EVALUATION OF METHYL ISOTHIOCYANATE AS A TOXIC AIR CONTAMINANT

$$H_3C-N=C=S$$

Part C—Human Health Assessment

California Department of
Pesticide Regulation

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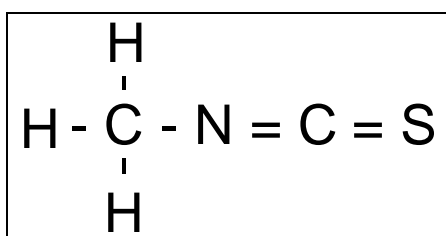# Department of Pesticide Regulation

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Part C
Health Assessment

Medical Toxicology Branch
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RISK ASSESSMENT AND RISK MANAGEMENT: DEFINITIONS

The following definitions of risk assessment and risk management are quoted directly from <u>Casarett and Doull's Toxicology</u>, 5th edition, Chapter 4, page 75 (Faustman and Omenn, 1996). The authors cite as background a National Research Council publication entitled, <u>Risk Assessment in the Federal Government: Managing the Process</u> (Washington, DC; National Academy Press, 1983).

> *Risk assessment is the systematic scientific characterization of potential adverse health effects resulting from human exposures to hazardous agents or situations. This type of assessment includes qualitative information on the strength of the evidence and the nature of the outcomes, quantitative assessment of the exposure and the potential magnitude of the risks, and a description of the uncertainties in the conclusions and estimates.*
>
> *Risk management refers to the process by which policy actions to deal with the hazards identified in the risk assessment process are chosen. Risk managers consider scientific evidence and risk estimates, along with statutory, engineering, economic, social, and political factors, in evaluating alternative regulatory options and choosing among those options.*

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I.   SUMMARY

There are currently only 2 registered products, both wood preservatives, that list methyl isothiocyanate (MITC) as an active ingredient. In 1999, reported use of MITC totaled 620 pounds in California, representing a tiny fraction of the MITC released into the atmosphere. Agricultural use rates in California of metam sodium, the major parent compound of MITC, rose steadily from 5.9 million pounds in 1990 to 15.3 million pounds in 1995 to 17.3 million pounds in 1999. Metam sodium is converted on a mole-to-mole basis to MITC, which, based on the relative molecular weights of the 2 compounds, results in a conversion rate of approximately 60% by weight. It is thus estimated that an average of 9.2 million pounds per year of MITC were released into the air from agricultural applications of metam sodium in the 1995-1999 period. Another compound, dazomet, a thiadiazine used as an anti-microbial and soil sterilant, also produces MITC upon degradation. However, dazomet use rates under agricultural settings are minor compared to those of metam sodium, never rising above 23,000 pounds for any year between 1990 and 1999. This assessment addresses the potential health effects from exposure of the general public to MITC in ambient and application site air from the agricultural use of metam sodium. A parallel document will address, in addition to those populations and scenarios just indicated, the risks to workers of MITC exposure (DPR, 2002c). Chronic exposure risks will also be dealt with in that document.

MITC entered the public consciousness following the accidental spill of 19,500 gallons of 32.7% metam sodium from a railroad tank car into the Sacramento River on July 14, 1991. This occurred at the Cantara Loop, 6 miles north of the town of Dunsmuir and 45 miles north of Shasta Lake. Exposure of the Dunsmuir area population to airborne MITC evolving from the river resulted in numerous visits to local healthcare facilities, largely due to complaints of eye and respiratory irritation, nausea, headache, dizziness, vomiting and shortness of breath. More persistent symptoms, including a chemically-induced asthma known as reactive airways dysfunction syndrome (RADS), were also documented. Reliable measurements of MITC air concentrations in the affected localities were not available during the first three days after the accident. Once they did become available, the highest of these measurements was 125 ppb, registered at Dunsmuir on Day 4 post spill. Attempts to estimate peak MITC air concentrations during the first 3 days were based on Gaussian plume modeling. There was substantial uncertainty associated with the parameters required to generate the estimates using the Gaussian approach, leading to substantial uncertainty in the estimates themselves. The parameters included the metam sodium-to-MITC conversion rate, the flux of MITC from the water surface, and the meteorologic conditions. The Office of Environmental Health Hazard Assessment estimated the peak 1-hour time weighted average concentration to be 1300 ppb for a distance of up to 100 meters from the river, dipping to 340 ppb at 500 meters. DPR's maximal 1-hour time weighted average estimates were 4500 ppb at 100 meters and 1240 ppb at 500 meters downwind from the river. The Metam Sodium Task Force conducted a screening level sensitivity analysis, estimating maximum 1-hour time weighted average air concentrations of MITC at the river's edge to be between 3 ppm and 650 ppm.

