Re-evaluation of Drinking-Water Guidelines for Diisopropyl Methylphosphonate

Subcommittee on the Toxicity of Diisopropyl Methylphosphonate, Committee on Toxicology, Board on Environmental Studies and Toxicology, National Research Council

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Preface

THERE HAS BEEN a long-standing disagreement between the U.S. Army and the tate of Colorado over the appropriate drinking-water guideline for diisopropyl methylphosphonate (DIMP), a groundwater contaminant at the U.S. Army's Rocky Mountain Arsenal. The disagreement is over the 100-fold difference between the guideline established by the U.S. Environmental Protection Agency and the one promulgated by Colorado.

In response to a request from the Army, the National Research Council (NRC) has conducted an independent evaluation of new studies on the toxicity of DIMP and a re-evaluation of the federal and state drinking-water guidelines for DIMP. This report is intended to provide information to help assess clean-up efforts at the arsenal.

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that assist the NRC in making the published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their participation in the review of this report: Maureen Feuston, Sanofi Pharmaceuticals, Inc., Malvern, Pennsylvania; Curtis

Klaassen, University of Kansas Medical Center, Kansas City, Kansas; Loren Koller, Oregon State University, Corvallis, Oregon; and Ernest Eugene McConnell, ToxPath, Inc., Raleigh, North Carolina.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by Harihara Mehendale, University of Louisiana at Monroe, Monroe, Louisiana, appointed by the Commission on Life Sciences, who was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

We gratefully acknowledge the individuals who provided background material and gave presentations to the subcommittee: Thomas Bucci, Pathology Associates International; Edward Calabrese, University of Massachusetts; Timothy Kilgannon, Rocky Mountain Arsenal; Raj Goyal, Edward La Rock, and Ellen Mangione, Colorado Department of Public Health and Environment; and William Wustenburg, Alternet Medical.

We are also grateful for the assistance of the NRC staff in preparing the report. Staff members who contributed to this effort are Carol Maczka, senior program director for the Toxicology and Risk Assessment Program; Kulbir Bakshi, senior program director of the Committee on Toxicology; Kate Kelly, technical editor; Leah Probst and Emily Smail, project assistants; and Mirsada Karalic-Loncarevic, information specialist. We especially wish to recognize the contributions of project director Susan Pang, who coordinated the project and contributed to the preparation of the subcommittee's report.

Finally, we would like to thank all the members of the subcommittee for their dedicated efforts throughout the development of this report.

John A. Moore, D.V.M.
Chair, Subcommittee on the Toxicity of Diisopropyl Methylphosphonate
Bailus Walker, Jr., Ph.D., M.P.H.
Chair, Committee on Toxicology

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Executive Summary

DIISOPROPYL METHYLPHOSPHONATE (DIMP) is a groundwater contaminant at the U.S. Army's Rocky Mountain Arsenal in Colorado. DIMP is a chemical by-product resulting from the manufacture and detoxification of the nerve agent GB (Sarin), which was produced at the arsenal from 1953 to 1957. For some time, there has been disagreement between the Army and the State of Colorado regarding the appropriate drinking-water contaminant guideline for DIMP. In 1989, the U.S. Environmental Protection Agency (EPA) established a drinking-water guideline of 600 micrograms per liter (μg/L), but the State of Colorado promulgated a lower guideline of 8 μg/L. The reason for the difference is that different studies were used to calculate the guidelines. Colorado used one-generation reproductive toxicity study in mink, whereas EPA used a subchronic toxicity study in dogs. EPA did not use the mink study, which reported an increase in female mortality, citing natural high mortality in captive mink and uncertainties about the relevance of mink to human health assessment. Colorado disagreed with EPA's assessment, contending that EPA used inappropriate data to assess mortality rates in captive mink and that mink have extrapolative relevance to humans.

To help resolve the disagreement, a two-generation reproductive study in mink was conducted. The Army asked the National Research Council (NRC) for an independent evaluation of that 1997 study and a re-evaluation of the drinking-water guideline for DIMP. The NRC as

signed this task to the Committee on Toxicology, which convened the Subcommittee on the Toxicity of Diisopropyl Methylphosphonate, a multidisciplinary group of experts. The subcommittee evaluated the two-generation reproductive study, as well as other studies relevant to assessing the toxicity of DIMP. Of particular relevance were a subchronic toxicity study in mink and a comparative metabolism study in mink and rats. Data on the use of mink as a predictive model in toxicology also were reviewed.

The subcommittee evaluated the biology and physiology of mink and found no scientific basis to preclude the use of mink in quantitative human-health risk assessments. In fact, the weight of evidence on DIMP indicates that two studies in mink—the two-generation reproductive toxicity study and the subchronic toxicity study—provide the most appropriate database for deriving a drinking-water guideline for DIMP.

The subcommittee concludes that neither EPA's nor Colorado's drinking-water guideline for DIMP is based on the best currently available data. The subcommittee considers the 1997 two-generation reproductive study to be the best available study for deriving a drinking-water guideline, because it involved the most relevant exposure duration (13 months) and because the most biologically meaningful findings of the study, Heinz body formation (protein aggregates in oxidatively-stressed red blood cells) and cholinesterase (ChE) inhibition, are consistent with the results of the subchronic toxicity study in mink.

Introduction

DIMP IS A GROUNDWATER contaminant at and near the Army's Rocky Mountain Arsenal in Adams County, Colorado. It was released into the environment as a result of the manufacturing process and neutralization of GB (Sarin), which was manufactured at the arsenal between 1953 and 1957. There has been a long-standing controversy between the Army and the State of Colorado regarding the appropriate drinking-water guideline for DIMP. EPA recommends a guideline (lifetime health advisory) of 600 μ g/L, whereas the State of Colorado has promulgated a lower guideline of 8 μg/L. The reason for the difference is that different studies were used to calculate the drinking-water guidelines. Colorado used a one-generation reproductive toxicity study in mink (Aulerich et al. 1979) that reported increased mortality in female mink. EPA did not use that study, citing natural high mortality in captive mink and uncertainties about the relevance of mink to human health assessment. Instead, EPA used a 90-day toxicity study in dogs (Hart et al. 1980).

