver tumors in rats (Kommineni et al. 1979; Russfield et al. 1975; Stula et al. 1975), and bladder tumors in dogs (Stula et al. 1977) as discussed in Section 2.2.2.8.

Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

MBOCA is 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).

One epidemiological study examined the incidence of cancer in workers exposed to MBOCA. Workers who were occupationally exposed to MBOCA for a median employment period of 3.2 months (between 1968 and 1981) were examined for bladder tumors (Ward et al. 1990). Although the route of exposure was not specified in the report, the most likely exposure pathways were inhalation and dermal. The oral route cannot be excluded, however, since some of the inhaled dust to the lungs could be subsequently swallowed. Low-grade papillary transitional cell tumor of the urinary bladder was diagnosed by cystoscopy in 2 out of 200 examined workers, and 1 was diagnosed with a full-blown papillary bladder tumor. Two of the men were less than 30 years old. The interval between the time of first exposure and the initiation of the study averaged 11.5 years, while the latency period for most bladder carcinogens is about 20 years (Ward et al. 1990). Since bladder carcinoma in young men is very uncommon, this finding increases the concern that MBOCA has a potential for causing bladder cancer in humans. A limitation of this study is that there were no controls (there are no data on the incidence of bladder tumors diagnosed by cystoscopy in an asymptomatic nonexposed population). There was also no evidence for statistical associations regarding bladder tumor incidence in exposed workers. There is also no information on the daily dose of MBOCA to which the workers were exposed or about the route of exposure. Further investigations of this occupational exposure revealed the possibility of coexposure to other chemicals such as 4,4'-methylenedianiline, 4-chloro ortho-toluidine, aniline, and ortho-toluidine (Hogan 1993; Ward 1993). The dose and duration of exposure to these chemicals is not known. It is therefore difficult to estimate the exact contribution of MBOCA to the observed carcinogenic effects in the occupationally exposed workers.

No studies were located on cancer in animals after acute or intermediate oral exposure to MBOCA. Several studies in rats, mice, and dogs reported finding a carcinogenic response after chronic exposure to MBOCA. Lungs, liver, breast, and urinary bladder were the main target organs for cancer in these species.

A dose-dependent increase in lung tumors was observed in Sprague-Dawley rats fed 12.5, 25, or 50 mg/kg/day of MBOCA for 18 months; the incidence of lung tumors was 23%, 37%, and 70%, respectively (Kommineni et al. 1979). A similar observation (presence of lung adenocarcinomas) was made in Charles River rats fed 50 mg/kg/day of MBOCA for 2 years (Stula et al. 1975). In another study, male Charles River CD-1 rats were fed 25 or 50 mg/kg/day of MBOCA for 18 months. The authors reported finding lung adenocarcinoma in 1/22 and 1/19 animals, respectively (Russfield et al. 1975) although this finding was not statistically significant. In addition, 3 of 22 and 4 of 19 rats receiving 25 or 50 mg/kg/day of MBOCA, respectively, developed adenomatosis, which is a preneoplastic lesion (Russfield et al. 1975). A limitation of this study is the small number of animals on which the tumor incidence was based. All these studies were done with animals fed standardprotein diets. In contrast, the incidence of lung tumors in rats was lower if animals were fed MBOCA in a protein-deficient diet. In rats fed 6.25, 12, or 25 mg/kg/day of MBOCA in a lowprotein diet for 18 months, the incidences of lung tumors were 6%, 15%, and 26%, respectively, while no tumors were found in control animals (Kommineni et al 1979). These numbers are less than half of the incidences reported for comparable doses of MBOCA given to rats fed a standard diet. Similar observations were made in rats fed a higher dose of MBOCA (50 mg/kg/day for 2 years); the incidence of lung tumors in rats fed a protein-deficient diet was roughly one-half of that observed in rats fed a standard-protein diet (Stula et al. 1975). In Wistar II rats fed an average of 54 mg/kg/day of MBOCA in a low-protein diet for 500 days, 32% of males and 12% of females developed lung tumors (Grundmann and Steinhoff 1970). 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.

MBOCA also induces liver tumors in rats. An increase in the incidence of hepatomas was found in Charles River CD rats fed 25 or 50 mg/kg/day of MBOCA in a standard-protein diet for 18 months; the incidences were 5% and 21%, respectively (not statistically significant) (Russfield et al. 1975) compared to 4% and 36% found in Sprague-Dawley rats fed the same diet and the same amount of MBOCA (Kommineni et al. 1979). Similar findings were made in random-bred female albino mice (derived from HaM/ICR mice in 1959) fed 130 or 260 mg/kg/day of MBOCA for 18 months and then observed for an additional 6 months while on a control diet. They had a significantly increased incidence of hepatomas, 43% in the 130-mg/kg/day group and 50% in the 260-mg/kg/day group (Russfield et al. 1975). The incidence of hepatomas in treated random-bred male albino mice (derived from HaM/ICR mice in 1959) was not significantly different from that in controls. These results indicate that in mice there is a gender difference regarding MBOCA-induced hepatomas; female mice are affected, and male mice are not affected. The effect of standard- and low-protein diets on the incidence of MBOCA-induced hepatocellular carcinomas was investigated in Sprague-Dawley and Charles River rats (Kommineni et al. 1979; Stula et al. 1975). The results are inconclusive. In Sprague-Dawley rats fed 12.5 or 25 mg/kg/day of MBOCA in a standard-protein diet for 18 months, the incidences of hepatocellular carcinomas were 3% and 4%, respectively; in rats fed the same amounts of MBOCA in a protein-deficient diet, the incidences were 0% and 18%, respectively (Kommineni et al. 1979). These results indicate that a protein-deficient diet did not reduce the MBOCA-induced incidence of hepatocellular carcinoma in Sprague-Dawley rats. When male Charles River rats were fed 50 mg/kg/day of MBOCA for 2 years, however, the incidences of hepatocellular carcinomas were 7% in rats fed a standard-protein diet and 52% in rats fed a lowprotein diet (Stula et al. 1975). This observation, that the protein-deficient diet accentuates the carcinogenic effects of MBOCA, was not seen in female rats that had 7% and 5% hepatocellular carcinomas when fed standard-protein and low-protein diets, respectively (Stula et al. 1975). However, in Wistar II rats fed an average dose of 54 mg/kg/day of MBOCA in a low-protein diet for 500 days, liver cancer was present in 88% of males and 72% of females (Grundmann and Steinhoff 1970). The results suggest that gender and strain of rats play a role in the effect of low-protein diet on MBOCA-induced incidence of hepatocellular carcinomas. 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. One beagle died approximately 3.4 years of pyelonephritis unassociated with MBOCA exposure. Of the five surviving dogs, 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). 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.

A statistically significant increase of malignant mammary tumors was found in female Sprague-Dawley rats fed 50 mg/kg/day of MBOCA in a low-protein diet for 2 years (Stula et al. 1975). A similar finding was made in male rats fed 25 mg/kg/day of MBOCA in a low-protein diet for 18 months; the incidence of mammary tumors was 6% (Kommineni et al. 1979). When rats were fed a standard diet, the incidence of mammary adenocarcinoma was 28% at 50 mg/kg/day of MBOCA and 11% at 25 mg/kg/day (Kommineni et al. 1979). Zymbal's gland carcinomas were found in 12% of rats fed a low-protein diet and in 7% of rats fed a standard-protein diet; both diets contained 25 mg/kg/day MBOCA (Kommineni et al. 1979). The results in rats fed MBOCA in low- and standard-protein diets indicate that there is a dose-related increase in the incidence of lung and mammary carcinomas, while the incidence of hepatocellular carcinoma is not dose related (Kommineni et al. 1979).

Other tumor types were also found after chronic oral administration of MBOCA. The incidence of hemangiosarcomas was 4% and 8% in Sprague-Dawley rats fed 25 mg/kg/day of MBOCA in standardprotein or low-protein diets, respectively (Kommineni et al. 1979). In another study, vascular tumors (generally subcutaneous hemangiomas and hemangiosarcomas) were present in randomly bred male albino mice (derived from the HaM/ICR strain in 1959) fed 130 or 260 mg/kg/day of MBOCA for 18 months; the incidences were 23% and 40%, respectively (Russfield et al. 1975). In female mice, vascular tumors (43%) were present only in the group treated with the high dose of 260 mg/kg/day of MBOCA.

The incidence of pituitary adenomas (including adenocarcinomas) in Sprague-Dawley rats fed a standard-protein diet was reduced, especially in animals treated with high doses of MBOCA (Kommineni et al 1979). Rats fed 12.5, 25, or 50 mg/kg/day of MBOCA for 18 months had 36%, 25% (statistically significant), and 4% (statistically significant) incidences of pituitary adenomas, respectively. The incidence of pituitary adenomas in the control group was 42% (Kommineni et al. 1979). A statistically significant decrease in pituitary tumors was also observed in female, but not male, ChR-CD rats (Charles River Cesarean Delivered, Sprague-Dawley origin, random-bred albino rats) fed a standard-protein diet with 50 mg/kg/day of MBOCA for 2 years (Stula et al. 1975). This decrease in incidence of pituitary tumors was not as consistent in Sprague-Dawley rats fed a lowprotein diet; the incidences were 16%, 12% (statistically significant), and 20% in animals fed 6.25, 12.5, and 25 mg/kg/day, respectively, for 18 months (Kommineni et al. 1979). In the control group, the incidence of pituitary adenomas was 23%. neither the certainty of these findings nor the mechanism by which they could occur are known at the present time.

The precise mechanism of chemical carcinogenesis is not known, but it is thought that in some instances it involves the formation of chemical adducts in the genetic material through covalent binding of nucleophilic sites by electrophilic parent compounds or their activated electrophilic metabolites (Cheever et al. 1991). Therefore, it seems that the capacity of MBOCA to form adducts with tissue DNA (Cheever et al. 1990) hemoglobin (Cheever et al. 1991; Sabbioni and neumann 1990) and globin and serum albumin (Cheever et al. 1991) in rats may play a role in its toxicity and carcinogenicity.

The lowest doses that produced tumors (CELs) are presented in Table 2-1 and plotted in Figure 2-l.

TABLE 2-1. Levels of Significant Exposure to 4,4'-Methylenebis(2-chloroanaline) MBOCA - Oral

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
CHRONIC EXPOSURE								
Death								
1	Rat	(F)	18 mo 1 x/d				25 (23/50 males died after 72 weeks)	Kommineni et al. 1979
Systemic								
2	Rat		18 mo	Resp			50 (adenomatosis)	Russfield et al. 1975
				Other		25 (6.5% lighter than control)		
3	Rat	(F)	2 yr ad lib	Hepatic			50 (hepatocytomegaly, fatty change, necrosis, fibrosis, bile duct proliferation)	Stula et al. 1975
4	Dog	(C)	9 yr 5d/wk 1x/d	Hepatic		10[b] (increased GPT, nodular hepatic hyperplasia)		Stula et al. 1977

TABLE 2-1. Levels of Significant Exposure to 4,4'-Methylenebis(2-chloroanaline) (continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
Cancer								
5	Rat	(F)	2 yr ad lib				50 (CEL-lung adenocarcinoma, liver hepatocellular adenomas and carcinomas, mammary fibroadenomas and adenocarcinomas)	Stula et al. 1975
6	Rat	(F)	500 d 1x/d				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
7	Rat	(F)	18 mo 1 x/d				6.25 (CEL-6% incidence of lung adenocarcinomas) 12.5 (CEL-5% incidence of Zymbal's gland carcinoma, 15% lung adenocarcinomas) 25 (CEL-26% incidence of lung adenocarcinomas; 6% incidence of mammary adenocarcinomas; 12% incidence of Zymbal's gland carcinoma; 18% incidence of hepatocellular carcinomas; 8% incidence of hemangiosarcomas)	Kommineni et al. 1979

TABLE 2-1. Levels of Significant Exposure to 4,4'-Methylenebis(2-chloroanaline) (continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
8	Rat	(F)	18 mo 1 x/d				12.5 (CEL-23% incidence of lung adeno-carcinoma tumors; 8% incidence of Zymbal's gland carcinoma) 25 (CEL-37% incidence of lung adeno-carcinomas; 11% incidence of mammary adenocar-cinomas) 50 (CEL-70% incidence of lung adeno-carcinomas; 28% incidence of mammary adenocarcinomas; 22% incidence of Zymbal's gland carcinomas; 36% incidence in hepatic carcino-mas; 8% incidence of skin hemangio-sarcomas)	Kommineni et al. 1979
9	Rat		18 mo				25 (CEL-12% incidence of lung adeno-matosis; 4% incidence of bladder transition cell tumor)	Russfield et al. 1975

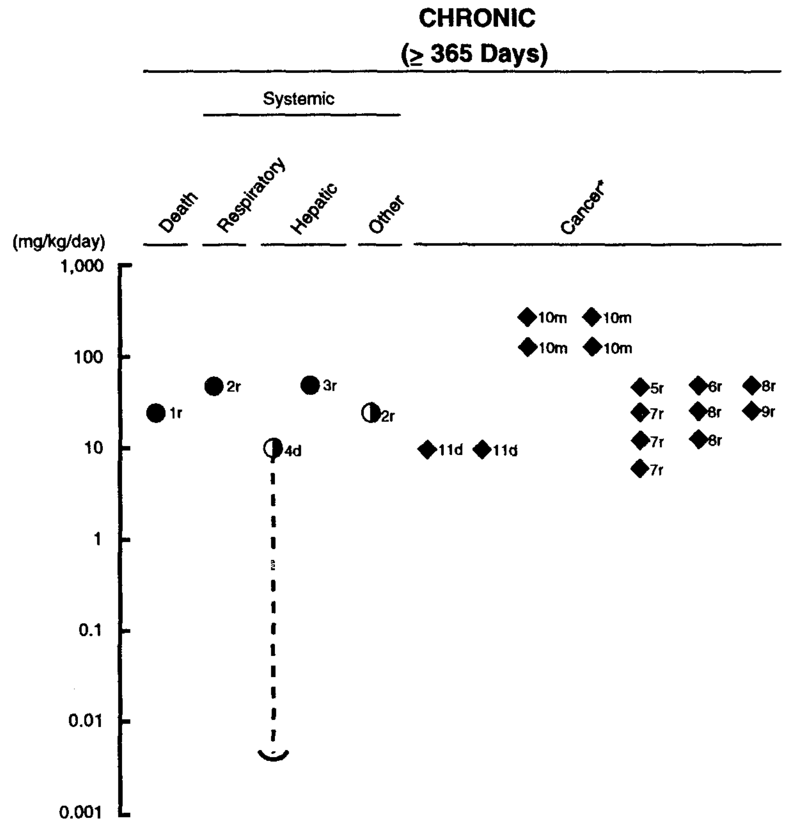
TABLE 2-1. Levels of Significant Exposure to 4,4'-Methylenebis(2-chloroanaline) (continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
10	Mouse	(F)	18 mo				130 (CEL-hepatomas in 43% of females) 260 (CEL-hepatomas in 50% of females) 130 (CEL- subcutaneous hemangiomas and hemangiosarcomas in 23% of males) 260 (CEL-subcutaneous hemangiomas and hemangiosarcomas in 43% females and 40% males)	Russfield et al. 1975
11	Dog	(C)	9 yr 5d/wk 1x/d				10 (papanicolaou IV and V - indicating neoplasia of genitourinary system) 10 (CEL- combined transitional cell carcinoma and adenocarcinoma of the urethra - 1/5 dogs; papillary transitional cell carcinoma of the urinary bladder - 3/5 dogs)	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)

ad lib = ad libitum; (C) = capsule; CEL = cancer effect level; d = days; (F) = feed; GPT = glutamate pyruvate transaminase; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; papanicolaou = cytologic evaluation for cancer detection and diagnosis using a multichromatic stain; wk = week(s); x = time(s); yr = year(s)

FIGURE 2-1. Levels of Significant Exposure to 4,4'-Methylenebis (2-chloroaniline) (MBOCA) - Oral



Key

r Rat
m Mouse
d Dog

● LOAEL for serious effects (animals)
◑ LOAEL for less serious effects (animals)
◆ CEL - Cancer Effect Level (animals)

Minimal risk level for effects other than cancer

The number next to each point corresponds to entries in Table 2-1.