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Some residents of the town of Earlimart, California were exposed to airborne MITC following an illegal sprinkler application of metam sodium to an adjacent field on November 13, 1999. Symptoms included eye, respiratory tract, and skin irritation, as well as possible exacerbations of asthmatic conditions. The presence or absence of persistent symptoms (*eg.*, RADS) is currently unknown. One hour average air concentration estimates based on Gaussian plume modeling fell between 0.5 and 1 ppm for the neighborhood closest to the field. These were likely to be lower bound estimates.

Pharmacokinetics. Because no inhalation pharmacokinetics studies have been published in the open literature or submitted to the State of California, the metabolic fate of MITC was assessed mainly by an oral exposure study. Eighty eight to 96% of the orally administered MITC was absorbed within 24 hours. In that time period, 80-82% was excreted in the urine, <1-2% in the feces and 6-15% in the expired air (as CO_2). The rest was excreted in the expired air as unmetabolized MITC (<1-2%) or carbonyl sulfide + carbon disulfide (<1%), or remained bound to tissues (1-3% after 168 hours). Thyroid, liver, kidneys, whole blood and adrenals were sites of tissue distribution. The major metabolites in the urine were N-acetyl-cysteine and cysteine conjugates. No unmetabolized MITC was detected in the urine.

Acute toxicity. The most relevant acute toxicity endpoint identified was eye irritation, established in an experimental study conducted with human volunteers. The critical air concentration NOEL was 220 ppb for exposure to the isolated eye region using special goggles. It was based on the observation of eye irritation at 800 ppb. A NOEL for an absorbed dose was not calculated for the study because MITC was not inhaled by the subjects. The irritating effect of MITC on ocular and respiratory tissues was also evident in animal studies, and in humans exposed to MITC either after the 1991 Sacramento River spill or after agricultural use. The air concentration NOEL of 220 ppb was the critical NOEL used for evaluation of potential short-term risk to residents and bystanders (adults and children) from exposure to airborne MITC.

The acute toxicity of MITC has also been demonstrated in animal studies. Acute oral exposure of rats between 25 and 300 mg/kg in one study led to sedation, dyspnea, altered body positions, ruffled fur, crying, spasms and exophthalmos. The LD_{50} in that study was 55 mg/kg in females and 82 mg/kg in males. Similar clinical signs were noted upon dermal exposure of rats at a dose range of 60-600 mg/kg (LD_{50} = 181 [F] and 225 [M] mg/kg) and rabbits at a dose range of 50-300 mg/kg (LD_{50} = 202 [F] and 145 [M] mg/kg). Other studies have shown MITC to be a powerful irritant to both skin and eyes. Acute inhalation studies in animals have yielded conflicting results, but do identify a potentially very damaging route of exposure. The most reliable studies in Sprague-Dawley rats show a 1-hour LC_{50} of 633 ppm and a 4-hour LC_{50} of 180 ppm. Clinical signs in the former study included hyperactivity followed by hypoactivity, eye irritation, dyspnea and convulsions.

Subchronic toxicity. Subchronic toxicity was evident in the critical 4-week Wistar rat inhalation study, which was conducted according to a 6-hours/day, 5-days/week exposure regimen. This study

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established a LOEL at the low dose of 1.7 ppm based on evidence of nasal epithelial atrophy at that dose. Signs and symptoms at the intermediate dose of 6.8 ppm included nasal epithelial atrophy, a rise in polymorphonuclear granulocytes (considered evidence for sub-histopathologic lung damage), and clinical signs. The latter, which were of unclear toxicologic significance, included somnolence, eye closure, and ruffled fur. Signs of severe respiratory tree irritation and, possibly, damage, were observed at the high dose of 34 ppm. These included bronchopneumonia, emphysema, bronchial and tracheal epithelial proliferation, rhinitis, and focal metaplasia, in addition to increases in lung weight and decreases in body weight. Haber's Law, which assumes that the longer the exposure time, the less MITC is required to elicit a particular response, was invoked to convert the 6-hours/day, 5-days/week exposure regimen to 24-hours/day, 7-days/week. This resulted in an estimated LOEL of 300 ppb. A default uncertainty factor of 3 was then used to calculate an estimated critical subchronic NOEL of 100 ppb. A LOEL of 2 mg/kg/day was reported in a 3-month oral gavage study in rats based on stomach lesions, liver inflammation, spermatogenic disturbance, and alteration in adrenal and ovary weights. These effects were considered slight at that dose, but increased in severity at 10 and 40 mg/kg/day. A NOEL of 0.7 mg/kg/day was established in a 3-month mouse oral gavage study based on reduced body weight gain and increased liver weight at 1 mg/kg/day. At higher doses, toxic effects included thickening of the forestomach lining, inflammation of the liver, testicular / spermatogenic disturbances and decreased ovary weights.