In 1990, the NRC Committee on Toxicology (COT) evaluated the scientific basis for the two drinking-water guidelines for DIMP. COT found the data from the one-generation mink study to be compromised because of inadequate testing and reporting procedures, and it concluded that EPA's drinking-water guideline for DIMP was an appropriate interim drinking-water guideline until additional research was

done. COT recommended that another reproductive toxicity study in mink be conducted. In 1997, a two-generation reproductive toxicity study (Bucci et al. 1997) in mink was completed, and the Army requested that NRC independently review the study and other recent publications on DIMP to determine whether the interim drinking-water guideline supported by NRC in 1990 is still valid. If it is not, the subcommittee was asked to make scientific recommendations for developing an appropriate guidance level.

Evaluation

THE SUBCOMMITIEE REVIEWED the one-generation (Aulerich et al. 1979) and two-generation (Bucci et al. 1997) reproductive toxicity studies in mink, as well as other data relevant to assessing the results of those studies. Of particular relevance were a subchronic toxicity study in mink (Bucci et al. 1994) and a comparative metabolism study in mink and rats (Weiss et al. 1994). The toxic potential of some metabolites of DIMP and data on the use of mink as a predictive model in human toxicology also were reviewed. The collective information was used to re-evaluate the state and federal drinking-water guidelines.

ONE-GENERATION REPRODUCTIVE TOXICITY STUDY

In the study by Aulerich et al. (1979), four groups of 24 female and 6 male dark variety mink were administered feed containing DIMP at concentrations of 0, 50, 150, and 450 parts per million (ppm). The animals were housed in outdoor commercial-style mink ranch sheds, and control animal sheds were interspersed with those of the test animals. The mink were about 3 months old at the beginning of the study, and treatment was continued for 49 weeks, which included one reproductive cycle. Daily intake of DIMP was calculated to be 0, 11, 37, and

95 milligrams per kilogram of body weight per day (mg/kg-day) based on mean feed consumption and mean body weight.

The most important finding was an increase in mortality among females; 2 of 23 (9%), 3 of 24 (13%), and 5 of 24 (21%) died in the low-, mid-, and high-dose groups, respectively, whereas no deaths occurred in the control group. No treatment-related effects were observed with regard to body weight, feed consumption, hematocrit values, hemoglobin parameters, and leukocyte counts, nor were any effects observed on reproduction. Gross and histologic examinations of the test animals showed no consistent changes, and there was no significant difference in organ weight compared with controls.

In 1990, the NRC found several weaknesses in the Aulerich et al. (1979) study, including insufficient details about experimental conditions and observations and inadequate pathology examinations. The original laboratory data were no longer available for clarifying those issues. The subcommittee recognizes these weaknesses, but it acknowledges that the purpose of the Aulerich et al. (1979) study was "to determine the toxicity and tissue residue accumulation of DIMP in wildlife" found at DIMP-contaminated sites—mallard ducks, bobwhite quail, and mink. The study was not designed to determine dose-response effects in mink.

There is uncertainty about whether the increased mortality observed in female mink was DIMP related. EPA cited a report that natural mortality for first-year mink in commercial fur ranches can be as much as 6% annually (Kennedy 1952). However, the subcommittee does not recommend using that report to make a judgment about background mortality rates in mink. It would be preferable to use historical control mortality rates at the testing facility for comparisons, particularly the rates from the year of the study. For example, Aulerich et al. (1979) also reported results from a study on dicyclopentadiene that was done concurrently with the DIMP study, using mink from the same lot. Four of 24 (17%) mink died in the control group of that study.

Another factor that was unclear in the DIMP study was the exact date of each death. This is important because most of the deaths occurred between January 17 and June 30. That period includes the mating, gestation, whelping, and lactation periods, which are stressful periods for female mink. Mink are excitable animals and additional stress is known to adversely affect their health. For example, lactating female mink are reported to suffer from nursing disease, a condition character

ized by anorexia, lethargy, dehydration, ataxia, a reluctance to move, and from several biochemical changes (Schneider and Hunter 1993). The disease is thought to result from the physiologic stress of milk production and nursing of offspring. Because the specific dates of the deaths in the DIMP study were not recorded, it is impossible to ascertain whether nursing stress could have been a factor in the deaths. However, the results of the two-generation reproductive study in mink (Bucci et al. 1997) (discussed below) suggest that the deaths were not related to DIMP.

TWO-GENERATION REPRODUCTIVE TOXICITY STUDY

A two-generation reproductive toxicity study by Bucci et al. (1997) was conducted as a follow-up to the Aulerich et al. (1979) study. Although that study has been described as a "replication" of the Aulerich study, it was clearly not intended to be so. The purpose of the Bucci et al. (1997) study was to conduct a more comprehensive evaluation of the effects of DIMP in mink over two generations, with an emphasis on effects in females. The study differs from that by Aulerich et al. (1979) in many ways: Bucci's group used a different strain of mink, different housing conditions, higher doses of DIMP, longer exposure duration (13 months for F_1 [first-filial] generation), modern testing procedures, and more toxicity end points. Table 1 compares the key data from the two studies.

In the Bucci et al. (1997) study, the parental generation (F_0) consisted of three groups of 35 female and 7 male Ranch Wild mink fed DIMP in their diet at concentrations of 150, 450, and 2500 ppm. Two replicate control groups were fed untreated feed. A second control group was used because of reported and anecdotal descriptions of reproductive problems with ranch-bred mink, including spontaneous abortions, kit mortality, and sudden death of dams. The animals were housed in a laboratory, and the lighting was adjusted weekly to approximate the outdoor sunrise and sunset times. The parental animals were treated for approximately 1 month before mating. Harem mating was used to produce F_1 offspring, which were divided into groups of 35 females and 13 males, weaned on the corresponding diets of their parents. The F_1 mink were mated similarly to produce the second generation (F_2).