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

2.2.3 Dermal Exposure

2.2.3.1 Death

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

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, hematological, musculoskeletal, or hepatic effects in humans or animals after dermal exposure to MBOCA. The systemic effects observed after dermal exposure are discussed below.

Gastrointestinal Effects. Extremely limited information is available regarding gastrointestinal effects of MBOCA after dermal exposure. In a case study of an accidental occupational exposure, a worker complained of feeling ill in the stomach after his face was sprayed with molten MBOCA that also entered his mouth (Hosein and Van Roosmalen 1978). He wore overalls, gloves, and safety glasses but no respirator or face shield. He washed his face and eyes immediately after the incident. no further information was given about his condition. As with other occupational exposure studies, there was no information on the dose to which he was exposed. Another limitation of this study is the likelihood that some of the MBOCA was inhaled or ingested.

No information was located regarding gastrointestinal effects in animals after dermal exposure to MBOCA.

Renal Effects. In a retrospective bladder cancer incidence study conducted among 532 workers exposed to MBOCA from 1968 to 1979 and 20 workers who were first employed in 1980 and 1981, 385 participated in the urine screening test (Ward et al. 1990). Exfoliative cytology revealed that 21 urine samples contained atypical cells. no cytology readings were positive for cancer. Sixteen urine samples were positive for heme (Ward et al. 1990). Almost 90% of the exposed workers were males, and the median duration of exposure was 3.2 months. This study is important because it gathered information on a relatively large group of workers exposed to MBOCA and alerted medical professionals to the limitations of follow-up procedures used after MBOCA exposure. In the course of the screening, one of the participants who had negative cytology and was negative for the presence of heme in the urine was diagnosed with a tumor of the urinary bladder. Because of this development, cystoscopy was introduced as a part of the follow-up procedure, and two additional lowgrade papillary tumors of the bladder were detected. The analysis of data on free urinary MBOCA from workers in companies of the PMA confirmed the importance of biological monitoring (Lowry and Clapp 1992). Cumulative results for 1985-1990 show an increase in percentage of urinary samples containing below 25 μg/L of MBOCA (from 77% in 1985 to 86% in 1990) and a decrease in percentage of samples with over 5 μg/L of MBOCA (from 12% in 1985 to 8% in 1990). These findings indicate that wearing protective clothing, using MBOCA in pellets not powder, monitoring indoor air for MBOCA, and other protective measures may play an important role in controlling occupational exposures.

Five hours after an acute exposure in which a worker was accidentally sprayed in the face with molten MBOCA, a urine sample contained 220 mg/L of protein, indicating possible damage to renal tubules (Hossein and Van Roosmalen 1978). There was only a trace amount of protein in the urine of this worker 11 hours after the accident. The two urine specimens collected over the course of 24 hours after the accident had low specific gravity indicating possible transient damage to the renal tubules.

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

Dermal/Ocular Effects. Very limited information is available regarding dermal/ocular effects after dermal exposure to MBOCA. In a case study, one worker was accidentally sprayed in the face with molten MBOCA. Some of the compound entered his mouth. The worker complained of burning face and eyes shortly after exposure (Hossein and Van Roosmalen 1978). The worker was wearing gloves and safety glasses but no respirator or face shield. Therefore, it is likely that the exposure may have also occurred by inhalation or oral absorption. The limitations of this study are that no further information was provided regarding the worker's condition, the dose of MBOCA, or the precise exposure route, and that only one exposed individual was described. In another case of accidental exposure to approximately 11.3 L of molten MBOCA, the worker described a burning sensation on his skin. He was sprayed over his chest, abdomen, and extremities (NIOSH 1986a; Osorio et al. 1990). Some exposure by inhalation and ingestion was also likely in this case.

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

No studies were located regarding the following health effects in humans or animals after dermal exposure to MBOCA:

2.2.3.3 Immunological Effects
2.2.3.4 Neurological Effects
2.2.3.5 Reproductive Effects
2.2.3.6 Developmental Effects
2.2.3.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans following dermal exposure to MBOCA. Radiolabeled MBOCA was applied to a 36-cm^2 shaved skin patch on the dorsal side of rats at a dose of 75 mg/kg. DNA adduct formation was determined for various tissues at intervals ranging from 1 to 29 days after exposure (Cheever et al. 1990). Throughout the study, less than 14% of the applied dose was absorbed. Adduct formation was about 100-fold less than after oral exposure to the same dose, but adducts were found in the same tissues with the same order of binding (liver > bladder > lymphocytes). This indicates that MBOCA was absorbed through the skin to react with DNA in target organs. Two studies of the genotoxicity of MBOCA in *D. melanogaster* also involved concomitant oral and dermal exposure and were discussed under oral exposure in Section 2.2.2.7.

Other genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

A limited number of epidemiological studies were located that examined the incidence of cancer in workers exposed to MBOCA. Workers who were exposed to MBOCA for a median employment period of 3.2 months (between 1968 and 1981) were examined for bladder tumors (Ward et al. 1990). After cystoscopy revealed a papillary cell tumor in one worker, a low-grade papillary transitional cell tumor of the urinary bladder was diagnosed by cystoscopy in 2 of the remaining 200 workers examined. Two of the men were less than 30 years old. The interval between the time of first exposure and the initiation of the study averaged 11.5 years, while the latency period for most bladder carcinogens is about 20 years (Ward et al. 1990). Since bladder tumors in young men are very uncommon, this finding increases the concern that MBOCA has a potential for causing bladder cancer in humans. A limitation of this study is that there were no controls (there are no data on the incidence of bladder tumors diagnosed by cystoscopy in an asymptomatic nonexposed population). The daily dose of MBOCA to which workers were exposed, was only estimated by assigning an exposure score of 0-20. Further investigation of this occupational exposure raised a possibility of coexposure to other chemicals such as 4',4-methylenedianiline, 4-chloro *o*-toluidine, aniline, and *o*-toluidine (Hogan 1993; Ward 1993). The dose and duration of exposure to these chemicals could only be estimated on the basis worker recollection. It is therefore difficult to estimate the exact contribution of MBOCA to carcinogenic effects observed in occupationally exposed workers.

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

2. 3 TOXICOKINETICS

Limited information is available regarding absorption of MBOCA. Following acute inhalatiomdermal exposure to very low concentrations of MBOCA, absorption appears to be rapid, based on the presence of MBOCA in the urine of occupationally exposed workers (Ichikawa et al. 1990). The urine of a worker accidentally exposed to molten MBOCA, contained 1,700 ppb of MBOCA 9 hours later indicating absorption and distribution following dermal and/or inhalation exposure (NIOSH 1986a; Osorio et al. 1990).

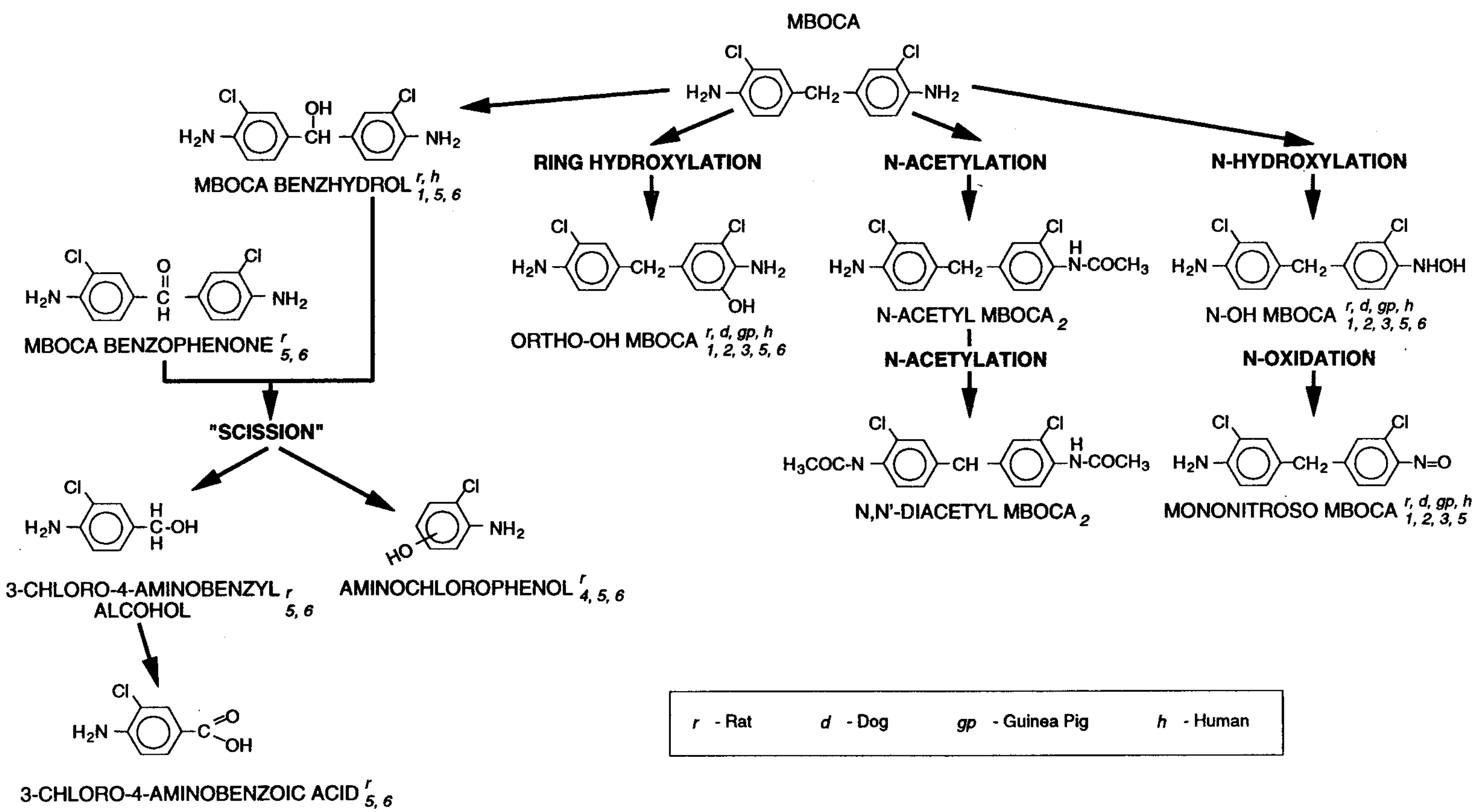
The distribution of MBOCA in laboratory animals is relatively uniform. In rats, the highest amount of MBOCA and its metabolites (total ^{14}C-radioactivity) was found in the liver following an acute P.O. injection or intermediate, gavage, exposure (Cheever et al. 1991). The tissue distribution of radioactivity following the acute exposure, was as follows: liver > kidney > lung > spleen > urinary bladder > testes > brain > lymphocytes (Cheever et al. 1991). The highest concentration of radioactivity in dogs following acute dermal exposure to radiolabeled MBOCA was in the bile (Manis et al. 1984). Detectable concentrations were also found in the liver, kidney, and fat. These results suggest that the distribution of MBOCA is similar in rats and dogs following oral and dermal exposures. A distribution study in rats following a single intravenous exposure to MBOCA, further support to these observations (Tobes et al. 1983).

MBOCA metabolism can proceed via several pathways: *N*-acetylation, *N*-hydroxylation, which may be followed by n-oxidation, and ring hydroxylation (see Figure 2-2). Some of these processes may be followed by conjugation. These are considered important pathways with regard to carcinogenicity (as cited in Cheever et al. 1991). Results of *in vitro* studies suggest that MBOCA rapidly penetrates the skin but is not metabolized in the process (Chin et al. 1983).

After inhalation of low levels, excretion of MBOCA has been monitored by measuring the amount in urine. Excretion occurs rapidly during the first 48 hours (NIOSH 1986a). Following an accidental exposure to 11.34 L of molten MBOCA, 1,700 ppb of MBOCA was found in the worker's urine four hours later (NIOSH 1986a; Osorio et al. 1990). This result suggests the rapid excretion of MBOCA after acute dermal and/or inhalation exposure. It is not clear, however, if urine is indeed the primary route of excretion in humans, since no information was presented on the amount of MBOCA contained in the feces. In rats that received a single oral dose of 10 mg/kg of radiolabeled MBOCA, 60% of the radioactivity was found in the feces (Farmer et al. 1981; Groth et al. 1984).

Little information is available on the kinetics of urinary MBOCA excretion in humans. One study found that 23 hours after an acute occupational exposure to MBOCA, 50% of this material was excreted in the urine (Osorio et al. 1990). However, other work (NIOSH 1986b) indicates that

FIGURE 2-2. Proposed Metabolic Pathway of 4,4'-Methylenebis (2-chloroaniline) (MBOCA)*



* Derived from Butler et al. 1989 (1); Cheever et al. 1991 (2); Chen et al. 1989 (3); Farmer et al. 1981 (4); Kuslikis et al. 1991 (5); Morton et al. 1988 (6).

clearance may be much slower, especially at higher exposures. The duration of exposure may also be a factor.

The precise mechanism of carcinogenicity for MBOCA is not completely understood. Some evidence suggests, however, that the ability to form adducts with tissue DNA (Cheever et al. 1990; Sabbioni and neumann 1990) hemoglobin (Cheever et al. 1988, 1990, 1991; Chen et al. 1991; Sabbioni and neumann 1990) and globin and serum albumin (Cheever et al. 1991) in rats following MBOCA exposure may play a role in its carcinogenicity.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Studies that directly measure absorption in humans following inhalation exposure to known concentrations of MBOCA were not located. 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 results showed that the preshift and postshift urine MBOCA levels of the exposed workers, including the worker with the highest level of exposure, did not significantly differ. Forty-eight hours after the exposure, urinary MBOCA levels were still similar to preshift levels; the authors interpreted this finding as evidence that the biological half-life of urinary MBOCA is relatively long (Ichikawa et al. 1990). 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). The limitations of this study include the small sample size and the probability that dermal exposure occurred as well.