Chronic toxicity and oncogenicity. No chronic inhalation toxicity studies of MITC were identified in the published literature or in documents submitted to DPR. Chronic exposure of rats and mice through the drinking water resulted in decrements in body weight gain. These were likely secondary to decreased water consumption, which was, in turn, due to unpalatability. The chronic NOELs in rats and mice, 0.46 and 2.74 mg/kg/day, were based on signs largely, if not completely, dependent on the decreased water consumption.

In dogs exposed to MITC by gavage, an apparent NOEL for MITC of 0.4 mg/kg/day was based on a plethora of signs both irritative and systemic in nature. Severe pathology noted at the mid dose was most likely due to overexposure to the corn oil vehicle. Unfortunately, a precise evaluation of this study has not been possible because it is not currently available to DPR.

The 2-year rat drinking water study provided weak evidence that MITC may have induced mammary fibroadenomas and carcinomas in females. A small increase in subcutaneous fibromas was also noted at the high dose, though it was unclear if MITC was responsible for the rise. The 2-year mouse drinking water study provided evidence that MITC may have induced cutaneous fibrosarcomas in both sexes. However, neither long-term drinking water study provided data that were sufficiently strong with regard to oncogenesis to trigger a quantitative oncogenic risk evaluation. In mice, various serum chemical, hematologic and histologic alterations were also noted at the top 2 doses, with amyloid degeneration in the kidney possibly increased at all doses in males and ovarian cysts in high dose females. However, the significance of these histologic observations was obscured by the lack of a historical control data base.

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Genotoxicity. The results from gene mutation assays using two different microorganisms (*Salmonella typhimurium*, *Escherichia coli*) were negative for MITC mutagenicity. However, none of the studies was acceptable to DPR due to various deviations from TSCA guidelines. In one acceptable mammalian cell assay, Chinese hamster V79 cells exposed to a range of MITC concentrations, ±S9 activating microsomes, showed no increase in mutation frequency in two trials. Cytogenetic investigations of possible chromosome effects provided no evidence for induction chromosomal aberrations in human lymphocytes. A weakly positive aberration response was registered in Chinese hamster V79 cells. Tests for DNA damage, and sister chromatid exchange were negative.

Reproductive toxicity. A 2-generation drinking-water study and a 3-generation oral gavage study were conducted to determine if MITC had effects on reproductive function in rats. While a decrease in pre-weaning viability was noted in the F_1 pups at all doses in the 2-generation study, lack of dose-responsiveness and statistical significance made it unlikely that MITC exposure was responsible. MITC was therefore not considered to be a reproductive toxicant.

Teratogenicity. Both maternal and fetal toxicity were evident in the teratogenicity studies. Maternal toxicity was expressed as decrements in body weight and food consumption at as low as 5 mg/kg in both species. Thickened maternal stomach lining was evident at 25 mg/kg in rats. Decreases in fetal size and weight were noted in rats at 25 mg/kg and in rabbits at 5 mg/kg, with a separate study showing embryo toxicity and reduction in 24-hour survival at 10 mg/kg. However, MITC was not a considered a developmental toxicant because doses which affected fetal parameters were associated with maternal toxicity.

Immunotoxicity. Immune toxicity was noted in one 5-day mouse gavage study, though the MITC doses examined were generally higher than those determining the critical subchronic NOELs. Significant changes in thymus weight (~50% of control) and thymus cell subpopulations were reported in mice given 45 mg/kg/day. Total WBC numbers were increased (~175% of control) at 30 mg/kg/day. The percentage of blood neutrophils was increased (~200% of control), and the percentage of blood lymphocytes was decreased (~80-90% of control) at 15 and 45 mg/kg/day. Flow cytometric analysis of thymus cells at 45 mg/kg/day indicated a decrease (~90% of control) in $CD4^+CD8^+$ thymocytes and an increase (~140% of control) in the percentage (but not absolute number) of $CD4^-CD8^-$ thymocytes. These results raised the possibility that immunotoxicity could result from longer-term exposures to lower doses of MITC. Unfortunately, no studies were available to assess the extent of immunotoxicity when exposure occurs by inhalation.