TABLE 1 Comparison of Key Data on Female Mink in Reproductive Studies

Study Design and End Point	Aulerich et al. (1979)	Bucci et al. (1997)	
Test animal	Dark variety mink	Ranch Wild mink (*Mustela vison*)	
Generation (age at beginning of treatment)	F_0 (3 months)	F_0 generation (9 months)	F_1 generation (in utero)
Daily dose, mg/kg-day	0, 11, 37, 95	0, 0, 26, 85, 461	0, 0, 20, 57, 329
Treatment duration	12 months	4 months	13 months
Mortality	0/0, 2/23, 3/24, 5/24	0/35, 1/35, 2/35, 2/35, 1/35	1/35, 1/35, 2/35, 1/35, 3/35
Blood changes	None	High-dose group: • Decreased red-blood-cell count • Increased eticulocytes, mean cell volume, Heinz body count	High-dose group: • Increased Heinz body count
Clinical chemistry findings	Not done	High-dose group: • Decreased cholinesterase in plasma, whole blood, red blood cells	High-dose group: • Decreased cholinesterase in plasma, whole blood, red blood cells
Reproductive effects	None	None	None

Necropsy and microscopic examination findings	None	<u>High-dose group</u>: • Increase in spleen weight, average absolute weight of the spleen, ratio to body weight and brain weight[a] • Increase in hematopoietic cell proliferation in the spleen	<u>High-dose group</u>: • Increase in mean ovarian follicle count [b]

[a] These findings were anticipated because they correlate with the shortened life span of Heinz-body-containing red blood cells.

[b] Only the high-dose and control animals were examined. It is unclear how to interpret this finding because the ovaries were histologically normal and breeding was unaffected.

Chemical characterization of DIMP indicated that it was at least 97% pure, and target concentrations and stability in the feed were within acceptable limits. Based on feed consumption and body weight, the daily mean consumption of DIMP by the F_0 generation was 26, 85, and 461 mg/kg-day for females and 15, 47, and 285 mg/kg-day for males; in the F_1 generation, DIMP consumption was 20, 57, and 329 mg/kg-day for females and 16, 45, and 262 mg/kg-day for males.

The subcommittee found several weaknesses in the number of animals used and the study's dosing regimen for the F_0 animals. Specifically, the number of male animals used in the F_0 generation (seven) was too low for an adequate statistical evaluation of male reproductive toxicity end points. In addition, premating exposure typically covers the period of spermatogenesis in male animals. In F_0 mink, premating exposure was about 30 days long; spermatogenesis takes about 65 days in mink. The treatment duration for the F_1 generation (13 months) was more than adequate to evaluate any potential reproductive issues.

Toxicity was assessed using standard parameters of periodic body weight, food consumption, and clinical examination. Clinical chemistry and hematologic analyses also were performed on the parents and kits. Indices of puberty (vaginal opening and prepuce separation) were not conducted on the kits to minimize handling, as the dams were reported to be excitable. In both generations, all parental animals and two kits per sex per litter underwent gross necropsy, and blood samples were taken from 8 to 13 kits per litter for clinical chemistry and hematology evaluations. Semen analyses were performed on F_1 males in the high-dose group (20 males are indicated, but the protocol required only 13 to be selected) and in 10 control males. Blood and brain ChE activity was measured in parental females, but ChE activity was measured only in blood for parental males and kits.

In the F_0 generation, mortality was observed in 2 of 35 (6%), 2 of 35 (6%), and 1 of 35 (3%) females in the low-, mid-, and high-dose groups, respectively, and only one female in the two control groups (0% and 3%) died. The females were pregnant at the time of death, except for one mid- and one high-dose mink that died before mating. Most of the animals that died exhibited a syndrome of lack of appetite, lethargy, weight loss, fatty liver, atrophied thymus, kidney degeneration, and, occasionally, gastrointestinal bleeding. The syndrome appeared to be stress-related, with the dams unable to meet the high metabolic demands of milk production in the late lactational period or un

able to meet the physiologic challenges of pregnancy. Bucci et al. (1997) stated that the small number of animals affected across the treatment groups could be considered an indication that effects were unrelated to DIMP treatment.

In the F_1 parental animals, mortality occurred in 2 of 35 (6%), 1 of 35 (3%), and 3 of 35 (9%) females in the low-, mid-, and high-dose groups, respectively, and one female mink died in each of the two control groups (3% in each). One high-dose mink was pregnant at the time of death, one low-dose mink died of a seizure disorder at the age of 3 months, and the remainder died before mating. Six of the deaths, including the two in the control groups, were attributed to stress associated with anesthesia used during blood collection. Recovery from sedation in these animals took up to 4 hours, compared with 15-20 minutes in the F_0 mink. Bucci et al. noted that the prolonged sedation was likely due to the larger dose of anesthesia given to the F_1 animals, which were heavier than the animals in the F_0 generation (anesthetic dose was based on body weight). One parental male died during the study, an F_1 mink from the mid-dose group, whose death was also attributed to the anesthesia treatment.

There appears to be a discrepancy in the number of deaths reported for the litters. The report states that three F_1 kits (from different litters) and three F_2 kits (from one control litter) died, whereas the report appendix on pup viability reported many more deaths. For example, for the F_1 pups, 18 of 208 died in the first control group, 33 of 196 died in the second control group, 10 of 124 died in the low-dose group, 20 of 212 died in the mid-dose group, and 32 of 185 died in the high-dose group. For the F_2 pups, 27 of 161 died in the first control group, 22 of 106 died in the second control group, 38 of 109 died in the low-dose group, 33 of 111 died in the mid-dose group, and 13 of 131 died in the high-dose group. In any case, the subcommittee found no evidence to conclude that the deaths were related to DIMP. It is unclear what caused the deaths.