In another study, 13 urine samples were collected from workers occupationally exposed to MBOCA on a Monday morning after 2 days away from work. The results showed that there was no detectable MBOCA in the urine of workers who had low MBOCA exposure(s) during the previous week. Only the urine from the worker with the highest MBOCA urine concentration (30 μg/L) the week before had detectable MBOCA. In most cases, this clearance of MBOCA after the weekend suggested that MBOCA had a relatively short retention time in the body (Clapp et al. 1991). As in the previous study, the exposure route(s) probably included inhalation and dermal. In another study of occupational exposure (combination of inhalation and dermal exposures), levels of MBOCA in the urine were determined in workers over a period of 22 months. The mean concentrations of MBOCA during that time ranged from 5.3 to 43.8 μg/L (NIOSH 1986b). Further analysis of the data revealed that urine samples with highest levels of MBOCA came from workers that are in direct daily contact with MBOCA, such as mixers and molders (NIOSH 1986b). One of the samples from a mixer was analyzed during the week (average urine MBOCA level was 29.9 μg/L) and after a 2-day weekend (average urine MBOCA level was 8.9 μg/L). This demonstrated that MBOCA was not cleared from the urine of a worker who had relatively high levels of MBOCA during the previous week (NIOSH 1986b). 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. Alternatively, MBOCA may be excreted at different rates depending upon exposure conditions including both the rate and duration of exposure. This question needs further examination.

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

2.3.1.2 Oral Exposure

No studies were located regarding absorption in humans after oral 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% remains unabsorbed in the feces (Groth et al. 1984).

2.3.1.3 Dermal Exposure

Studies that directly measure MBOCA absorption in humans following dermal exposure to known concentrations of this compound were not located. However, absorption was measured 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 levels of MBOCA ranged from 0.0002 to 0.0089 mgim”. The results showed that the preshift and postshift urine MBOCA levels did not significantly differ among exposed workers. Forty-eight hours after the exposure, the urinary MBOCA levels were similar to preshift levels suggesting that the biological half-time of MBOCA is relatively long (Ichikawa et al. 1990). The amount of MBOCA measured in urine was much higher than the amount(s) of inhaled MBOCA. The authors concluded that occupational exposures occur by other routes in addition to inhalation (in this case dermal exposure) and are important contributors to total body burdens of MBOCA. The limitations of this study include the small sample size and the probability that dermal exposure had occurred as well.

In another study, 13 urine samples were collected from workers occupationally exposed to MBOCA on a Monday morning after 2 days away from work. The results showed that there was no detectable MBOCA in samples that had low MBOCA levels during the past week. Only the sample that came from the worker who had the highest average MBOCA urine concentration (30 μg/L) the week before had detectable MBOCA (Clapp et al. 1991). These results suggest that the higher initial dose of MBOCA the longer it takes to be excreted. In another occupational study the exposure resulted from a combination of inhalation and dermal routes. The levels of MBOCA in the urine of exposed workers were determined over a period of 22 months. The mean concentrations of MBOCA ranged from 5.3 to 43.8 μg/L (NIOSH 1986b). A detailed data analysis revealed that the urine samples with the highest MBOCA levels were obtained from mixers and molders, i.e., workers that are in direct daily contact with MBOCA (NIOSH 1986b). Urinary measurements of MBOCA were obtained for one worker a mixer over a 5-day work week and over a 2-day weekend. The average urine MBOCA level during the week was approximately 30 μg/L; the level dropped to 8.9 μg/mL over the weekend. This finding suggests that MBOCA was not completely excreted via urine in a worker who had relatively high levels of MBOCA during the preceeding week (NIOSH 1986b). The reasons for the different clearance rates reported in the studies described (Clapp et al. 1991; Ichikawa et al. 1990; NIOSH 1986b) are unclear. It is possible that due to the small sample size the results may merely reflect individual differences. It is also possible that MBOCA may be removed at different rates depending on the length and rate of exposure.

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).

Using radiolabeled MBOCA and fresh human neonatal foreskin organ cultures, the absorption and penetration of MBOCA through a 7 mm x 7 mm area was evaluated over 4 hours (Chin et al. 1983). 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, while 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).

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution in humans or animals after inhalation exposure to MBOCA.

2.3.2.2 Oral Exposure

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

Twenty-four hours after oral administration of 28 μmol/kg of radioactive [methylene-^{14}C-]MBOCA to male Sprague-Dawley rats, the highest level of radioactivity was in the liver. The tissue distribution of total radiolabels (i.e., MBOCA plus its metabolites) was as follows: liver > kidney > lung > spleen > urinary bladder > testes > brain > lymphocytes (Cheever et al. 1991). In another study, the distribution in female Wistar rats 24 hours after gavage with radioactive MBOCA was similar: liver > intestine > lung > kidney > blood > stomach > spleen > uterus (Sabbioni and neumann 1990). The remaining radioactivity was recovered in urine and feces. After 28 consecutive oral doses of radioactive MBOCA, 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). The results show that, after either acute or intermediate oral administration, the highest amount of MBOCA and its metabolites accumulates in the liver. The results also show that after 28 doses, accumulation in the liver increased more than 100-fold.

Similar findings in terms of liver accumulation were found in a study in which two female LAC:Porton rats were fed 10 mg/kg of ^{14}C-MBOCA, and MBOCA's distribution was measured 48 hours later (Farmer et al. 1981). Again, liver had the highest amount of radioactivity, followed by white fat, blood, and kidney. This study is limited because it used only two animals. In another acute study, radiolabeled MBOCA was administered by gavage to rats by gavage (Morton et al. 1988). Forty-eight hours later, 64-87% of the administered radioactivity was recovered in the urine and feces. Excretion occurred primarily in the first 24 hours with feces containing 1.5-3 times the radioactivity of the urine. These results are consistent with previous reports that in rats MBOCA is extensively metabolized and rapidly excreted.

2.3.2.3 Dermal Exposure

Although no studies were located regarding distribution in humans after dermal exposure to MBOCA, it appears that it is rapidly eliminated from the body following dermal exposure. Nine hours after an accidental spill, the urine of a worker exposed to 11.34 L of molten MBOCA contained 1,700 ppb of the chemical (NIOSH 1986a; Osorio et al. 1990). The biological half-life of MBOCA in the human body based on the amount excreted into the urine was estimated to be 23 hours (Osorio et al. 1990). The authors assumed a one-compartment model and estimated that about 94% of the initial MBOCA would be eliminated within 4 days. They concluded that urinary excretion of MBOCA was rapid, a finding that is consistent with the observations made by Hosein and Roosmalen (1978). Although this study is limited because only one case was presented, it is important because it indirectly demonstrates that after dermal exposure of humans a large portion of MBOCA is distributed by the circulatory system to the kidneys (Osorio et al. 1990).

In a study using beagle dogs dermally exposed to 0.4 mg of radioactive MBOCA/cm^2, no measurable radioactivity was detected in blood or plasma up to 24 hours later (Manis et al. 1984). The highest concentration of radioactivity 24 hours post-exposure was found in the bile. no unmetabolized MBOCA was present in the bile. Detectable concentrations of radioactivity were found in the liver, kidney, fat, and lung. Urinary excretion of unmetabolized MBOCA was a small but consistent fraction, 0.4-0.5%, of the total urinary radioactivity, and the authors concluded that this may be a useful index of acute exposure. These results support the hypothesis that dermal absorption is a viable entry for MBOCA. They also demonstrate that the circulatory system distributes MBOCA to the liver, kidneys, fat, and lungs. In this study, intravenously exposed animals were used as controls in order to obtain a 100% absorbed dose baseline for comparison with the pharmacokinetic data obtained for dermal exposure.

2.3.2.4 Other Routes of Exposure

One hour after a single intravenous dose of 0.49 mg/kg of radiolabeled MBOCA to rats, radioactivity was found in the tissues in the following order: small intestine > liver > fat tissue > lungs > kidneys > skin > adrenals (Tobes et al. 1983). Similar results in terms of liver accumulation were obtained in rats 48 hours after an intraperitoneal injection of 1 mg/kg of radioactive MBOCA (Farmer et al. 1981). However, only l-2% of MBOCA and 95-97% of radiolabeled metabolites were found in the urine indicating rapid and extensive MBOCA metabolism in the rat. The finding that liver has the highest amount of radiolabel 24 hours after a single exposure is consistent with the findings from other intraperitoneal exposure studies (Cheever et al. 1991). Similar results were obtained in dogs after intravenous administration of MBOCA (Manis et al. 1984).

2.3.3 Metabolism

2.3.3.1 Inhalation Exposure

Analysis of 23 urine samples from workers occupationally exposed to MBOCA showed that all contained MBOCA, but only 10 of 23 contained *N*-acetyl MBOCA. The ratio of *N*-acetyl MBOCA to parent MBOCA ranged from 0.005 to 0.09 indicating that *N*-acetyl MBOCA is a relatively minor urine metabolite in humans (Cocker et al. 1988). Further analysis shows that the urine of MBOCAexposed workers also contains a major hydrolysis-labile metabolite, probably β-*N*-glucuronide (Cocker et al. 1990). This metabolite, upon hydrolysis, yields two to three times more MBOCA than the amount of parent compound present in urine after exposure to MBOCA. Another metabolite detected in the urine is a heat-labile conjugate of n-acetyl MBOCA (Cocker et al. 1990). In another study of occupational exposure, 35% of the MBOCA metabolites were excreted as conjugates in the urine (Osorio et al. 1990).

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

2.3.3.2 Oral Exposure

No studies were located regarding metabolism in humans after oral exposure to MBOCA. 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. These are important pathways with respect to the carcinogenicity of MBOCA (Cheever et al. 1991). Some of these metabolic conversions can be followed by conjugation. Carcinogenicity probably involves activation and adduct formation by MBOCA. A scheme of MBOCA metabolism and excretion in animals is presented in Figure 2-2. The microsomal P-450 enzyme system is involved in n-hydroxylation of MBOCA, which is considered to be an activation step related to adduct formation (Morton et al. 1988). Microsomes from phenobarbital-treated rats had an increased rate of MBOCA hydroxylation (Morton et al. 1988). Such effects were not obtained after treatment with methylcholanthrene. These findings were confirmed using human liver microsomes and purified rat liver cytochrome P-450 monooxygenases showing that the activation of MBOCA is catalyzed by the phenobarbital-inducible enzymes (Butler et al. 1989). More recently, it was shown that much higher binding of MBOCA to hemoglobin occurred in phenobarbital-treated rats (Cheever et al. 1991). These results suggest that enzymatic induction by phenobarbital leads to increased capacity of MBOCA and/or its metabolites to covalently bind to proteins and probably to DNA.

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).

The metabolism of MBOCA was also investigated *in vitro* by incubating human and rat liver microsomes with ^{14}C-MBOCA. The formation of metabolites was quantified using appropriate chemically synthesized standards (Morton et al. 1988). 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 2-2). 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 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). 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 2-2). 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).

2.3.3.3 Dermal Exposure

MBOCA metabolites were investigated in urine samples from workers occupationally exposed to MBOCA (Cocker et al. 1988, 1990). 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 less than 10% of the level of MBOCA recovered in urine of exposed workers. Skin absorption of MBOCA was considered an important factor in both 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).

Studies in dogs (Manis et al. 1984) and rats (Groth et al. 1984) indicate that MBOCA is rapidly metabolized following dermal exposure to ^{14}C-MBOCA and that urinary levels of unmetabolized MBOCA represents 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.

The major DNA adduct of MBOCA was identified by a combination of *in vivo* and *in vitro* studies (Silk et al. 1989). Male rats derived from a Wistar strain were exposed intraperitoneally to 89 μmol/kg of radioactive MBOCA or 141 μmol/kg of acetyl-MBOCA. Twenty-four hours later, animals were sacrificed, and their liver DNA was extracted and analyzed for the amount of bound radioactivity and DNA adduct formation. Results showed that the cleavage of the bond between the methylene bridge and one aromatic nucleus of MBOCA resulted in the formation of *N*-(deoxyadenosine-8-yl)-4-amino-3-chlorobenzyl alcohol, the major *in vivo* adduct. Two other adducts were also formed but were not characterized.

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

MBOCA has been analyzed and detected in the urine of exposed humans. nine hours after an accidental spill, the urine of a worker exposed to 11.34 L of molten MBOCA contained 1,700 ppb of MBOCA (NIOSH 1986a; Osorio et al. 1990). The biological half-life of MBOCA in the urine was estimated to be approximately 23 hours (Osorio et al. 1990). This was determined by measuring the levels of MBOCA in urine 2 weeks after exposure ceased. Assuming a one-compartment model, about 94% of the initial dose of MBOCA would be eliminated within 4 days. In another report, after an acute occupational exposure to molten MBOCA, a worker complained of burning eyes and face. Hot, liquid MBOCA was sprayed over his face and some entered his mouth (Hosein and Van Roosmalen 1978). The worker was monitored and his urine was analyzed for MBOCA over a 3-week period. Five hours after the spill, the level of MBOCA in the urine was 3.6 mg/L (3,600 ppb). MBOCA was rapidly excreted during the first 18 hours after exposure. Twenty-four hours after exposure, urine MBOCA level was down to 0.03 mg/L (30 ppb), and 3 weeks later it was below that level (Hosein and Van Roosmalen 1978). It was unclear from this study whether urine was the primary route of MBOCA excretion in humans following dermal exposure since no information was presented on the amount of MBOCA measured in feces.

Analysis of urine samples from 34 exposed workers showed that the urine levels of MBOCA were proportional to the exposure levels. Workers exposed to the highest levels of MBOCA (MBOCA mixers) had the highest level of the compound in the urine (NIOSH 1986b). Ten out of 10 mixers had a urinary MBOCA level of >5 μg/L and 5 of 10 had >50 μg/L of MBOCA in their urine. Urine samples from workers in the molding department (intermediate exposure) had levels of MBOCA ranging Erom <5 to 50 μg/L. All trimmers and supervisors (the lowest exposure group) had no detectable levels of MBOCA in the urine. The body clearance of MBOCA was tested in 13 urine samples from workers who were absent from exposure for 48 hours. Only one of the 13 workers, an MBOCA mixer, had a detectable MBOCA urine level (average: 8.9 μg/L) (NIOSH 1986b). The level of MBOCA in the air was <3 $\mu g/m^3$ most of the time, and the results of the wipe test ranged from 0.035 to 53 μg. The limitations of this study are that precise exposure levels and the exact routes of exposure were not given and that there were too few data points for any general conclusions to be made. 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. The study authors concluded that the biological half-time of urina MBOCA is relatively long. The breathing zone levels of MBOCA were 0.0002-0.089 mg/m^3. Limitations of this study include a small sample size and lack of controls.