Risk characterization. Risk characterization for non-oncogenic endpoints requires knowledge of the toxicity endpoints and the expected exposures. Monitoring of MITC levels in towns near regions of heavy agricultural metam sodium use ("ambient" levels) and near fields that have been recently treated ("application site" levels) demonstrated the potential for exposure of the general public. (Not all of the applications were performed under conditions which would be considered compliant with more recent

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Technical Information Bulletins (TIBs) and product labels for metam sodium. Several applications would be currently illegal because they did not use a post-application water seal; they were included in this document for comparative purposes. An additional study was conducted under conditions that were possibly consistent with the presence of an inversion layer. The results of this study should be interpreted with caution.) The risk of incurring adverse effects from these exposures was expressed as the margin of exposure (MOE), defined as the ratio of the NOEL value established in animal or human studies over the human exposure dose. Generally, a MOE of >100, which assumes not only that there is a 10-fold variation in susceptibility within the human population, but also that humans are 10 times more sensitive than the most sensitive animal, is considered adequate to protect humans from the effects in question. A MOE of >10 is sufficient if human experimental data provide the critical NOEL value.

Because the critical acute NOEL value for MITC is eye irritation (NOEL = 220 ppb), it was necessary only to know the estimated short-term ambient and application site air concentrations to calculate the acute MOEs (had the critical NOEL been based on a systemic endpoint, absorbed dose calculations would have been necessary for the exposure term). Results of short-term ambient air monitoring for MITC from 3 studies (2 from Kern County utilizing several receptor sites, one from Lompoc) were used in the estimation of 1-hour, 8-hour and 24-hour ambient residential / bystander exposures. These concentrations ranged from 0.1 to 14.6 ppb, yielding MOEs between 15 and 2200. Results of application site air monitoring from 4 soil injection studies and 3 fixed-set sprinkler studies were used in the estimation of 1-hour, 8-hour and 24-hour application site residential / bystander exposures. These concentrations ranged between 41 and 2853 ppb for 1-hour exposures, between 32 and 2348 ppb for 8-hour exposures, and between 12.7 and 1102 ppb for 24-hour exposures. No difference in the range of MITC air concentrations would be registered if only those exposure studies that were in compliance with the current TIBs for metam sodium were used. MOE ranges for 1-, 8-, and 24-hour application site exposures were <1 to 5, <1 to 7, and <1 to 17, respectively. (Removal from the analysis of those application site studies in which a water seal was not applied did not change the range of air concentration or MOE values. However, meteorologic conditions in one study, which registered the highest acute air concentrations and lowest MOEs, may have been consistent with the presence of an inversion layer, though this was not confirmed.)

MOEs for seasonal residential / bystander exposures were calculated using the 4-week rat estimated subchronic inhalation NOEL of 100 ppb. Results of ambient air monitoring from the same 3 studies as were used in the acute risk analysis study generated seasonal MITC concentration estimates of 0.0006-3.54 ppb. The corresponding ambient MOE range was 28-166,667. Because an MOE of 100 is generally considered to be protective of human health for adverse effects observed in animal studies, some potential for seasonal ambient health effects was indicated. Application site monitoring showed seasonal air levels to range between 2 and 80 ppb depending on site and distance from the application. These values resulted in MOEs between 1 and 50, signifying a seasonal human health risk under

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application site scenarios. (However, the caveats mentioned above for short-term exposure also apply for subchronic exposure.)

Reference Exposure Levels (RELs). The REL is defined by the Office of Environmental Health Hazard Assessment as "the concentration level at or below which no adverse health effects are anticipated for a specified exposure duration." RELs are based on the best available medical and toxicological studies and "are designed to protect the most sensitive individuals in the population by the inclusion of margins of safety." The REL for acute effects of MITC was calculated by dividing the critical NOEL, 220 ppb, by 10 to account for intrahuman variability when the NOEL was determined in a human study. Because the eye irritation NOEL was stable at 1, 4 and 8 hours in the critical laboratory study, the REL was relevant for potential exposure times of up to 8 hours. The resultant value, 22 ppb, was well below many of the anticipated acute exposure levels established in the application site air monitoring studies (see above), indicating a potential human health hazard. Estimated ambient exposure estimates for 1 and 8 hours did not exceed 14.6 ppb. A human health concern was therefore not indicated under those conditions.