The subcommittee notes that several types of medical interventions were performed on the mink, including subcutaneous injections of lactated Ringer's solution to prevent dehydration in wasting animals, addition of palatable foods to the mink ration to encourage feeding, the occasional use of antibiotics, and treatment with phosphoric acid for “wet belly” (urinary incontinence and skin inflammation). Such treatments are used on commercial ranch farms and were probably justified to en

sure that enough animals survived to produce two generations, but the subcommittee concludes that the interventions confound the reported mortality rates and make it difficult to compare rates with other studies in which such interventions were not used.

Another shortcoming of the study was that data from pregnant and nonpregnant females were combined, which makes it difficult to determine whether observed effects might have been due to DIMP, pregnancy, or a combination of them. For example, no differentiation was made between uterine weight for pregnant and nonpregnant females, even though pregnancy, whelping, and lactation cause significant physiologic changes.

The F_0 generation produced significantly smaller numbers of litters in the low-dose group (19 litters compared with 32 in the control group). In the absence of a dose-related trend, it is unlikely that this was due to DIMP. Furthermore, one of the F_1 control groups had a similarly small number of litters (19 litters). In general, the number of litters obtained in the DIMP-treated $_1$ group was lower than in the F_0 generation, but this was not considered important because one F_1 control group also produced a similarly low number of litters. All other litter parameters in the DIMP groups were similar to controls.

Hematologic and clinical chemistry changes were observed in the F_0 and F_1 generations. In the F_0 generation, decreases in red-blood-cell count and increases in reticulocyte numbers, mean cell volume, and Heinz body counts (protein aggregates in oxidatively stressed red blood cells) were observed in the females in the high-dose group. Heinz body increases also were observed in the F_1 females, although the effect was less marked. Bucci et al. (1997) concluded that DIMP likely induced oxidative damage to hemoglobin (production of Heinz bodies) and affected red blood cells were removed from circulation prematurely, which stimulated the bone marrow and spleen to increase red-blood-cell production. The subcommittee agrees that this is a reasonable conclusion. Such changes were not observed in animals from the lower-dose groups, and F_0 males did not exhibit any biologically significant hematologic changes. In the F_1 males, there was a small elevation in Heinz bodies at the highest dose. Only minor changes were observed in the F_1 and F_2 male kits at the highest dose.

In the F_0 females, plasma ChE activity was decreased in all dose groups, but only in the high-dose group was the decrease greater than 20%. In F_1 females, plasma ChE activity was reduced at only the high

dose (30%). Red-blood-cell ChE was reduced to a small degree at the mid and high dose (7%-8%) in the F_0 females and only at one time period in the high-dose F_1 females (4%). There was no change in brain ChE activity. In males, the responses also were small, with less than 20% ChE inhibition noted only in the high-dose group. Similarly, in the kits, ChE changes were small and confined to the highest-dose group.

At necropsy and histologic examination of the F_0 generation, spleen weight was found to be increased, spleen weight ratios to body weight and to brain weight were also increased, and there was evidence of an increase in hematopoietic cell proliferation. These findings were attributed to the shortened life span of Heinz-body-containing red blood cells, because the spleen removes damaged red blood cells from circulation and is involved in the production of replacement red blood cells. No similar changes were found in the F_1 generation, but an increase in mean ovarian follicle count was reported. The significance of this finding is unclear because only the control and high-dose groups were examined, the ovaries were histologically normal, and breeding was unaffected.

A decrease in uterine weight in female F_0 mink was noted in the low-and high-dose groups and prostatic and testicular weights were increased in the low-dose F_1 males. Because no significant microscopic changes were found in those organs during pathologic examination, the subcommittee did not consider the changes in organ weights to be adverse.

The subcommittee believes that the most biologically meaningful effects of DIMP were related to hematologic changes (Heinz body formation and reduced red-blood-cell count) and effects on ChE. These effects are considered biomarkers of exposure and not adverse effects. However, they are indicators for potential toxic effects. Thus, the subcommittee believes that the overall no-observed-effect level (NOEL) for the study was 450 ppm (approximately 57 mg/kg-day) for female mink, and the NOEL for reproductive toxicity was 2500 ppm (approximately 329 mg/kg-day) on the basis of F_1 females.

SUBCHRONIC TOXICITY STUDY

Bucci et al. (1994) conducted a 90-day feeding study of DIMP in mink, in which groups of 10 male and 10 female 12-month-old dark

brown Ranch Wild mink were fed DIMP at concentrations of 0, 50, 450, 2700, 5400, and 8000 ppm in the diet. The daily consumption of DIMP was 0, 8, 73, 400, 827, and 1136 mg/kg-day, respectively. Two additional groups were used as pair-fed controls for the two highest-dose groups. Clinical observations were performed twice a day, and body weight and food intake were recorded weekly. Blood samples for cell counts and chemical profiles (including plasma and red-blood-cell ChE activity) were obtained at weeks 0, 3, 7, and 13. Necropsy and microscopic examinations were performed on all animals.

The randomization of experimental animals, animal husbandry, and ad libitum feeding appear to have been acceptable. The subcommittee notes that the laboratory conditions do not represent what Ranch Wild mink would experience in commercial captivity or in nature. However, the controlled conditions under which they were held do not appear to have adversely affected the outcome or ability to interpret the study. DIMP fed to the experimental groups was provided in a well-described and analyzed commercial diet.

No deaths or clinical morbidity were observed during the study. The subcommittee assumed that no animal received veterinary medical treatment or therapy, as was done during the two-generation study (Bucci et al. 1997).

Animals fed the two highest doses had significantly lower body weight than did untreated control animals. When compared with pair-fed controls, there was no significant difference in body weight. However, significant weight loss in male and female mink fed DIMP at 1136 mg/kg-day could be explained only partially by the use of pair-fed control animals. There is a strong indication that decreased food consumption, related to the palatability of the feed containing that dose of DIMP, was the cause of weight loss in the group. However, the tendency for the pair-fed control animals to compensate for restricted feeding when later provided ad libitum feeding weakened the argument that decreased feed consumption rather than DIMP toxicity was the only reason for the weight loss.