The MBOCA levels in urine have been used to assess occupational exposure of workers engaged in the manufacture of polyurethane elastomers (Thomas and Wilson 1984). The objective of the study was also to identify areas that could be improved in order to reduce the exposure. Between 12 and 15 workers were followed over a 5-year period, and the exposure levels were reported as the monthly average amount of nmol MBOCA/mmol creatinine. The results of this biological monitoring program indicated that urine levels of MBOCA slowly but steadily decreased. Between July and September of 1978, the average level was 50 nmol MBOCA/mmol creatinine. Although the individual variations were present, by February 1980 the average level had fallen to 5 nmol MBOCA/mmol creatinine. Some of the measures that helped reduce the exposure to MBOCA were improved ventilation system, use of protective clothing, dry cleaning of clothing, improvement of the general hygiene in the factory, and special attention to workers with consistently high urinary MBOCA levels (Thomas and Wilson 1984).

N-acetyl MBOCA is a minor metabolite that was found in the urine of occupationally exposed workers (Cocker et al. 1988). Also present was a major labile metabolite, probably β-*N*-glucuronide (Cocker et al. 1990). After hydrolysis, this conjugate yields unmetabolized MBOCA. In an occupational exposure study of 122 workers from 19 different plants (one MBOCA manufacturer, 18 MBOCA users) urine MBOCA levels were determined in 215 samples collected mainly at the end of the shift (Ducos and Gaudin 1983). The highest urine levels of MBOCA were found in workers from the manufacturing plant: the highest mean urinary MBOCA level among the 12 workers was 600 μg/L or 450 μg/g relative to creatinine. This level was greatly reduced (to an average of 62 μg/g of creatinine) following manufacturing and processing modifications related to increased workers protection and good manufacturing practices. The levels of urinary MBOCA in workers of the other 18 businesses varied greatly and were highest in workers who had the greatest contact with MBOCA. The individual urinary MBOCA levels ranged from undetectable to a maximum of 940 μg/L (510 μg/g of creatinine). However, if the values outside of the detection limits (l-1400 μ/L) are excluded from the calculation, the urinary MBOCA averages are low varying from 4 to 318 μg/L (2-174 μg/g of creatinine) (Ducos and Gaudin 1983). The results indicate that the urinary MBOCA levels greatly depended on the frequency of MBOCA use, the quantities used, the form of MBOCA (granules, solid, or in solution), methods of manufacturing, and from work practices in general. Individual factors also affect the excretion levels. The predominant metabolite in urine was monoacetylated MBOCA present at low concentration of <1/10 of the total. Another metabolite, *N,N'*-diacetyl MBOCA was also identified in one of the workers. This was an unexpected finding because of the poor water solubility of diacetyl MBOCA. The identification of diacetyl MBOCA is important because it is possible that from *N,N'*-diacetyl MBOCA, *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 that has been proposed as one of the mechanisms involved in carcinogenesis.

One proposed mechanism of MBOCA excretion suggests that very little MBOCA is excreted into the bladder as the free amine since most of the MBOCA in the renal perfusate may be present in the form of the β-*N*-glucuronide conjugate (Cocker et al. 1990). Urine contains β-glucuronidase, and therefore, β-*N*-glucuronide may undergo some hydrolysis in the bladder. This is proposed as the source of free MBOCA in the urine.

2.3.4.2 Oral Exposure

No detailed studies were located regarding excretion in humans following oral MBOCA exposure. After an acute occupational exposure to molten MBOCA, one worker complained of burning eyes and face. Hot, liquid MBOCA was sprayed over his face and some entered his mouth (Hosein and Van Roosmalen 1978). The worker's urine was monitored for MBOCA excretion over a 3-week period. MBOCA was found to be rapidly excreted during the first 18 hours following exposure, reaching a maximum concentration of 3.6 mg/L; 6 hours later, (24 hours post-exposure), the urine MBOCA level was down to 0.03 mg/L. At 3 weeks, only trace amounts of MBOCA were detected in urine (Hosein and Van Roosmalen 1978).

A single oral dose of 10 mg/kg of radiolabeled MBOCA was administered to female LAC:Porton rats. Forty-eight hours later, the majority of the radioactivity was excreted in the feces (60%), while the liver retained the most radioactivity (2%) (Farmer et al. 1981). Similar observations were made in Sprague-Dawley rats given 12 mg/kg of radiolabeled MBOCA (Groth et al. 1984). 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 up to day (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-life of MBOCA was 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).

2.3.4.3 Dermal Exposure

Nine hours after an accidental spill, the urine of a worker exposed to 11.34 L of molten MBOCA contained 1,700 ppb of MBOCA (NIOSH 1986a; Osorio et al. 1990). The biological half-life of MBOCA in the urine was approximately 23 hours (Osorio et al. 1990). This was evaluated by monitoring the MBOCA urine levels for 2 weeks after exposure. Assuming a one-compartment model, about 94% of the initial dose of MBOCA would be eliminated within 4 days. In another report, after an acute occupational exposure to molten MBOCA, one worker complained of burning eyes and face. Hot, liquid MBOCA was sprayed over his face and some entered his mouth (Hosein and Van Roosmalen 1978). The worker was monitored and his urine analyzed for MBOCA over a j-week period. MBOCA was rapidly excreted within the first 18 hours following exposure, reaching a maximum concentration of 3.6 mg/L. Six hours later, (24 hours post-exposure), the MBOCA level in urine was down to 0.03 mg/L, dropping to trace amounts 3 weeks later (Hosein and Van Roosmalen 1978). In another report on occupational exposure, 340 analyses were performed on urine samples from 150 workers from 19 factories (Ducos and Gaudin 1983; Ducos et al. 1985). Samples were usually collected at the end of the shift. The levels of MBOCA ranged from undetectable (<0.5 μg/L) to 1,600 μg/L. The highest urine MBOCA levels were present in 12 workers from a factory that handled crystallized MBOCA. In a similar 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 (Edwards and Priestly 1992). There was no information on the levels of MBOCA that employees were exposed to, on the duration of exposure, or on the route. Based on the type of work, it is likely that the main route of exposure was dermal. The urinary output of thioether was evaluated because thioethers are metabolic end products of the pathway involving mercapturic acid. The thioethers, therefore, were thought to be potential, nonspecific, markers of exposure to electrophilic xenobiotics such as rubber monomers or petroleum.
They have not been used as markers for MBOCA exposure. The urinary thioether levels were similar in pre- and post-work samples ($p>0.05$) and did not correlate with urinary MBOCA levels, indicating that they are not useful for estimating occupational MBOCA exposure. Although there were no significant differences in urinary MBOCA levels between the three groups of employees (supervisors, laboratory personnel, process workers), the results reflect the potential exposure levels. The median values of MBOCA in the post-work urine of process workers and supervisors were 9.33 and 1.05 μmol mol^{-1} of creatinine, respectively. There was no MBOCA in the urine samples from 10 control workers. The small amount of urinary MBOCA in occupationally exposed workers suggests that other major metabolites, such as β-*N*-glucuronide conjugate (Cocker et al. 1990) may be present in the urine. From the results of all of the exposure studies it is not clear, however, if urine is the

primary excretion route in humans after dermal exposure to MBOCA since no information was provided regarding the amount of MBOCA in the feces.

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).

2.3.4.4 Other Routes of Exposure

Feces and urine were major excretion routes in rats after acute exposure to radiolabeled MBOCA by stomach intubation (Morton et al. 1988) or intravenous injection (Tobes et al. 1983). At 48 hours after exposure to 44-58 mg/kg of radiolabeled MBOCA by intubation, 64-87% of radioactivity was recovered in the urine and feces (Morton et al. 1988). A similar observation was made in rats 48 hours after a single intravenous dose of 0.51 mg/kg; feces contained 73% of the total cumulative dose of radiolabeled MBOCA (Tobes et al. 1983). These results indicate that total elimination of ^{14}C following intubation or intravenous injection of ^{14}C-MBOCA to rats was similar to that observed following oral dosing, which also occurred primarily in the feces (Farmer et al. 1981; Groth et al. 1984). After a single intraperitoneal injection of 1,13, or 100 mg/kg of MBOCA, the compound was excreted most rapidly within the first 24 hours. Rats receiving the highest concentration of MBOCA produced three times more urine during the first 24 hours than did controls (Farmer et al. 1981). Studies with intravenous administration of MBOCA in the dog found that the major route of excretion was through the urine (Manis et al. 1984). Within 24 hours after intravenous injection of ^{14}C-MBOCA, 46% of the dose had been excreted in the urine and 32% in the bile, accounting for 78% of the total dose. This indicates that often substantial differences occur between species and that route of exposure determines the route of elimination of MBOCA.

In an attempt to use thioether as a potential urinary indicator for MBOCA exposure, 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. There were no significant differences noted between the two experimental groups. As in the study with occupationally exposed workers, the urinary thioether levels were not affected by MBOCA treatment indicating that thioether was not a useful indicator for MBOCA exposure (Edwards and Priestly 1992).

The *in vivo* formation of MBOCA-hemoglobin adducts was determined in blood samples of male Sprague-Dawley rats intravenously injected with 0.04, 0.2, or 1.0 μmol/kg of n-hydroxy-MBOCA (Chen et al. 1991). The injection of the lowest dose of *N*-hydroxy-MBOCA resulted in formation of 0.9 ng/50 mg of MBOCA-hemoglobin adducts 24 hours after the injection. This level remained almost unchanged (0.8 ng/50 mg) 1 week after exposure indicating a relatively long half life of this adduct. A similar observation was made in guinea pigs injected subcutaneously with 4, 20, or 100 mg/kg of MBOCA. Detectable levels of MBOCA-hemoglobin adducts were still present

10 weeks after exposure to the lowest dose of MBOCA. In guinea pigs, the life span of MBOCA-hemoglobin adducts was similar to that of red blood cells, 60-80 days.

2.3.5 Mechanisms of Action

The precise mechanism of action of MBOCA is not completely understood. However, the capacity of MBOCA to form adducts with hemoglobin (Cheever et al. 1988, 1990, 1991; Chen et al. 1991; Sabbioni and neumann 1990) tissue DNA (Cheever et al. 1990) and globin and serum albumin (Cheever et al. 1991) in rats may play a role in its carcinogenicity. The adduct formation is especially important in elucidating the carcinogenicity of MBOCA. Two metabolites were identified in the urine of one occupationally exposed worker: n-acetyl MBOCA and *N,N'*-diacetyl MBOCA (Ducos and Gaudin 1983). The finding of n,n'-diacetyl MBOCA was unexpected (because of its poor water solubility) and important. It can be assumed, using benzidine metabolism as a model, that from *N,N'*-diacetyl MBOCA, *N*-hydroxy-*N,N*'-diacetyl MBOCA can be formed. *N*-hydroxy-*N,N'*-diacetyl MBOCA is important because it can directly bind to nucleic acids. Therefore, the confirmation of its existence would elucidate the biochemical basis for adduct formation. This finding is in agreement with a recently proposed mechanism for chemical carcinogenesis that may involve the formation of chemical adducts in DNA through covalent binding. This proposition was based on the fact that MBOCA produces DNA adducts in rat liver at levels characteristic of genotoxic carcinogens (Kugler-Steigmeier et al. 1989). Since it is known that MBOCA can bind to rat liver DNA (Cheever et al. 1990), and that liver is one of the primary target organs for development of tumors after exposure of rats to MBOCA (Kommineni et al. 1979; Russfield et al. 1973, it may be assumed that MBOCADNA adducts play a role in the development of tumors. The mechanism of hemoglobin adduct formation by *N*-oxidized MBOCA metabolites was recently investigated in viva and *in vitro* using human and rat hemoglobin (Chen et al. 1991). Male Sprague-Dawley rats were injected intraperitoneally with 0.5-50 mg/kg of MBOCA, and male English short hair guinea pigs were injected subcutaneously with 4-100 mg/kg of MBOCA. In the same study, treatment of rats with either phenobarbital or β-naphthaloflavone (BnF) resulted in induction of cytochrome P-450 as evidenced by increased enzymatic activity of two P-450 isozymes. This induction caused increased *ortho*-hydroxylation of MBOCA that was not statistically significant and resulted in the formation of various metabolites. Two of those 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. This *in vitro* binding occurred in a dose-related manner and could be inhibited by cysteine and glutathione but not by oxidized glutathione or methionine (Chen et al. 1991). Therefore, the administration of MBOCA to rats resulted in a dose-related formation of hemoglobin adducts that were detectable 24 days after treatment. Similar observa tions were made in guinea pigs; the life span of MBOCA-hemoglobin adducts followed that of re cells in the guinea pig and was approximately 60-80 days.

One of the major DNA adducts of MBOCA was identified by a combination of *in vivo* and *in vitro* studies (Silk et al. 1989). Male rat derived from a Wistar strain were injected intraperitoneally with 89 µmol/kg of radioactive MBOCA or 141 µmol/kg of acetyl-MBOCA. Twenty-four hours later, animals were sacrificed, and their liver-DNA was extracted and analyzed for the amount of bound radioactivity and nature of adducts. Results,showed that the cleavage of the bond between the methylene bridge and one aromatic nucleus of MBOCA resulted in the formation of the major *in vivo* adduct *N*-(deoxyadenosine-8-yl)-4-amino-3-chlorobenzyl alcohol. Two other adducts also formed after intraperitoneal injection were not identified. In an attempt to further elucidate the DNA adduct formation, DNA was isolated from liver, lung, and kidney of ^{14}C-MBOCA-treated Charles River CD rats and compared to *in vitro* *N*-hydoxy-MBOCA-treated rat DNA (Segerback and Kadlubar 1992).

Rats were orally treated with 95 μmol/kg body weight of radiolabeled MBOCA and sacrificed 1, 3, 7, and 21 days later. In both *in vivo* and *in vitro* experiments, two major peaks were identified containing two adducts: *N*-(deoxyadenosine-8-yl)-4-amino-3-chlorobenzyl alcohol and n-(deoxyadenosine-S-yl)-4-amino-3-chlorotoluene. These two major peaks were present in both liver and kidney DNA samples. The results confirmed the existence of one adduct found in a similar study (Silk et al. 1989) and identified the second one. The study found that the binding of *N*-hydroxy-MBOCA to DNA can be inhibited by ascorbic acid, glutathione, nitrosobenzene, and methyl viologen but not by nitromethane,p-nitrobenzylpyridine or methionine. 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).

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 doses ranging from 7.5 nmol/mL (noncytotoxic) to 15.5 nmol/mL (cytotoxic) (Kuslikis et al. 1991). Since GJC is important in controlling cell proliferation, and many known tumor promoters inhibit GJC, GJCinhibition assays have been proposed as short-term screens for promoters.