The subchronic REL of 1 ppb was generated by dividing the 24-hour critical subchronic rat inhalation NOEL of 100 ppb by an uncertainty factor of 100 (10-fold to account for intrahuman variability and 10-fold for the assumption that humans are more sensitive than animals). Seasonal ambient time-weighted average MITC concentration determinations indicated some cause for human health concern. A health concern clearly existed for application site scenarios where the REL values were almost always exceeded.

A chronic REL value was estimated in the eventuality that use patterns or air monitoring at some future time would indicate a potential for chronic exposure. In the absence of a chronic inhalation toxicity study, the 24-hour chronic REL was estimated by dividing the subchronic REL by a default uncertainty factor of 10, yielding a value of 0.1 ppb.

Toxicity of metam sodium. Exposure to metam sodium, the parent compound of MITC, is projected to occur only under occupational scenarios and only by the dermal route. Toxicity studies show both local irritative and systemic effects. Chronic exposure of rodents through the drinking water was associated with an increased incidence of angiosarcoma (a type of malignant vascular tumor). Metam sodium was also clastogenic and embryotoxic. Occupational MOEs fell between 1724 and 3333 for acute systemic exposure, between 679 and 1333 for acute local dermal (irritation) exposure, and between 909 and 2500 for seasonal systemic exposure. The risk to workers for development of angiosarcoma fell between 1.71×10^{-6} and 5.14×10^{-6} when expressed as the maximum likelihood estimate and between 3.70×10^{-6} and 1.11×10^{-5} when expressed as the 95% upper bound estimate. These estimates were based on the assumption that metam sodium acted through a linearized, multistage, non-threshold-dependent mechanism. However, experimental support for this assumption was considered to be incomplete.

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    <u>Toxicity of breakdown products of metam sodium and MITC.</u> Breakdown of metam sodium and MITC in the environment results in measurable air levels of at least 2 gases of great potential toxicologic significance: methyl isocyanate (MIC) and hydrogen sulfide (H_2S). The contribution of these compounds to the toxicity profile of humans exposed to MITC in the field is not adequately understood. Methyl isocyanate, the chemical responsible for the deaths of up to 5000 people in the aftermath of the Bhopal disaster in 1984, is a severe pulmonary irritant with LC_{50} levels in animals of 6-12 ppm. A host of toxicologic effects on other tissues and organ systems were evident both in studies on Bhopal victims and on laboratory animals. Calculations carried out in this document based on the potential for human eye irritation resulted in a conditional acute 1-hour REL value of 0.98 ppb. In the only study in which MIC was measured after a metam sodium application, levels as high as 2.5 ppb were measured (this study would not have satisfied current TIBs and product labels, however). Correcting for recovery and application rate, the theoretical level under the conditions of that study was 5.4 ppb, 5-fold greater than the acute REL.

    H_2S is a metabolic poison as well as a mucus membrane and respiratory irritant. Symptoms commonly reported after accidental human exposures include dyspnea, sore throat, coughing, chest pain and signs of pulmonary obstruction. Little is known of the H_2S levels following metam applications. In one study, uncorrected measurements of H_2S after application of metam sodium showed levels reaching 76 ppb at 1-4 hours post application. The ACGIH TLV and the Cal OSHA PEL for H_2S are set at 10,000 ppb. The short term exposure limit (STEL) is 15,000 ppb. The ATSDR acute and intermediate minimum risk levels (MRLs) of 70 and 30 ppb, respectively, are derived from observations of respiratory effects in humans and mice. They incorporate uncertainty factors based on possible variations in human sensitivity, LOAEL-to-NOAEL extrapolation, and species-to-species extrapolation. The California Ambient Air Quality standard is 30 ppb for a 1-hour average.