DIMP at doses of 827 mg/kg-day and greater was associated with a significant decrease in hematocrit and hemoglobin and a significant increase in the number of reticulocytes. The authors suggest that the reticulocytosis was due to oxidative injury to red blood cells induced by the ingestion of high doses of DIMP. Splenic hematopoiesis was seen at necropsy in animals fed the highest dose of DIMP. These altered values

were reversible with nearly complete return to baseline after a 4-week recovery period.

DIMP caused a greater than 20% inhibition of plasma ChE at doses of 400 mg/kg-day and greater but did not cause significant inhibition of red-blood-cell ChE. ChE concentrations returned to normal within the first week of recovery.

On the basis of the significant reductions in plasma ChE, the subcommittee believes that 73 mg/kg-day and 400 mg/kg-day are the NOEL and LOEL (lowest-observed-effect level), respectively, for this study.

COMPARATIVE METABOLISM STUDY

Weiss et al. (1994) conducted a comparative pharmacokinetic study of DIMP in mink and rats to determine whether there are marked differences between the two species. The subcommittee found the study to be inadequate for making quantitative assessments about the metabolism of DIMP in mink compared to rats; however, some qualitative information is provided. Below, the subcommittee evaluates the study and discusses some noteworthy points about the metabolism of DIMP.

DIMP was obtained with a carbon-14 (^{14}C) label in the methyl carbon at a purity of 97%. Two groups of eight adult Ranch Wild mink of each sex were administered DIMP by gavage at doses of 27 or 170 mg/kg. A third group of mink was treated with the lower dose intravenously. In the test with rats, groups of eight Sprague-Dawley rats of each sex were administered DIMP by gavage at doses of 66 or 660 mg/kg, and another group of rats was treated intravenously with DIMP at the lower dose. The high doses in the oral tests were the LD_{10}s (lethal doses to 10% of test animals) for the respective species. Radioactivity measurements were taken for whole blood, urine, and feces.

The mink and rats received 40 millicuries (mCi)- ^{14}C-DIMP/kg orally and 20 mCi- ^{14}C-DIMP/kg intravenously. The subcommittee noted that those levels of radioactivity are high for a typical disposition study. The radioactive DIMP was diluted with unlabeled DIMP to achieve the desired doses, which means that the specific activity of DIMP differs among the dose group. This is important to the interpretation of the report data, which present counts per minute in blood or excreta.

DIMP is hydrolyzed to isopropyl methylphosphonate (IMPA) and isopropanol in equal amounts (Figure 1). Because the methyl carbon

atom was labeled with ^{14}C for the disposition and kinetic studies, only IMPA carries the label after hydrolysis. IMPA and DIMP were both measured by reverse phase-high performance liquid chromatography and identified by gas chromatography-mass spectrometry and nuclear magnetic resonance.

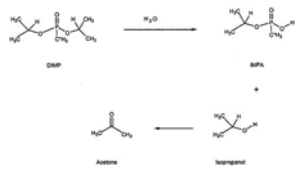

FIGURE 1 Metabolic pathway for hydrolysis of DIMP to IMPA and isopropanol.

The subcommittee found it difficult to assess the concurrent process of distribution, metabolism, and excretion because the data were presented using different time scales. Three types of data for each species were presented graphically. First, radioactivity counts in blood were plotted for up to 24 hours after dosing for mink and rats. Second, cumulative radioactivity in excreted urine for both species and both sexes was presented for up to 140 hours. Third, the time courses for DIMP and IMPA in plasma for mink and rats, respectively, were presented for up to only 8 hours. In the figures, only point estimates (presumably the mean values for the test groups) are shown. No standard errors or standard deviations were provided. Because of the scatter in data points in the mink tests, reporting standard errors or standard deviations would have been informative.

Observations in Mink

The data on total radioactivity in whole blood of mink indicates that clearance of DIMP at the high dose in males is considerably less efficient than is clearance at the lower dose (Weiss et al. 1994). Most of the dose (more than 70% for all treatment groups) was eliminated in urine in the first 5 days. Urinary elimination increased rapidly and reached a plateau in the first day for the oral doses.

Of the radioactivity measured in urine from the high-dose mink, 98-100% was IMPA, with a minor contribution of DIMP. At the lower doses, all urine radioactivity was associated with IMPA. The apparent half-life of DIMP in blood was 12-14 minutes, although it was not clear how this value was calculated. It might have been estimated from the total radioactivity curve, or plasma elimination of DIMP after intravenous injection could have been used for the calculation. The data used to create the intravenous elimination curves are sparse and inadequate to make a reliable half-life estimate.

The study reports that the plasma profiles and area under the curve (AUC) estimates indicate nonlinear conversion of DIMP to IMPA with saturation kinetics, but it does not specify the basis for the AUC calculations. The curves for IMPA production are unusual: At the high dose, there are continuous concentrations of IMPA over 400 minutes, whereas at the low dose, there is a higher relative production of IMPA and then an appreciable loss over the study time. Comparison of those two data sets indicates some degree of nonlinearity. In the absence of a more quantitative analysis, it is difficult to gauge the effect that this nonlinear behavior would have in cases of more prolonged exposure.

Observations in Rats

The elimination of ^{14}C-DIMP from rat blood was slower than in the mink, with appreciable radioactivity still exhibited in plasma at 24 hours. Male rats appeared to have more rapid clearance of radioactive constituents from blood than did females. Again the absence of standard error data makes comparisons difficult. In the urinary elimination study, male rats excreted a larger percentage of the dose in the urine than did females. The time required to excrete radioactive constituents

in rats was longer than in mink. The excretion curves have a positive slope in the rat throughout most of the observation period and appear to be biphasic, especially in females. The mink curves were flat after 48 hours.