2.4 RELEVANCE TO PUBLIC HEALTH

Exposure to MBOCA contaminated soil or water at hazardous waste sites is possible via the oral or dermal routes. Inhalation and dermal exposure to this compound in air are less likely because MBOCA is not volatile. There is no information regarding the level that would be of concern to a population living in the vicinity. Occupational or accidental exposure may occur in industries using MBOCA as a curing agent in manufacturing processes. It is not known if MBOCA and/or its metabolites can be released from polyurethane (MBOCA is used as a curing agent for castable liquid polyurethane elastomers) (HSDB 1991; IARC 1974; Sax and Lewis 1987).

Acute-duration exposure to high levels of MBOCA may cause eye and skin irritation in humans (Hosein and Van Roosmalen 1978). Intermediate and chronic exposures to MBOCA may lead to tumors of the urinary bladder. Of 200 occupationally exposed workers, 3 were found to have urinary bladder tumors (Ward et al. 1990). This finding is supported by results obtained in dogs (Stula et al. 1977). Results in other animal species also show that MBOCA is a potential carcinogen and that other target organs include the lung, liver, breast, and Zymbal's gland in rats (Kommineni et al. 1978; Russfield et al. 1975; Stula et al. 1975) and lung, liver, and vascular system in mice (Russfield et al. 1975). It is not possible to estimate if these tumors would be of concern to humans since there is no information available.

Limited information indicates that MBOCA is rapidly absorbed in humans and that the amount absorbed through skin and inhalation is in proportion to the dose (Chin et al. 1983; Cocker et al 1988, 1990; Ichikawa et al. 1990; NIOSH 1986b). No information is available regarding distribution and rate of metabolism in humans. However, toxicokinetic data obtained in animals indicate that MBOCA is rapidly absorbed, distributed, and excreted within 24-48 hours (Farmer et al. 1981; Groth et al. 1984). The bioaccumulation of MBOCA is unlikely because of its rapid metabolism and urinary excretion. Of potential concern, however, is the fact that MBOCA readily forms adducts with body proteins and DNA. Studies in rats and guinea pigs suggest that MBOCA-hemoglobin adducts have a biological life similar to that of red blood cells and may remain in the body for many weeks.

No information was located regarding reproductive or developmental effects in humans or animals.

There is no information in humans regarding MBOCA retention or any delayed effects. Information on the effects of exposure to MBOCA in humans comes from case reports of accidentally or occupationally exposed workers in manufacturing facilities. In one such study, low-grade papillary tumors of the urinary bladder were observed in 3 out of 200 workers more than 8 years following occupational exposure to MBOCA (Ward et al. 1990). Studies in progress include assessing the incidence of bladder carcinomas in workers occupationally exposed to MBOCA and the role of adducts in MBOCA-induced carcinogenicity (see Section 2.93). Animal studies support the hypothesis that MBOCA and/or its metabolites have carcinogenic potential. MBOCA metabolites bound to DNA were found following oral (Cheever et al. 1990; Kugler-Steigmeier et al. 1989) or dermal (Cheever et al. 1990) exposures in rats.

No studies were located regarding the genotoxicity of MBOCA in humans *in vivo.* Results from *i*n *vivo* animal studies support the conclusion that MBOCA is a mutagen; mixed results from some assays can probably be attributed to incomplete testing.

Minimal Risk Levels for MBOCA

Inhalatio*n *MRL3

No inhalation MRLs have been derived for any duration of exposure because of lack of data.

Oral MRL8

No acute-duration oral MRL has been derived because of lack of data.

No intermediate-duration oral MRL has been derived because of lack of data.

An MRL of 0.003 mg/kg/day has been derived for chronic-duration oral exposure to MBOCA. This is based on a LOAEL of 10 mg/kg/day for hepatic effects in dogs (Stula et al. 1977).

Death. no increased mortality from any cause has been reported in humans who have been exposed to MBOCA. Decreased lifespan was observed in rats following chronic oral exposure to MBOCA (Kommineni et al. 1979). Data are insufficient to assess the effects that prolonged exposure to MBOCA may have on populations that are potentially at risk.

Systemic Effects

Respiratory Eflects. no data are available regarding adverse effects of MBOCA on the respiratory tract in humans.

Lung adenomatosis, a preneoplastic lesion, was observed in rats following chronic oral exposure to 25 or 50 mg/kg/day of MBOCA (Russfield et al. 1975). MBOCA may induce changes in the lungs that can progress to cancerous lesions. Since information on respiratory effects following exposure to MBOCA is limited, the impact of findings in animals on public health is difficult to assess. For more information, see the discussion of Cancer, below, in this section.

Gastrointestinal Eficts. Extremely limited information is available regarding adverse gastrointestinal effects in humans after exposure to MBOCA. A worker complained of feeling ill in the stomach after being sprayed in the face with molten MBOCA (Hosein and Van Roosmalen 1978). No further information was given on his condition or the level of exposure. Evidence is insufficient to assess the relevance of this finding to public health.

Hematological Eflcts. no information is available regarding adverse hematological effects in humans after exposure to MBOCA.

However, no changes in hemoglobin, hematocrit, erythrocyte, or mononuclear leukocyte counts were observed in beagle dogs following 9 years of exposure to MBOCA (Stula et al. 1977). This study is of limited usefulness because blood analysis was done only twice a year, so that changes between analyses were probably not recorded. The significance of this finding with regard to human health cannot be assessed.

Hepatic Efìcts. no information was located in humans regarding adverse hepatic effects following exposure to MBOCA.

Hepatic effects such as elevated liver enzyme levels, nodular hepatic hyperplasia, fatty changes, necrosis, fibrosis, and bile duct proliferation were observed in rats and dogs following chronic exposure to MBOCA (Stula et al. 1975, 1977). Chronic exposures to high levels of MBOCA at hazardous waste sites may induce similar adverse hepatic effects.

Renal Effects. Following an acute exposure in which a worker was sprayed in the face with molten MBOCA, protein was detected in the urine within the first 5 hours after exposure, suggesting that damage to renal tubules occurred (Hosein and Van Roosmalen 1978). Damage to the renal tubules was also indicated by a decrease in specific gravity of the urine collected the next day. Although the amount of MBOCA to which the worker was exposed was not known, it may have been substantial, since the urine MBOCA level was 3.6 mg/L 5 and 11 hours after the incident. It is unknown whether exposure to these high levels would occur at hazardous waste sites. In another study, exfoliative cytology revealed that 21 out of 385 urine samples collected from workers exposed to MBOCA contained atypical cells in urine sediment (Ward et al. 1990). None of the cytology readings were positive for cancer. Sixteen out of the 385 urine samples were positive for heme (Ward et al. 1990). The median duration of employment was 3.2 months. Although this study is limited because it lacks information on exposure levels, it is important because it alerted the medical community to the inadequacies of the procedures normally used following MBOCA exposure. Since one of the participants (who had negative cytology and was negative for heme) was diagnosed with a low-grade papillary tumor of the bladder, cystoscopy was introduced as a part of the follow-up procedure for workers exposed to MBOCA, and two additional cases of bladder tumors were detected subsequently. Therefore, exposure to MBOCA at hazardous waste sites may cause renal damage that may lead to cancer.

Studies on chronic exposure of dogs to MBOCA support the findings in humans (Stula et al. 1977). In dogs treated orally with an average of 10 mg/kg/day of MBOCA for 9 years, urine sediment cytology revealed dysplastic changes indicative of neoplasia of the genitourinary system.

Dermal/Oculur Effects. Burning of the face and eyes developed shortly after a worker was accidentally sprayed with molten MBOCA (Hosein and Van Roosmalen 1978). He was wearing gloves and safety glasses but no respirator or face shield. A similar burning sensation was described by a worker who was also accidentally sprayed over his upper body and extremities with molten MBOCA (Osorio et al. 1990). The general population is not likely to be involved in such exposures at hazardous waste sites. However, in cases of occupational accidents, exposure to MBOCA may cause adverse dermaliocular effects.

Immunological Effects. no studies were located in humans or animals regarding immunological effects following exposure to MBOCA.

Neurological Effects. no studies were located regarding adverse neurological effects in humans after exposure to MBOCA by any route.

Cystic hyperplasia of the pituitary gland was found in one out of six dogs after 8.3 years of MBOCA exposure (Stula et al. 1977). This change was considered to be unrelated to chronic MBOCA treatment. It is difficult to predict if analogous effects would occur in individuals or populations living near hazardous waste sites.

Reproductive Effects. no studies were located regarding reproductive effects in humans or animals following exposure to MBOCA.

Developmental Effects. no studies were located regarding developmental effects in humans or animals after exposure to MBOCA.

Genotoxic Effects. no studies were located regarding the genotoxicity of MBOCA in humans *in vivo.*

In vivo animal studies 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. Small increases in the SLRL values were observed in D. melanogaster adults fed a 7.5millimolar MBOCA solution for 3 days (Vogel et al. 1981).

MBOCA induced gene mutations at the thymidine kinase (TK) locus in mouse lymphoma cells (Caspary et al. 1988; Myhr and Caspary 1988). Unscheduled DNA synthesis (UDS) was induced in HeLa cells (Martin and McDermid 1981), in rat primary hepatocytes at $\geq$10 µmol (McQueen et al. 1981; Mori et al. 1988; Williams et al. 1982) and in hamster (McQueen et al. 1981) and rabbit (McQueen and Williams 1987) hepatocytes. The concentration that tested positive in the mouse was 50 µmol (McQueen et al. 1981). Sensitivity to MBOCA showed species-specific variations: rat > mouse > hamster > rabbit (McQueen et al. 1981, 1983). Because hepatocytes have their own metabolic activation systems, no exogenous metabolic activation is needed. In assays using attachment independence as an end point, MBOCA, at concentrations near the LC_{50}, transformed baby hamster kidney (Daniel and Dehnel 1981; Styles 1981), rat embryo (Dunkel et al. 1981; Traul et al. 1981), and Balb/3T3 cells (Dunkel et al. 1981). Transformation assays have not been evaluated as thoroughly as some other genotoxicity assays. In an interlaboratory comparison, one laboratory found equivocal evidence that nonactivated MBOCA induced sister chromatid exchange in Chinese hamster ovary cells at a dose of 5.0 µg/mL. The response is considered equivocal because the dose-response curve was inconsistent. The result was not confirmed by the second laboratory. In the presence of S9 activation, positive results were obtained by one laboratory at 50 µg/mL. The other laboratory observed the beginning of a dose-response curve, but the high-dose (30 µg/mL) results did not meet the testing laboratory's criterion for a positive response. In a chromosome aberration assay testing activated and nonactivated concentrations up to 5 and 30 µg/mL, respectively, neither laboratory found a positive result (Galloway et al. 1985). Another study (Perry and Thomson 1981) found no evidence of sister chromatid exchange at up to cytotoxic doses (100 μg/mL), but no doses were tested in the probable sensitive range (>10 and <100 μg/mL). Thus, MBOCA is clastogenic in some systems but not others.

In another study, MBOCA was classified as a clastogen on the basis of results from an in viva micronucleus bone marrow assay (Katz et al. 1981). Evaluations using a two-phase micronucleus assay and intraperitoneal dosing of $B6C3F_1$ hybrid mice were incomplete, but the results suggested that MBOCA is an in viva clastogen at doses of >32 mg/kg (Sa amone et al. 1981) or 50% of the 1 LD_{50} (Katz et al. 1981). Results were inconclusive because of toxicity during the first phase of testing in which mice were dosed at 0 and 24 hours with 80% of the LD_{50} (51 mg/kg), and results were evaluated at 48 hours. In the second phase of testing, mice were dosed with 32 or 51 mg/kg and sampled 48 hours later, or in a separate test, dosed with 32 or 48 mg/kg and sampled 36 hours later. Results were negative at 32 mg/kg when sampled at 48 hours but positive at the same dose in a separate assay when sampled at 36 hours. Results were positive at the higher dose for both sampling times. Multiple sampling times were not performed in any assay (Salamone et al. 1981). In another study, results in the micronucleus test were negative at doses up to 50% of the LD_{50} (32 mg/kg) using CD-1 mice (dosing was done intraperitoneally at 0 and 24 hours and sampling at 30 hours) (Tsuchimoto and Matter 1981). Results from in viva testing are summarized in Table 2-2.

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 doses ranging from 7.5 nmol/mL (noncytotoxic) to 15.5 nmol/mL (cytotoxic) (Kuslikis et al. 1991). Since GJC is important in controlling cell proliferation, and many known tumor promoters inhibit GJC, GJCinhibition assays have been proposed as short-term screens for promoters.

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In vitro testing has provided clear and convincing evidence that MBOCA is mutagenic in the *Salmonella typhimurium*/mammalian microsome mutagenesis assay and that the mutagenic effect requires exogenous metabolic activation (Baker and Bonin 1981; Cocker et al. 1985; Dunkel et al. 1984; MacDonald 1981; Martire et al. 1981; McCann et al. 1975; Messerly et al. 1987; Rao et al. 1982). Although not all investigators used each tester strain, the general result is that MBOCA is mutagenic only in strains TA98 and TA100, 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 and TA100 contain this plasmid, but strains TA1535, TA1537, and TA1538 do not (Ames et al. 1975). 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 (Matsushima et al. 1981). The plasmid carries genes involved in an "errorprone" DNA repair system that introduces mutations as it removes DNA damage (Walker 1984). S9 derived from dog and human liver could activate MBOCA to a form mutagenic to strain TA100 but only in a protocol using a fluctuation assay (Cocker et al. 1985). These data are presented in Table

TABLE 2-2. Genotoxicity of 4,4'-Methylenebis(2-chloroaniline) (MBOCA) *In Vivo*

Species (test system)	End point	Results	Reference
Mouse CD1 (intraperitoneal)	Micronucleus formation	–	Tsuchimoto and Matter 1981
Mouse $B6C3F_1$ (intraperitoneal)	Micronucleus formation	+	Salamone et al. 1981
Mouse $B6C3F_1$/BR (intraperitoneal)	Micronucleus formation	+	Katz et al. 1981
Drosophila melanogaster (oral, dermal)	Wing spot test	+	Kugler-Steigmeier et al. 1989
D. melanogaster (oral, dermal)	Sex-linked recessive lethal mutation	+	Vogel et al. 1981
D. melanogaster (inhalation, occupational)	Sex-linked recessive lethal mutation	+	Donner et al. 1983
Rat (Sprague-Dawley; oral)	DNA adduct formation	+	Cheever et al. 1990
Rat (Sprague-Dawley; oral)	DNA adduct formation	+	Kugler-Steigmeier et al. 1989
Rat (Sprague-Dawley; dermal)	DNA adduct formation	+	Cheever et al. 1990
Rat (Wistar-derived strain; intraperitoneal)	DNA adduct formation	+	Silk et al. 1989

– = negative result; + = positive result; DNA = deoxyribonucleic acid

TABLE 2-3. Genotoxicity of 4,4'-Methylenebis(2-chloroaniline) (MBOCA) *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium TA100[a] (reverse mutation)	Gene mutation	+	No data	Cocker et al. 1986
S. typhimurium TA100 (histidine reversion)	Gene mutation	+	-	Cocker et al. 1985
S. typhimurium TA100 (histidine reversion)	Gene mutation	+	No data	Hesbert et al. 1985
S. typhimurium TA98, TA100 (histidine reversion)	Gene mutation	+	-	Dunkel et al. 1984
S. typhimurium TA100 (histidine reversion)	Gene mutation	+	-	Messerly et al. 1987
S. typhimurium TA100 (histidine reversion)	Gene mutation	+	No data	Ichinotsubo et al. 1981a
S. typhimurium TA100 (histidine reversion)	Gene mutation	+	-	McCann et al. 1975
S. typhimurium TA100 (histidine reversion)	Gene mutation	+	No data	Kugler-Steigmeier et al. 1989
S. typhimurium TA98 (histidine reversion)	Gene mutation	+	No data	Rao et al. 1982
S. typhimurium TA98, TA100 (histidine reversion)	Gene mutation	+	-	Brooks and Dean 1981
S. typhimurium TA98, TA100 (histidine reversion)	Gene mutation	+	-	Baker and Bonin 1981
S. typhimurium TA98, TA100 (histidine reversion)	Gene mutation	+	-	Martire et al. 1981
S. typhimurium TA98, TA100 (histidine reversion)	Gene mutation	+	-	Garner et al. 1981
S. typhimurium TA98, TA100 (histidine reversion)	Gene mutation	+	-	MacDonald et al. 1981
S. typhimurium TA98, TA100 (histidine reversion)	Gene mutation	+	-	Simmon and Shepherd 1981
S. typhimurium TA98, TA100 (histidine reversion)	Gene mutation	+	-	Nagao and Takahashi 1981
S. typhimurium TA1535, TA98, TA100 (histidine reversion)	Gene mutation	+	No data	Trueman 1981

TABLE 2-3. Genotoxicity of 4,4'-Methylenebis(2-chloroaniline) (MBOCA) *In Vitro (continued)*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
S. typhimurium TA98, TA100, (histidine reversion)	Gene mutation	+	-	Venitt and Crofton-Sleigh 1981
S. typhimurium TA100 (histidine reversion)	Gene mutation	+	-	Hubbard et al. 1981
S. typhimurium TA1538 (histidine reversion)	Gene mutation	+	-	Gatehouse 1981
S. typhimurium TA100 (histidine reversion)	Gene mutation	+	-	Rowland and Severn 1981
S. typhimurium TM677 (contains pKM101; 8-azaguanine^r)	Gene mutation	+	No data	Skopek et al. 1981
Escherichia coli WP2 uvrA (tryptophan reversion)	Gene mutation	-	-	Gatehouse 1981
E. coli WP2 uvrA/pKM101 (tryptophan reversion)	Gene mutation	+	-	Matsushima et al. 1981
E. coli WP85, WP2 uvrA(P) (tryptophan reversion)	Gene mutation	+	-	Venitt and Crofton Sleigh 1981
E. coli 58-161 envA (lambda lysogen)	Phage lambda induction (SOS induction)	+	No data	Thomson 1981
E. coli P3478 (polA$^-$)/W3110 (polA$^+$)	Differential killing	-	+	Rosenkranz et al. 1981
E. coli JC2921 (recA$^-$), JC5519 (recBC$^-$)	Differential killing	+	No data	Ichinotsubo et al. 1981b
E. coli WP67, CM871	Differential killing	-	+	Tweats 1981
Bacillus subtilis rec$^-$	Differential killing	+	+	Kada 1981
Eucaryotic organisms:				
Fungi:				
Saccharomyces cerevisiae XV185-14C (auxotroph reversion)	Gene mutation	-	-	Mehta and Von Borstel 1981
S. cerevisiae XII	Mitotic recombination	-	-	Kassinova et al. 1981
S. cerevisiae D4	Mitotic gene conversion	-	-	Jagannath et al. 1981

TABLE 2-3. Genotoxicity of 4,4'-Methylenebis(2-chloroaniline) (MBOCA) *In Vitro (continued)*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
S. cerevisiae JD1	Mitotic gene conversion	+	+	Sharp and Parry 1981
S. cerevisiae D6	Mitotic aneuploidy	+	+	Parry and Sharp 1981
Mammalian cells:				
Chinese hamster ovary	Chromosomal aberrations	–	–	Galloway et al. 1985
Chinese hamster ovary	Sister chromatid exchange	+/–	+/–	Galloway et al. 1985
Chinese hamster ovary	Sister chromatid exchange	–	–	Perry and Thomson 1981
HeLa cells	Unscheduled DNA synthesis	+	–	Martin and McDermid 1981
Rat primary hepatocytes	Unscheduled DNA synthesis	No data	+	McQueen et al. 1981
Rat primary hepatocytes	Unscheduled DNA synthesis	No data	+	Williams et al. 1982
Rat primary hepatocytes	Unscheduled DNA synthesis	No data	+	Mori et al. 1988
Mouse primary hepatocytes	Unscheduled DNA synthesis	No data	+	McQueen et al. 1981
Hamster primary hepatocytes	Unscheduled DNA synthesis	No data	+	McQueen et al. 1981
Rabbit primary hepatocytes	Unscheduled DNA synthesis	No data	+	McQueen and Williams 1987
Primary hamster embryo cells	Single strand DNA breaks	No data	+	Casto 1983
Human male embryonic lung cells	Single strand DNA breaks	No data	+	Casto 1983
Mouse lymphoma (L5178Y TK+/-)	Forward gene mutation	+	–	Myhr and Caspary 1988
Mouse lymphoma (L5178Y TK+/-)	Forward gene mutation	+	–	Caspary et al. 1988
RLV-infected rat embryo ($2FR_450$)	Transformation (attachment independence)	No data	+	Traul et al. 1981

TABLE 2-3. Genotoxicity of 4,4'-Methylenebis(2-chloroaniline) (MBOCA) *In Vitro (continued)*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
RLV-infected rat embryo ($2FR_450$)	Transformation (attachment independence)	No data	+	Dunkel et al. 1981
Balb/3T3 mouse cells	Transformation (attachment independence)	No data	+	Dunkel et al. 1981
Baby hamster kidney (BHK21 C13)	Transformation (attachment independence)	+	+	Daniel and Dehnel 1981
Baby hamster kidney (BHK21)	Transformation (attachment independence)	+	No data	Styles 1981
C3H2K cells/MLV	Viral integration enhancement	No data	–	Yoshikura and Matsushima 1981
Human bladder explant culture	DNA adduct formation	No data	+	Stoner et al. 1988
Dog bladder explant culture	DNA adduct formation	No data	+	Stoner et al. 1988

[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; DNA = deoxyribonucleic acid; MLV = Moloney mouse sarcoma-leukemia virus; RLV = Rauscher leukemia virus; SOS induction = induction of an error-prone repair system; TK = thymidine kinase

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 of ≥5 μg/plate (Kuslikis et al. 1991). 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 a deactivating step. neither n-acetyl nor *N*,*N*-diacetyl derivatives were mutagenic in *S. typhimutium* in the absence of activation (Hesbert et al. 1985). In the presence of S9 activation, the mutagenic activity of the acetylated derivatives is less than that of the parent compound (Cocker et al. 1986; Hesbert et al. 1985).

DNA adducts have been found following oral (Cheever et al. 1990; Kugler-Steigmeier et al. 1989; Segerback and Kadlubar 1992) and dermal (Cheever et al. 1990) administration of radiolabeled MBOCA to rats, following the incubation of radiolabeled MBOCA with explants of dog and human bladder urothelium (transitional cell epithelium) (Stoner et al. 198) and incubation of rat DNA and radiolabeled *N*-hydroxy-MBOCA (Segerback and Kadlubar 1992). 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 adduct formation in dog bladder tissue is of particular note, since MBOCA has been found to cause bladder tumors in dogs (Stula et al. 1977). The *in vivo* and *in vitro* studies identified two other major rat DNA MBOCA adducts, n-(deoxyadenosin-8-yl)-4-amino-3-chlorobenzyl alcohol and *N*-(deoxyadenosin-8-yl)-4-amino-3 chlortoluene (Segerback and Kadlubar 1992). The finding of DNA adducts in human bladder tissue suggests similar processes may occur in humans.

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

Cancer. Since MBOCA has been suspected of being a human carcinogen, epidemiological studies have examined the incidence of cancer in workers exposed to MBOCA. Three out of 200 examined workers exposed to MBOCA for an average of 3.2 months were found to have low-grade papillary tumors of the urinary bladder (Ward et al. 1990). The latency period for most bladder cancers is about 20 years; therefore, the detection of bladder tumors in this study 11.5 years (mean latency period for the entire cohort) after first exposure to MBOCA provides suggestive evidence that MBOCA is a suspected human carcinogen. Two of the men affected with bladder tumors were less than 30 years old. Since it is known that the occurrence of bladder tumors in young men is very uncommon, this finding increases the concern that MBOCA has a potential for causing bladder tumors in humans. However, there was no evidence of recurrent bladder lesions in the above cases after the initial discovery. It is also difficult to estimate the biological and statistical significance of these bladder lesions because of the small sample size. One important outcome of this study is the introduction of cystoscopy as a part of the monitoring protocol for workers exposed to MBOCA. The limitation of the study is that there are no data on bladder cancer incidence diagnosed by cystoscopy in an asymptomatic nonexposed (control) population. The importance of biological monitoring programs was also evident in the occupational study/review of urinary free MBOCA levels in workers from PMA member companies (Lowry and Clapp 1992). The data have been collected for over 15 years, and the study summarized them for 1985-1990. The analysis of 7,844 urine samples confirmed that after the introduction of biological monitoring it was possible to reduce the urinary MBOCA levels by using effective exposure control and protection in the workplace. The reduced percentage of samples containing >50 μg/L of MBOCA (from 12% in 1985 to 8% in 1990) and increased percentage of urine samples with <25 μg/mL (from 77% in 1985 to 86% in 1990) further supported the importance of biological urinary MBOCA monitoring.

The probable carcinogenicity of MBOCA has been a concern for some time. In 1972, the British rubber industry imposed upon itself a ban on the use of MBOCA because it was a suspected human carcinogen (Parkes 1979). The initiation of a biological monitoring program that would examine exposure levels and help establish Environmental or other pertinent controls as preventive measures has also been suggested (Ward et al. 1986). This biomonitoring program would also maintain a system to follow the workers exposed to MBOCA in order to detect possible neoplastic changes and initiate treatment.

Chronic exposure studies in animals support the findings in humans. The main target organs for cancer development were the urinary bladder, lungs, liver, and breasts. The urinary bladder was a target organ in beagle dogs fed MBOCA for 9 years; three of the five surviving dogs developed papillary transitional cell carcinoma of the urinary bladder; another dog had a combined urethral adenocarcinoma and transitional cell carcinoma (Stula et al. 1977). This study, in spite of the use of a small number of animals, shows that the ingestion of MBOCA over 9 years was associated with the incidence of carcinomas of the urinary bladder and urethra. Several other chronic exposure studies in rats demonstrate the ability of MBOCA to cause lung cancer (Kommineni et al. 1979; Stula et al. 1975). A significant increase in the incidence of liver tumors after chronic exposure to MBOCA was found in rats (Kommineni et al. 1979; Russfield et al. 1975) and in female mice (Russfield et al. 1975). The rats fed MBOCA for 2 years had an increased incidence of mammary adenocarcinomas (Kommineni et al. 1979). Other tumor types were also observed after chronic oral administration of MBOCA. The incidence of hemangiosarcomas was 8% in rats (Kommineni et al. 1979); vascular tumors were present in male random-bred albino mice (Russfield et al. 1975); and hemangiosarcomas were present in both male and female mice (Russfield et al. 1975). Tumor incidence was affected by gender in mice; no data regarding gender specificity are available for rats or dogs.

The precise mechanism of action of MBOCA is not completely understood, but it is thought that in some instances it involves the formation of chemical adducts in the genetic material through covalent binding of nucleophilic sites by electrophilic compounds or activated metabolites (Cheever et al. 1991). Therefore, it seems that the capacity of MBOCA to form adducts with tissue DNA (Cheever et al. 1990), hemoglobin (Cheever et al. 1990, 1991; Sabbioni and neumann 1990) and globin and serum albumin (Cheever et al. 1991) in rats may play a role in its toxicity and carcinogenicity. The adduct formation is especially important in elucidating the carcinogenicity of MBOCA. It was recently proposed that chemical carcinogenesis may involve the formation of chemical adducts in DNA through covalent binding based on the fact that MBOCA produces DNA adducts in rat liver at levels characteristic of genotoxic carcinogens (Kugler-Steigmeier et al. 1989). Since it is known that MBOCA can bind to rat liver DNA (Cheever et al. 1990) and that liver is one of the primary target organs for development of tumors after exposure of rats to MBOCA (Kommineni et al. 1979; Russfield et al. 1975), it may be assumed that MBOCA-DNA adducts play a role in the development of tumors. Further support for the carcinogenic potential of MBOCA comes from the study showing that MBOCA binds to DNA from human and dog urinary bladder tissue (Shivapurkar et al. 1987).

The mouse skin assay was used in one study to evaluate the carcinogenicity of MBOCA in the presence of 12-*o*-tetradecanoylphorbol-U-acetate (TPA) as a tumor promoter (nesnow et al. 1985). SEnCAR mice were exposed to 0, 0.1, 1, 10, 100, or 200 mg of MBOCA and 1 week later to 2 μg of TPA biweekly for 26 weeks. No mouse skin papillomas were observed at any dose level, but the authors stressed that this finding was based on the limited dose levels tested in the study. There are several limitations to this study; it was not clear if TPA was administered to control animals, if MBOCA was applied to shaved skin, or if was the area was protected after treatment; in addition, no other parameters aside from tumor initiation were examined during the 26-week period.

There is some evidence that MBOCA itself may be a tumor promoter. MBOCA caused a statistically significant dose-dependent inhibition of GJC at doses ranging from 7.5 nmol/mL (noncytotoxic) to 15.5 nmol/mL (cytotoxic) under *in vitro* conditions (Kuslikis et al. 1991). Since GJC is important in controlling cell proliferation, and known tumor promoters inhibit GJC, GJC-inhibition assays have been proposed as short-term screens for promoters. The use of GJC-inhibition assays in individuals living in the vicinity of hazardous waste sites would provide needed information for better understanding of potential control of the tumor initiation process.

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are indicators of events in biologic systems. They have been classified as markers of exposure, markers of effect, and markers 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). Preferred exposure biomarkers are either the substance proper or substance-specific metabolites in readily obtainable body fluid(s) or excreta. Several factors may confound the use and interpretation of exposure biomarkers: (1) the body burden of a substance may represent more than one exposure; (2) the substance being measured may be the metabolite of another substance (e.g., high levels of phenol in urine can result from exposure to several different aromatic compounds). Depending on the substance's properties (e.g., biologic half-life), and exposure conditions (e.g., duration and route of exposure), the substance and metabolites may have left the body by the time samples are collected. Individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium) may be difficult to identify. Biomarkers of exposure to MBOCA are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). Biochemical or cellular signs of tissue dysfunction and physiologic alterations are included within this definition. note that these markers are often not substance specific. Biomarkers of effect(s) may indicate potential health impairment(s) (e.g., DNA adducts). Biomarkers of effects caused by MBOCA are discussed in Section 2.5.2.