The reported ^{14}C DIMP half-life in rats was longer than in mink: 45 minutes versus 12-14 minutes. Analysis of data from oral and intravenous dose routes indicate a first-pass effect for DIMP in the rat. Again, the basis of these calculations was not provided in the paper. The urine from rats contained three metabolites, IMPA and two unidentified compounds. Based on other results measuring exhaled carbon dioxide, it appears unlikely that there is much metabolism of the methyl group. Hart (1976, as cited in Weiss et al. 1994) reported that only 0.05% of a dose of ^{14}C-DIMP was excreted in expired air. In general, the intravenous data in rats seem more consistent than similar intravenous data from the mink.

Weiss et al. (1994) concluded that there are similarities in absorption, metabolism, and excretion between mink, rats, and other species. Although this general conclusion is qualitatively supported by the data, some sex-related and species-related differences deserve comment. Female mink eliminate DIMP slightly slower than do males, as noted with the blood time course of total radioactivity and with the urinary elimination profiles. In addition, both sexes of mink clear DIMP and IMPA from their bodies faster than do male or female rats.

As noted above, a deficiency of this study was its failure to explain how several pharmacokinetic parameters, especially those used to draw conclusions about nonlinear metabolism, half-life, and first-pass metabolism, were calculated. Despite the absence of a more quantitative pharmacokinetic analysis, the general qualitative conclusions drawn by the authors are supported by the time-course curves. Nevertheless, the significance of differences in AUCs or half-life between sexes of from one species to another cannot be evaluated adequately without better information about the statistical treatment and variability in the data points.

Other Noteworthy Points

In mink, DIMP is rapidly converted to IMPA, which is then rapidly excreted in urine. Females clear DIMP from plasma more slowly than do males, and IMPA excretion in females takes slightly longer than in

males. Thus, the urinary excretion time courses are consistent with distribution of DIMP according to a one-compartment model with elimination by a saturable metabolic pathway. The urinary time course does not indicate multiple kinetic processes in the conversion of DIMP to IMPA in male mink. The data from female mink show some evidence of a slower elimination phase for IMPA in urine, which is consistent with a two-compartment distribution in female mink.

The urinary-elimination curves in the rat are quite different, showing biphasic elimination of IMPA. The most likely explanation is that the distribution of DIMP in rats is more consistent with a two-compartment model, and that there is significant storage of DIMP in a deep compartment. If this is the case, the two phases in urinary excretion represent initial metabolic clearance of DIMP from a central compartment by metabolism and then redistribution of DIMP to the central compartment from deep tissue stores, which in this case would most likely be fat. Comparison of the four groups indicates that the deep storage sites are largest in female rats, followed in order by male rats, female mink, and male mink. The kinetics of urinary elimination strongly suggest to the subcommittee that mink have considerably less body fat than do rats and that females of each species at their respective ages have more body fat than do males. These conclusions could be verified by application of a more quantitative pharmacokinetic model to assess the kinetic behaviors in these various groups.

Because of the biphasic behavior in rats, there should also be species differences in production and circulating concentrations of metabolites. The rat should have a more prolonged time course for metabolism of DIMP to IMPA and isopropanol. In mink, the production of metabolites from the single central compartment occurs more quickly. This difference could have some toxicologic importance if isopropanol and acetone have any contributing role in observed responses (see discussion below). In mink, larger concentrations of metabolites would be expected because little of the DIMP would be removed to storage compartments.

TOXIC POTENTIAL DIMP METABOLITES

Metabolism of DIMP to IMPA also creates equimolar amounts of isopropanol. The molecular weights of DIMP and isopropanol are 181 and 60 daltons, respectively. The highest dose of DIMP in the sub

chronic mink study (Bucci et al. 1994) was 8000 ppm in feed, which was equivalent to 1136 mg/kg-day. This represents a daily dose of isopropanol of 376 mg/kg-day (1136 × 60/181). Nearly all isopropanol produced will be converted to acetone. Because of this, alcohol and ketone metabolites should be considered within the evaluation of the toxic responses of high daily doses of DIMP. In rats, isopropanol at a dose of 1000 mg/kg-day caused early postnatal death of F_1 offspring in a reproductive toxicity study (Bevan et al. 1995). In a developmental toxicity study in rabbits (Tyl et al. 1994), isopropanol at 480 mg/kg-day led to toxicity (mortality, peripheral vasodilation of the ears, cyanosis) and reduced food intake in the pregnant females. The potential effects of isopropanol and acetone on pregnant and lactating mink are not known.

Mink are known to suffer from nursing disease. In this syndrome, dams unable to meet the heavy metabolic requirement of milk production late in lactation die from the negative nutritional balance (Schneider and Hunter 1993). In the two-generation mink study, Bucci et al. (1997) note a similar problem in high-producing dairy cattle, in diabetic humans with insulin-glucose imbalance, and in starvation from many causes. The syndrome has been better characterized for cattle and humans than for other species: In the absence of sufficient dietary glucose, the body metabolizes its fat and muscle protein. Metabolic byproducts of this abnormal state, including ketones, can further suppress appetite and exacerbate the condition (Bruss et al. 1989). Interactions between acetone accumulating from administration of DIMP (ketosis) and the stresses of lactation in the mink could combine to lead to adverse responses.

COMPARISON OF MINK AND OTHER TEST ANIMALS

Most major toxicologic investigations of DIMP used either mink or rats; one used dogs (Hart 1980). Those investigations yielded different results, raising the question of whether one species is more appropriate to assess the toxicity of DIMP in humans. The subcommittee reviewed the literature on mink to assess the differences between mink and other laboratory animal species. The subcommittee was particularly interested in whether basic mink biology or physiology provided any reason to exclude mink results from consideration in assessing data for a human drinking-water guideline.