A biomarker of susceptibility may indicate 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 either an intrinsic genetic characteristic, a preexisting disease that results in greater absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, Populations That Are Unusually Susceptible.

2.5.1 Biomarkers Used to Identify or Quantify Exposure to MBOCA

Exposure to MBOCA has commonly been determined by measuring levels of MBOCA or its metabolites in urine. no information on quantifiable dose threshold levels in humans was located. 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.

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. 19SS). 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,identifiedas5-hydroxy-3,3'-dichloro-4,4'-diamino-diphenylmethane-5-sulfate,wasthe 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; Sabboioni and newmann 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 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.

2.5.2 Biomarkers Used to Characterize Effects Caused by MBOCA

The available information suggests that the bladder is a target organ for MBOCA-induced carcinogenesis. Medical surveillance of occupationally exposed workers, currently in progress, may help to ascertain the incidence of MBOCA-induced bladder cancer. Cystoscopy may help identify new biomarkers and characterize early preneoplastic changes in the bladder.

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 halflife of MBOCA-globin adducts is greater than the half-life of MBOCA. Section 2.2 contains more detailed information on adverse effects of MBOCA. Additional information can be obtained from the CDC/ATSDR (CDC/ATSDR 1990) and Office of Technology Assessment (OTA 1990) reports listed in Chapter 8.

2.6 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. 19%). 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. 19S9). 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).

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population may have a heightened response to MBOCA compared with the general population. A population’s susceptibility may be determined by their genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These unique characteristics could result in a decreased ability to detoxify and excrete the xenobiotic material and metabolites (mainly hepatic, renal, and respiratory). We expect that the elderly, (with declining organ function), and infants and children with immature and developing organs, may be more susceptible to the effects of toxic substance exposure(s) than healthy adults. Populations at greater risk because of their atypically high exposures are discussed in Section 5.6, Populations With Potentially High Exposure.

Differences in species sensitivity to MBOCA have been explored *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. 198%). 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. 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.

An indication of DNA adduct formation following acute exposure to MBOCA was found in autoradiographs of postlabeled liver DNA from exposed rats (Endo and Hara 1991). Compared to control, the relative adduct level (RAL) was considerably higher in experimental samples than controls. However, further studies are needed to identify these rat liver DNA adducts. In an attempt to elucidate the DNA adduct formation, DNA was isolated from liver, lung, and kidney of ^{14}C-

MBOCA-treated rats and compared to *in vitro N*-hydoxy-MBOCA-treated rat DNA (Segcrback and Kaclluhar 1992). In both casts, two major peaks wcrc identified containing two adclucts: n-(deoxyadenosinc-8-yl)-4-amino-3-chlorobenzyl alcohol and N-(deoxyadenosine-8-yl)-4-amino-3-chlorotoluene. Although the study found that the binding of *N*-hydroxy-MBOCA to DNA can be inhibited by ascorbic acid, glutathione, nitrosobenzcne, and methyl viologen (but not by nitromethane. p-nitrobcnzylpyridine, or met hionine), the precise mechanism of single-ring MBOCA DNA adduct formation is not completely understood. Gluthatione (GSH) has also been shown to protect against hemoglobin adduct formation with oxidized MBOCA metabolites (Chen et al. 1991). Individuals with lowcrcd GSH levels brought about by oxidative stress or excessive exposure to GSH depleting xenobiotics (such as acetaminophen) could also be at increased risk following MBOCA exposure. More studies are needed to understand the mechanism and the intermediaries in the DNA adduct formation. In the cast of MBOCA, acetylation was not related to genotoxicity (McQueen et al. 1983). In a study performed in rabbits, the genotoxic response to MBOCA varied considcrnbly; the net number of radioactive grains per nucleus in individual rabbits ranged from 0 to 13.7.

Factors such as these may partially inlluence individual susceptibilities to MBOCA-induced carcinogcnicity. 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.

Workers in MBOCA manufacturing facilities have a higher risk because of their higher-level exposures to MBOCA. Worker exposure occurs primarily through inhalation and dermal contact. Other populations with higher risk are those that live near these facilities (if releases are not controlled) and family members of exposed workers (because contamination of the household occurs as a result of clothing) (Willams 1979).

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to MBOCA. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposure to MBOCA. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

No information has been located regarding specific treatment of humans or animals following exposure to MBOCA other than removal from the source of exposure. However, as in cases of most toxic exposures, supportive therapy dealing with symptoms is recommended. These supportive therapies include the monitoring of vital functions, as well as electrocardiographic monitoring, observation of vital signs, the intake and output of fluids, body temperature, and possibly pulmonary wedge pressure if indicated (HSDB 1991). Of special importance in minimizing absorption is the selection of protective clothing and gloves. The permeability of tight commercially available glove materials to methanolic solutions of aromatic amines was tested (Weeks and Dean 1977). The results indicate that there are considerable differences in the permeability of tested materials. Natural latex seemed to be most protective for 9.8 mg/mL MBOCA (1.0 weight percent) in methanol (Weeks and Dean 1977). The recommendation of how frequently the gloves should be changed will depend on the specific situation. The results of the study show that penetration of the glove material by the MBOCA solution was concentration dependent, and that highly concentrated MBOCA (9.8 mg/mL) penetrated the gloves within 2 hours, while MBOCA at a relatively low concentration did not penetrate for 35 hours (Weeks and Dean 1977). Biological monitoring of workers occupationally exposed to MBOCA is a preventive measure that can reduce potential toxic and carcinogenic effects. Among the other recommended procedures are monitoring MBOCA air levels and urinary MBOCA levels, doing wipe tests to check surface contamination that can potentially lead to skin exposure (Clapp et al. 1991; Lowry and Clapp 1992), and considering the performance of cystoscopy in exposed workers for early detection of bladder tumors (Mason and Volger 1990; Ward et al. 1990). Continued education of the work force about potential work hazards is also important (NIOSH 1986b).

2.8.2 Reducing Body Burden

MBOCA is not retained by the body since the majority of the compound is excreted within 24-48 hours after exposure (Hosein and Van Roosmalen 1978). The biological half-life of MBOCA in the urine was estimated at approximately 23 hours in a case of an accidental exposure (NIOSH 1986a). The same study assumed a one-compartment excretion model that would take 4 days to eliminate about 94% of MBOCA from the body. Similar results were obtained in rats after a single dose of radioactive MBOCA; 64-87% of the radioactivity was recovered in the urine and feces within 48 hours after exposure (Morton et al. 1988). It is not known if MBOCA metabolites are retained in the body and for how long. no information was located regarding reduction of body burden in humans or animals following exposure to MBOCA.

Because phenobarbital induces the microsomal cytochrome P-450 enzyme system responsible for MBOCA hydroxylation (Chen et al. 1991), phenobarbital treatment might increase the breakdown of MBOCA and speed its removal from the body. However, because MBOCA's metabolites are more toxic than the unmetabolized moiety, this intervention must be used with caution. Such treatment could also affect MBOCA adduct formation. While phenobarbital did not increase in. Go hemoglobin adduct formation in one study in rats (Chen et al. 1991), Cheever et al. (1991) found an increase in globin adduct formation (radioactivity) following phenobarbital induction. The major DNA adduct of MBOCA was identified in another study by a combination of *in vivo* and *in vitro* experiments (Silk et al 1989). Male rats derived from a Wistar strain were intraperitoneally exposed to either 89 μmol/kg of radioactive MBOCA or 141 μmol/kg of acetyl-MBOCA. Twenty-four hours later, animals were sacrificed and their liver DNA was extracted and analyzed for the amount of bound radioactivity and nature of adducts. Results showed that the cleavage of the bond between the methylene bridge and one aromatic nucleus of MBOCA resulted in the formation of the major *in vivo* adduct identified as *N*-(deoxyadenosine-8-yl)-4-amino-3-chlorobenzyl alcohol. Two other adducts also formed after intraperitoneal injection were not identified. These findings were confirmed and extended when DNA was isolated from liver, lung, and kidney of ^{14}C-MBOCA-treated rats and compared to *in vitro* *N*-hydoxy-MBOCA-treated rat DNA, and two major adducts were identified: *N*-(deoxyadenosine-8-yl)-4-amino-3-chlorobenzyl alcohol and *N*-(deoxyadenosine-8-yl)-4-amino-3-chlorotoluene (Segerback and Kadlubar 1992). Although the results showed that the adduct formation (binding of *N*-hydroxy-MBOCA to DNA) can be inhibited by ascorbic acid, glutathione, nitrosobenzene, and methyl viologen but not by nitromethane, P-nitrobenzylpyridine, or methionine, the precise mechanism of single-ring MBOCA DNA adduct formation is not completely understood. Therefore, more information on DNA adduct formation especially in human bladder, and possibly in liver, is needed since DNA adducts are considered to play a major role in MBOCA-induced carcinogenesis.

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

Existence of an antidote capable of reducing or alleviating MBOCA-induced toxicity has not been reported. Available information indicates that most harmful effects of MBOCA in humans come from its metabolites. Once the detailed mechanism of action of MBOCA is known, and more Information is gathered on the potential adduct inhibitors, it may be possible to interfere with DNA adduct formation and reduce the adverse effects.

2.9 ADEQUACY OF THE DATABASE

Section 104(i)(S) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of MBOCA is available. Where adequate information is not available, ATSDR, in conjunction with the national Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of MBOCA.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

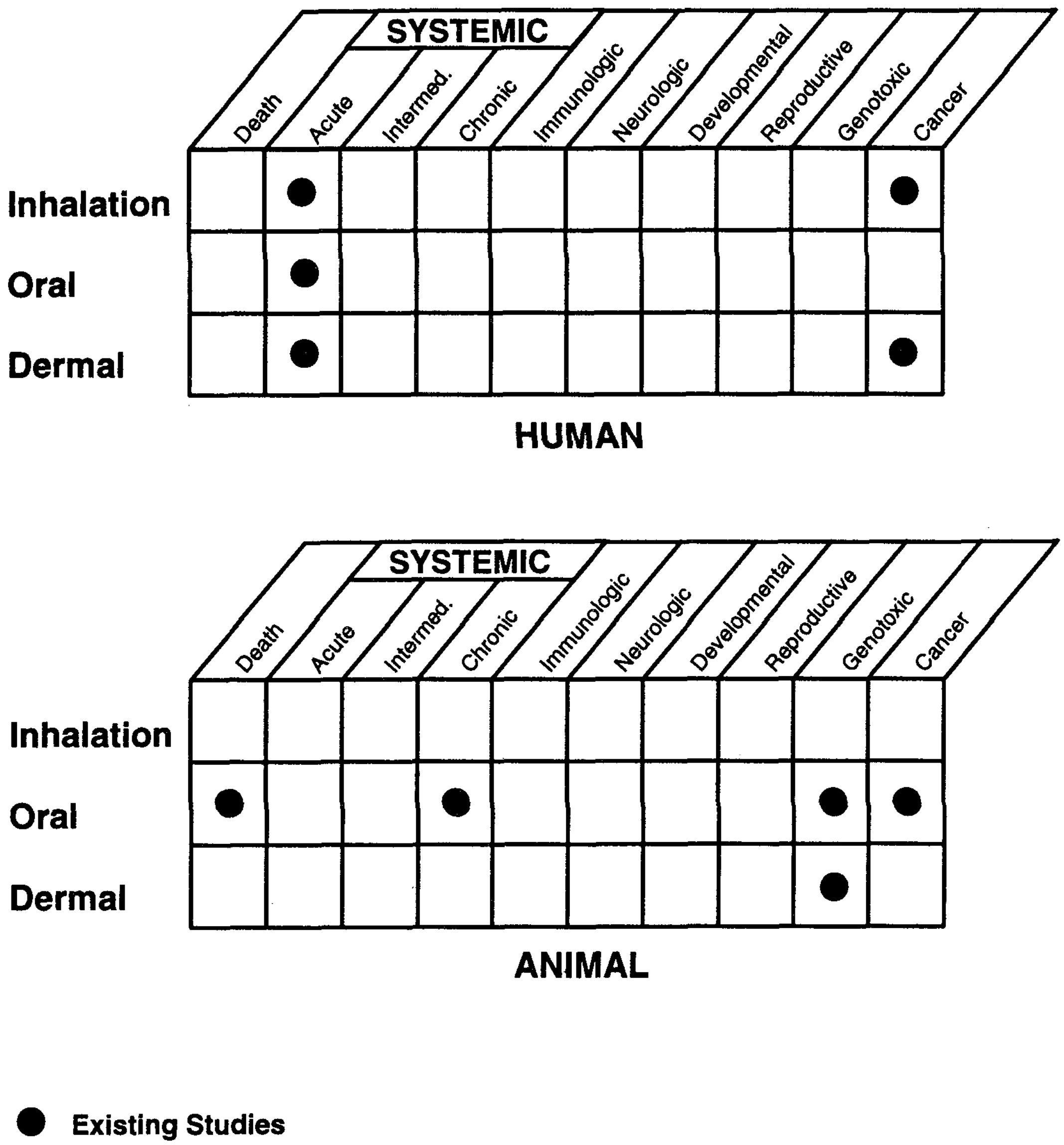
2.9.1 Existing Information on Health Effects of MBOCA

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to MBOCA are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of MBOCA. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs." A data need, as defined in ATSDR's Decision Guide for Identifying Substance-Specific Data needs Related to Toxicological Profiles (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

With regard to human health effects of MBOCA, the few available studies were either case reports of acute occupational exposure or involved intermediate or chronic epidemiological studies. Acute exposures to MBOCA were by the inhalation, oral, or dermal routes, although in some studies it was difficult to clearly define the exposure route. Intermediate exposures were by either inhalation and/or dermal contact; no intermediate oral exposure studies were located. Chronic exposure in humans occurred by inhalation and/or dermal contact; no chronic oral studies were located. no information is available regarding immunological, neurologic, reproductive, developmental, or genotoxic effects in humans by any route of exposure. Studies on cancer incidence in humans after inhalation and/or dermal exposure to MBOCA were located.

Virtually all of the data regarding the health effects of MBOCA in animals were obtained from studies in which MBOCA was administered orally. Extremely limited information is available regarding health effects in animals following inhalation or dermal exposure.