The biology of mink has been studied as a consequence of the fur industry and commercial use of the pelts. Mink, *Mustela vison,* belong to the family Mustelidae, as do martens, wolverines, skunks, and otters; ferrets and weasels are in the same genus.These carnivorous animals generally live near water and feed on vertebrates (e.g., frogs, snakes, birds, small mammals) and invertebrates (e.g., crayfish, mussels). Several important literature reviews cover most aspects of the biology of mink, such as husbandry, basic biology, reproduction, and nutrition (Aulerich et al. 1999; Eagle and Whitman 1987; Sundqvist et al. 1989; Tomson 1987). Table 2 compares several biological parameters of mink, rats, and dogs.

One major, and not insignificant, difference between mink and either rats or dogs is the extent to which populations or strains have been bred and maintained in captivity. Dogs and rats have been bred in captive conditions for many generations—over hundreds of years, in the case of dogs. The breeding is a form of artificial (genetic) selection in which some traits are selected for and maintained in a population; other traits are bred out. Breeding programs invariably and intention ally affect a range of biologic characteristics, many manifested in narrowing the phenotypic range displayed in a population. Although mink have been bred in captivity, the extent of artificial selection seems to be substantially less than in dogs or rats, based on the number of years or generations over which the species have been bred in captivity. In addition, mink farms have introduced some wild animals into the stocks over the course of their breeding programs (W. Wustenberg, Alternet Medical, personal communication, Nov. 1999). Mink also are held under conditions more similar to the natural environment than are dogs or rats, both of which are routinely held under controlled conditions in a laboratory.

TABLE 2 Several Biologic Parameters of Mink, Rats, and Dogs

	Mink	Rat	Dog
Body weight, kilograms	1-1.5	0.5	10
Age at maturity, months	8-12	3	6+
Life span, years	6-11	3	12-18
First litter, months	8-12	2	6-12

Another difference between mink and rodents or dogs is the mink's

semi-aquatic mode of life. As semi-aquatic animals, mink display some adaptations to the water, but generally are not as efficient in water as are their fully aquatic counterparts (Williams 1998). Mink are comparatively good swimmers and exhibit morphologic and physiologic adaptations for tolerating low temperatures. The insulating fur that makes mink commercially valuable is replaced by internal fat layers in fully aquatic mammals. The most significant adaptation is the greater metabolic response to swimming and living in water than observed in aquatic mammals. According to Williams (1998), the greater basal metabolism in water and while swimming indicate a higher metabolic maintenance cost in mink than in fully terrestrial animals.

Husbandry conditions in the DIMP studies in mink did not involve either swimming or aquatic exposure, so the specific responses of mink to semi-aquatic conditions are not invoked in interpreting results of the studies. However, the greater metabolic lability of this species is clearly consistent with the overall physiologic responses that comprise the "wasting" observed in farm-held animals.

The reproductive biology of mink has been the subject of investigation for husbandry applications as well as for studies in comparative biology. Sundqvist et al. (1989) conducted an extensive literature review and concluded that mink do not differ greatly from other mammals in their reproductive biology. Unlike rats and dogs, however, mink are strictly seasonal breeders, and they exhibit delayed implantation. These characteristics make husbandry and breeding more challenging, but they are not reasons to exclude reproductive experimental results obtained on mink.

Several reviews of mink biology have addressed the question of whether the mink is applicable and appropriate as an experimental animal in toxicology. A review by Calabrese et al. (1992) specifically considered the applicability of mink data for making predictions for protecting human health. Based on an extensive review of the literature that addressed metabolism and biochemistry, body morphometrics, physiologic functions and rates, and allometric relationships, those researchers concluded that the mink was typical of other mammals and that toxicologic results can be extrapolated to other animals and humans. They also noted that the practical reasons mink are not used in toxicology include long life span, seasonal reproductive cycle, difficult handling, and musk odor.

The subcommittee found nothing about the basic biology or physiol

ogy of mink to preclude it from being used as a predictive model of toxicity. Thus, the subcommittee concludes that data from mink studies can be used for quantitative human health risk assessments.

DRINKING-WATER GUIDELINES FOR DIMP

Table 3 outlines how the drinking-water guidelines for DIMP were determined by EPA and by the State of Colorado. EPA has established a drinking-water guideline (lifetime health advisory) for DIMP of 600 μg/L and Colorado promulgated a guideline of 8 μ g/L. The reason for the difference is that different studies were used to calculate the guidelines. Colorado used a one-generation reproductive toxicity study in mink (Aulerich et al. 1979), whereas EPA used a 90-day toxicity study in dogs (Hart et al. 1980).

Three studies in mink were conducted after EPA and Colorado made their determinations to assess whether the increase in female mink mortality in the Aulerich et al. (1979) study was DIMP-related—a two-generation reproductive toxicity study (Bucci et al. 1997), a subchronic toxicity study (Bucci et al. 1994), and a comparative metabolism study (Weiss et al. 1994). On the basis of the weight of evidence from those studies and other background information, the subcommittee did not consider the deaths in the Aulerich study to be DIMP-related. It appears likely that the deaths were due to the stresses of pregnancy, as evidenced by the stress-related deaths of pregnant mink in the two-generation reproductive study (Bucci et al. 1997) and the absence of morbidity or mortality in nonpregnant females in the subchronic toxicity study (Bucci et al. 1994).

The subcommittee concludes that neither EPA's nor Colorado's drinking-water guideline for DIMP are based on the best currently available data. The weight of evidence indicates that two new studies in mink—a two-generation reproductive toxicity study (Bucci et al. 1997) and a subchronic toxicity study (Bucci et al. 1994)—provide the most appropriate database for deriving a drinking-water guideline for DIMP. Those studies were conducted using modern testing methods and examined more end points than the Aulerich et al. (1979) study. The subcommittee considers the two-generation reproductive study to be the best available study for deriving a drinking-water guideline, because it involved the most relevant exposure duration (13 months for the F_1

generation) and because the most biologically meaningful findings of the study, Heinz body formation and ChE inhibition,[1] are supported by the results of the subchronic toxicity study in mink. The NOELs in the two studies were similar—56 mg/kg-day in the two-generation study (Bucci et al. 1997) and 73 mg/kg-day in the subchronic study (Bucci et al. 1994). Furthermore, studies with other species have reported similar or greater NOELs (75 mg/kg-day in a dog study, 135 mg/kg-day in a three-generation rat study, and 150 mg/kg-day in rat teratogenicity study [Hart et al. 1980]),[2] which gives the subcommittee greater confidence in using the two-generation study. Although the subcommittee finds that study to be more appropriate than either of the studies used by EPA or C11:37 AM 5/2/01olorado for deriving a drinking-water guideline, it does note the need for careful consideration of the uncertainties in the study due to the use of medical interventions and combining data from pregnant and nonpregnant animals. In addition, further consideration should be given to the metabolism of DIMP and to the effects of its alcohol and ketone metabolites.