FIGURE 2-3. Existing Information on Health Effects of 4,4'-Methylenebis (2-chloroaniline) (MBOCA)



2.9.2 Identification of Data Needs

Acute-Duration Exposure. Populations close to hazardous waste sites that contain MBOCA would be exposed to MBOCA for short periods of time, most likely be through the dermis, although inhalation and ingestion exposure(s) cannot be excluded. Limited information on acute exposure in humans comes from reports on accidental exposure by skin absorption and inhalation (Hosein and Van Roosmalen 1978). In this case, eyes and skin were affected since the worker was sprayed on the face with molten, liquid MBOCA. A small amount of protein in his urine indicated that the kidney was a target organ for MBOCA toxicity. Insufficient information was available to calculate an acuteduration MRL for MBOCA.

No information was located regarding toxic effects in animals following acute exposure to MBOCA. Studies addressing toxic effects of MBOCA in animals after skin application and inhalation (since these are the most likely routes of exposure for human populations in the vicinity of hazardous waste sites) could provide needed information for estimation of possible acute toxicity of MBOCA in humans.

Intermediate-Duration Exposure. Limited information is available in humans regarding toxic effects of MBOCA following intermediate exposure by inhalation and/or dermal contact, and no information was located regarding oral exposure. In one study, exposure most likely occurred as a result of combined inhalation and dermal exposures, and the exposure duration was averaged at 3.2 months. The results showed that the urinary bladder is the major target organ in humans after intermediate-duration exposures to MBOCA. Papillary neoplasms (one carcinoma) of the urinary bladder were found in 3 out of 200 occupationally exposed workers (Ward et al. 1990). No studies of intermediate-duration exposure in animals were located to support this finding. However, results from chronic animal studies clearly support the findings in humans after intermediate exposure to MBOCA. Lung, liver, and mammary tumors were found in rats (Kommineni et al. 1979; Stula et al. 1975). Hepatomas and vascular tumors were present in mice (Russfield et al. 1973, while transitional cell carcinoma of the urinary bladder was found in dogs (Stula et al. 1977) after chronic exposure to MBOCA. Insufficient information is available to calculate an intermediate-duration MRL for MBOCA.

Animal studies examining the effects of intermediate-duration exposure to MBOCA via the inhalation and dermal routes are necessary to evaluate the likelihood of urinary bladder carcinoma in humans after intermediate exposure to MBOCA.

Chronic-Duration Exposure and Cancer. Epidemiological studies in humans following chronic exposure to MBOCA are very limited. No information is available regarding toxic effects in humans following chronic exposure to MBOCA.

One study of chronic occupational exposure in humans found low-grade papillary tumors of the urinary bladder in 3 out of 200 workers (Ward et al. 1990). This result suggests that the urinary bladder is the major target organ in terms of tumor incidence in humans following chronic exposure. This finding is supported by results obtained in dogs (Stula et al. 1977). Data in other animal species following oral exposure also support the finding that MBOCA is a potential carcinogen and show that other target organs include lungs, liver, breast, and Zymbal's gland in rats (Kommineni et al. 1979; Russfield et al. 1975; Stula et al. 1975) and lungs, liver, and vascular system in mice (Russfield et al. 1975). Although the animal data support those in humans following an occupational exposure (Ward et al. 1990) the report in humans is limited in that it is a case report without controls to allow the determination of a dose-response relationship and statistical significance of data. There was also potential of co-exposure to other chemicals. Further occupational exposure studies are needed to establish the carcinogenic potential of MBOCA in humans.

Currently, there are several studies in progress sponsored by NIOSH that are investigating different aspects of human exposure to MBOCA (FEDRIP 1991; also Section 2.93). One of the studies is involves the worker population exposed to MBOCA between 1969 and 1979. The principal investigator is E.M. Ward, and the objective of the study is to see if the occurrence of cancer can be linked to exposure to MBOCA. The second study concerns biomonitoring for populations occupationally exposed to aromatic amines (of which MBOCA is one). F.B. Daniel is the principal investigator for this study entitled "Biomonitoring for Populations Occupations Exposed to Aromatic Amines." Hemoglobin and DNA adduct formation by MBOCA will be analyzed in order to propose a methodology for monitoring workplace exposure. Also sponsored by NIOSH is a third study which involves on developing and evaluating biological monitoring methodologies for detecting the presence and uptake of MBOCA by urine. L.L. Lowry is the principal investigator for this project entitled "Biological Monitoring Methods Research and Evaluation." This investigator will also evaluate the effectiveness in predicting worker uptake of MBOCA and two other compounds. The NTP also has a study in progress on "At-y1 Amine Adducts in Blood as Indicators of Exposure." In this study, blood samples from 100 workers will be analyzed for hemoglobin o-toluidine adducts. MBOCA will be used to develop a high-performance liquid chromatography (HPLC) method for separation and isolation of mitochondrial or total aryl amine-DNA adducts. In addition, the *in vitro* activation of potential carcinogens will be studied, and a mathematical model for MBOCA distribution, metabolism, and adduct formation will be prepared. The overall objective of the project is to develop a more sensitive adduct isolation procedure to be used for biological monitoring. The contact person for this project is K. Cheever (NTP 1991a). A grant was awarded in September 1991 to the Michigan Department of Public Health to conduct an epidemiologic study under the title "Analytic Epidemiologic Study of MBOCA" (ATSDR 1991). The objective of the study is to determine if there is an association between exposure to MBOCA and occurrence of bladder cancer in workers, their family members, and residents who live near three NPL sites. Cystoscopy will be used in all cases with positive screening results. The information on all MBOCA-exposed members of the cohort will be maintained in a registry. A case-control study will be conducted to assess the association of MBOCA exposure and bladder cancer incidence. The contact person for this project is H. Humphrey from the Michigan Department of Public Health. Thus, there are sufficient on-going studies on biomonitoring of humans exposed to MBOCA.

Genotoxicity. There is considerable evidence that MBOCA metabolites are mutagenic in *S. typhinurium* strains TA98 and TA100 (Cocker et al. 1985; MacDonald 1981; Matsushima et al. 1981; Messerly et al. 1987; Kuslikis et al. 1991) and in mouse lymphoma cells (Caspary et al. 1988; Myhr and Caspary 1988) and induce UDS in HeLa cells (Martin and McDermid 1981) and in hepatocytes from rats (McQueen et al. 1981; Moriet al. 1988; Williams et al. 1982) mice, hamsters (McQueen et al. 1981), and rabbits (McQueen and Williams 1987). However, nonactivated MBOCA is not a mutagen. *In vitro* evidence indicates that human liver (Cocker et al. 1985) and bladder epithelium (Stoner et al. 1988) contain enzymes that can activate MBOCA to genotoxic derivatives. It is less clear whether MBOCA is a clastogen. Mixed results have been obtained in the *in vivo* micronucleus assay (Katz et al. 1981; Salamone et al. 1981; Tsuchimoto and Matter 1981) and in the *in vitro* assays for sister chromatid exchange (Galloway et al. 1985; Perry and Thomson 1981) while data regarding chromosome aberrations were negative (Galloway et al. 1985; Perry and Thomson 1981). Micronucleus assays done with multiple sampling times, and *in vivo* and *in vitro* chromosome aberration assays performed near cytotoxic levels would be useful in assessing the clastogenic potential of MBOCA. MBOCA metabolites bind to DNA in rat (Cheever et al. 1990; Kugler-Steigmeier et al. 1989), dog, and human (Stoner et al. 1988) bladder and liver tissues. Wide variations in the amount of material bound were observed in dog and human tissue explants (Stoner et al. 1988). Identifying the source of this variability might help in identifying sensitive populations. One study reported that MBOCA itself may be a promoter (Kuslikis et al. 1991); confirmation or negation of this result might be useful in evaluating the interactions of MBOCA with other chemicals.

Reproductive Toxicity. No studies were found regarding reproductive effects in humans or animals following exposure to MBOCA by any route. Thus, studies examining the effects on reproduction by any route of exposure would be useful.

Developmental Toxicity. No human or animal studies are available on developmental effects for any exposure route. Studies assessing postnatal survival after maternal exposure to MBOCA by all three routes would be very informative.

Immunotoxicity. No information on immunotoxicity after exposure to MBOCA by any of the three routes is available in humans or animals. Studies in laboratory animals following acute inhalation, oral, and dermal exposure to MBOCA would help define possible effects on antibody production and cellular immunity. This information would be useful for determining sensitive populations.

Neurotoxicity. No studies on neurotoxicity in humans following exposure to MBOCA were located. In one out of six female beagle dogs, cystic hyperplasia of the pars intermedia of the anterior pituitary gland was found after 8.3 years of treatment with MBOCA (Stula et al. 1977). Although this change was not present in any of the control dogs, it was not considered to be treatment related. Studies in animals following acute, intermediate, or chronic exposure to MBOCA by any route would be useful in establishing if the central nervous system is one of the target organs for MBOCA toxicity. These studies should include neurobehavioral screening tests such as a functional observation battery of tests, tests for motor activity, and schedule-controlled operant performance tests. neuropathology data from MBOCA-exposed animals would also be helpful.

Epidemiological and Human Dosimetry Studies. Human studies on MBOCA consist of either case reports of accidental exposure or follow-up studies of workers previously exposed to MBOCA. Exposures in both cases are mainly inhalation and dermal. Since MBOCA is a potential human carcinogen, the follow-up studies focused on monitoring the possible development of bladder carcinomas in workers exposed to MBOCA (Ward et al. 1990). At the present time, there are three studies in progress sponsored by NIOSH that are investigating different aspects of MBOCA exposure in humans (FEDRIP 1991) (see Section 2.93). One of the studies involves workers exposed to MBOCA; the second involves biomonitoring for populations occupationally exposed to aromatic amines (including MBOCA); and the third involves the development and evaluation of biological methodologies for monitoring the presence and uptake of MBOCA in urine. Thus, at the present time, there are sufficient studies on biomonitoring of humans exposed to MBOCA.

Biomarkers of Exposure and Effect

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

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

Effect. The urinary bladder is a target organ for MBOCA-induced carcinogenicity (Ward et al. 1990). Retrospective biological monitoring using cystoscopy would help identify new biomarkers necessary to characterize the preneoplastic state of the urinary bladder.

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

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

No information was located regarding metabolism in humans after oral exposure to MBOCA. Limited information is available regarding metabolism in humans following inhalation or dermal exposure (Cocker 1988, 1990; Ducos et al. 1985; Osorio et al. 1990) to MBOCA. Metabolism has been partially characterized in animals following oral exposure. *In vitro* studies investigating the capacity of MBOCA to form adducts characterized one of its metabolites, a product of cleavage between the methylene bridge and one of the aromatic nuclei, as a DNA adduct-forming metabolite, *N*-hydroxy-MBOCA (MBOCA-NHOH) (Silk et al. 1989). Several MBOCA metabolites were identified following *N*- and *o*-hydroxylation of MBOCA by the canine, guinea pig, and rat liver mixed-function oxidase systems (Chen et al. 1989). Because differences in metabolism may occur with differences in the route of exposure, more data on metabolism following inhalation and dermal exposures would be useful. Also needed is information on MBOCA metabolites in terms of their potential carcinogenic capacities.

No human data were located regarding excretion following oral exposure to MBOCA. There is, however, limited information on excretion in humans after inhalation and/or dermal exposure showing that the metabolites (*N*-acetyl MBOCA, β-*N*-glucuronide of MBOCA) and very limited amounts of parent MBOCA are excreted in the urine (Cocker et al. 1988; Ichikawa et al. 1990; NIOSH 1986b). Studies in rats show that, after acute oral exposure to radioactive MBOCA, the majority of the label is in the feces (Farmer et al. 1981; Groth et al. 1984). More information is needed on excretion rate in animals after exposure to MBOCA via all three routes in order to establish which is the major route of excretion.

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

Methods for Reducing Toxic Effects. Protocols need to be developed to abolish or alleviate adverse effects following exposure to MBOCA. As in most cases of toxic exposures, supportive therapy dealing with symptoms is recommended. Biological monitoring of workers occupationally exposed to MBOCA is a preventive measure that can reduce potential toxic and carcinogenic effects. Among the other recommended procedures are monitoring MBOCA air levels and urinary MBOCA levels, doing wipe tests to check surface contamination that can potentially lead to skin exposure (Clapp et al. 1991), and considering the performance of cystoscopy in exposed workers for early detection of bladder tumors (Mason and Volger 1990; Ward et al. 1990). Also important in minimizing absorption is the selection of protective clothing and gloves. The permeability of eight commercially available glove materials (with regard to methanolic solutions of aromatic amines) differed considerably, and the penetration of MBOCA was dependent on the concentration (Weeks and Dean 1977). Therefore, the recommendation for how frequently the gloves should be changed depends on the specific situation. Continued education of the work force about potential work hazards is also important (NIOSH 1986b).

2.9.3 On-going Studies

On-going studies regarding the health effects of MBOCA were reported in the Federal Research in Progress (FEDRIP 1991) database. The national Institute for Occupational Safety and Health (NIOSH) is the sponsoring institution of three studies involving MBOCA currently in progress. One project is entitled “Investigation of Workers Exposed to MBOCA” and the principal investigator is E.M. Ward from NIOSH. The main objective of this study is to see if the occurrence of cancer can be linked to exposure to MBOCA. To achieve this, the study will use biological markers (MBOCA in the urine and urinary bladder cytology) to develop more effective, sensitive, and specific early markers for occupationally related health conditions. F.B. Daniel from NIOSH is the principal investigator of the study on “Biomonitoring for Populations Occupations Exposed to Aromatic Amines.” The investigator will analyze hemoglobin and DNA adduct formation by MBOCA in order to propose a methodology for monitoring workplace exposure. L.L. Lowry from NIOSH is the principal investigator of the project entitled “Biological Monitoring Methods Research and Evaluation.” This study will develop and evaluate biological monitoring methods in urine for their effectiveness in predicting worker uptake of MBOCA and two other compounds. The NTP has a study in progress on “Aryl Amine Adducts in Blood as Indicators of Exposure.” In this study, blood samples from 100 workers will be analyzed for hemoglobin *o*-toluidine adducts. MBOCA will be used to develop a high-performance liquid chromatography (HPLC) method for separation and isolation of mitochondrial or total aryl amine-DNA adducts. In addition, the *in vivo* activation of potential carcinogens will be studied, and a mathematical model for MBOCA distribution, metabolism, and adduct formation will be prepared. The overall objective of the project is to develop a more sensitive adduct isolation procedure to be used for biological monitoring. The contact person for this project is K. Cheever (NTP 1991a). A grant was awarded in September 1991 to the Michigan Department of Public Health to conduct an epidemiologic study under the title “Analytic Epidemiologic Study of MBOCA” (ATSDR 1991). The objective of the study is to determine if there is an association between exposure to MBOCA and occurrence of bladder cancer in workers, their family members, and residents who live near three NPL sites. The screening regimen includes urine dipstick selftesting (using Hemastix kits) and collection of specimens for cytology. Cystoscopy will be used in all cases with positive screening results. The information on all MBOCA-exposed members of the cohort will be maintained in a registry. A case-control study will be conducted to assess the association of MBOCA exposure and bladder cancer incidence. The contact person for this project is H. Humphrey from the Michigan Department of Public Health.