[1]ChE inhibition is considered a biomarker of exposure to an anticholinesterase agent and not an adverse effect. However, it has been used as a "toxicity" end point in risk assessments for neurotoxicants, such as organophosphate pesticides (EPA 1997) and chemical-warfare agents (NRC 1999). Although brain and RBC ChE are typically considered better indicators than plasma ChE for potential effects on the nervous system, consistent effects in the Bucci et al. (1997) study were observed only in plasma.

[2]The exposure duration in these studies were 90-days in the dog study, 30 weeks in the three-generation rat study, and 10 days in the rat teratogenicity study.

TABLE 3 Drinking-Water Criteria for DIMP

	EPA: 600 μg/L	Colorado: 8 μg/L
Study	Hart et al. 1980	Aulerich et al, 1979
Test animal	Dog	Mink
Exposure	90 days	12 months
Toxicity end point	No adverse effects observed	Mortality
NOAEL or LOAEL	75 mg/kg-day (NOAEL)	11 mg/kg-day (LOAEL)
Uncertainty factors	10 - Intraspecies variability 10 - Interspecies extrapolation 10 - Less than lifetime duration 1000	10 - Intraspecies variability 10 - Interspecies extrapolation 10 - Less than lifetime duration 10 - LOAEL to NOAEL 10,000
Assumptions	Adult body weight, 70 kg Daily water consumption, 2 L Relative source contribution, 20%	Adult body weight, 70 kg Daily water consumption, 2 L Relative source contribution, 20%

Calculations	$RfD = \frac{75\ mg/kg\text{-}day}{1000} = 0.08\ mg/kg\text{-}day$ $DWG = \frac{(0.08\ mg/kg\text{-}day)\ (70\ kg)}{2\ L/d} = 2.8\ mg/L$	$RfD = \frac{11\ mg/kg\text{-}day}{10,000} = 0.0011\ mg/kg\text{-}day$ $DWG = \frac{(0.0011\ mg/kg\text{-}day)\ (70\ kg)}{2\ L/d} = 0.0385\ mg/L$
	(2.8 mg/L) (0.20) = 0.56 mg/L (rounded to 600 μg/L)	(0.0385 mg/L) (0.20) = 0.0077 mg/L (rounded to 8 μg/L)

DWG, drinking-water guideline; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; RfD, reference dose.

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Appendix

BIOGRAPHICAL INFORMATION ON THE SUBCOMMITTEE ON THE TOXICITY OF DIISOPROPYL METHYLPHOSPHONATE

JOHN A. MOORE *(Chair)* is president and chief executive officer of the Institute for Evaluating Health Risks, a nonprofit institution that serves government, industry, and the public on issues that address the health risks of chemicals. He received his D.V.M. from Michigan State University. Dr. Moore has held a number of distinguished positions in the government, including director of toxicology research and testing at the National Institute for Environmental Health Sciences, deputy director of the National Toxicology Program, and assistant administrator of the Office of Pesticides and Toxic Substances at the U.S. Environmental Protection Agency (EPA). He also served as acting deputy administrator of EPA for one year. Dr. Moore has held a number of elected positions in the Society of Toxicology, including president of the Risk Assessment Specialty Section. Among his many honors and achievements, Dr. Moore has received the highest federal award of Distinguished Executive.

MELVIN E. ANDERSEN is a professor in the Department of Environmental Health at Colorado State University. He received his Ph.D. in

biochemistry and molecular biology from Cornell University. He is widely known for his contributions in developing biologically realistic models of the uptake, distribution, metabolism, and biological effects of drugs and toxic chemicals and applying these models in safety assessments and quantitative health risk assessments.

PETER L. DeFUR is affiliate associate professor in the Center for Environmental Studies at Virginia Commonwealth University. Before his appointment at the university, he was a senior scientist at the Environmental Defense Fund. He received his Ph.D. in biology from the University of Calgary. His research interests include hormone disrupting chemicals in the environment and health and environmental risks from dioxin and related compounds.

PAUL M.D. FOSTER is a program director for endocrine, reproductive, and developmental toxicology at the Chemical Industry Institute of Toxicology. He received his Ph.D. in biochemistry and toxicology from Brunel University, Uxbridge, Middlesex, United Kingdom. His research interests include mechanisms of toxicity, target organ toxicity, and male reproductive physiology and its application to the study of toxic effects. Dr. Foster has served on a number of national and international committees reviewing reproductive toxicology, including committees of the U.S. National Toxicology Program, EPA, European Center for Ecotoxicology of Chemicals, and World Health Organization.

SIDNEY GREEN is associate professor of pharmacology at Howard University. He received his Ph.D. in pharmacology from Howard University. His research interests include genetic and systemic toxicology. Dr. Green was formerly director of toxicology at Covance Laboratories, Inc. He was also director of the Division of Toxicological Research at the U.S. Food and Drug Administration in the Center for Food Safety and Applied Nutrition and director of the Toxic Effects Branch in EPA's Office of Toxic Substances.

DAVID H. MOORE is director of Medical Toxicology Programs at Battelle Memorial Institute, Battelle Edgewood Operations. Dr. Moore received his D.V.M. from the University of Georgia College of Veterinary Medicine, and his Ph.D. in physiology from Emory University School of Medicine. His research interests are in the clinical effects of chemical warfare agents.