    Co-exposure to any combination of metam sodium, MITC, MIC, and H_2S (or other metam sodium breakdown products) could elicit additive or synergistic effects. These might be expected particularly in respiratory and ocular tissues, which are known to be sensitive to the irritative effects of these compounds in isolation. Unfortunately, as no clear experimental or epidemiologic data are available to suggest the presence of, or potential for, additive / synergistic interactions, it can only be said that such effects are plausible. Furthermore, meaningful estimation of the potential for toxicologic effects arising from simultaneous exposure, even in a single tissue, is contingent on careful measurements of the air levels of each chemical under both application site and ambient scenarios. As noted, only preliminary measurements are available to this date.

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II.   **BACKGROUND**

        A.   **Regulatory History**

Methyl isothiocyanate (MITC) is a general biocide used to control weeds, nematodes, and soil and wood fungi. As a soil fumigant it may be mixed with 1,3-dichloropropene. As a wood preservative, MITC is poured into small holes bored into utility poles and similar structures. MITC is also the active principle of the soil fumigant, metam sodium, evolving as a gaseous by-product after metam sodium application.

After March 1, 1996 the US EPA decreed that only certified pesticide applicators may purchase metam sodium sewer products used to control tree roots in sewer lines (US EPA, 1996). All metam sodium applications must be made by, or under the supervision of, a certified applicator.

DPR placed the agricultural use of metam sodium and MITC on the "restricted-use" pesticide list in 1994 following interim evaluation of the potential human risk from agricultural use (DPR, 1994). Pesticides on the list may be possessed and used only by persons who have obtained a use permit from the county agricultural commissioner. The permitting process allows commissioners to review the proposed site of application and, where necessary, require specific use practices to protect nearby sensitive areas, such as schools. Restrictions may include control measures such as buffer zones around application sites, reduced application rates, limitations on acreage treated, and laying down of a post-application water seal. In addition, applications during meteorological inversions are prohibited. If necessary the commissioner may deny a permit and prohibit use in the most **"**sensitive" neighborhoods.

        B.   **Mechanism of Action**

It is generally agreed that most of the pesticidal activity associated with metam sodium is due to MITC, its primary degradation product. Little is known of the mechanism of action of MITC. One possibility is that cellular toxicity is due to cyanide, with the implication that metabolic poisoning is involved. It has also been postulated that pesticidal action is dependent on metabolism "to the isothiocyanate radical (-N=C-S), which inactivates the -SH groups in amino acids contained within the individual pathogen cells" (Ware, 1994). Widespread inhibition of enzyme activities would be one consequence of the metabolic reduction of disulfide bonds. However, as is clear from many of the studies reviewed for this assessment, irritation of epithelial membranes is a prime component of the MITC toxicity profile. It is not clear if metabolic inhibition would account for such irritative reactions. Finally, whether or not cytochrome p450-dependent biotransformation plays a role in MITC toxicity is not currently known.

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III.   PHARMACOKINETICS

A.   Introduction

No FIFRA (Federal Insecticide, Fungicide and Rodenticide Act) guideline standard metabolism studies were submitted by the registrants. However, an oral-dose metabolism study in rats (Hawkins *et al*, 1987; reviewed in sections B-E below) and an intraperitoneal-dose metabolism study in rats and mice (Lam *et al*, 1993; reviewed in section G below) were available. In addition, a single-dose dermal absorption study in rats using metam was also examined (Stewart, 1992; reviewed in section F below). In the environment, and after oral administration to animals, the major metam sodium degradate is methyl isothiocyanate (MITC). For this reason, the pharmacokinetic fate of MITC and metam sodium were examined together in the Hawkins and Lam studies. The fate of orally administered dazomet was also examined in the Hawkins study (see section F below).

B.   Oral route

1.   Absorption

The oral-exposure metabolism study compared the absorption, tissue distribution, and excretion of metam sodium and MITC (Hawkins, *et al*, 1987). Rats were given radiolabeled metam sodium at 10 or 100 mg/kg, or MITC at 4.4 or 33 mg/kg by gastric gavage. Feces were collected at 24-hour intervals for up to 7 days. Expired air was collected at 24-hour intervals for up to 3 days, passing through a series of 3 traps capable of selectively capturing MITC, CO_2 and carbonyl sulfide + CS_2, respectively. Tissue levels were also determined at the end of the study. Percent recoveries of excreted metam and its metabolites are summarized in Table 1.

Urinary and expired air levels were used to estimate absorption after oral exposure. The data indicate that over 80% of the administered doses of metam was absorbed within 24 hours and about 90% was absorbed within 168 hours (*note*: these are minimal estimates, *i.e.*, estimates that don't consider contributions from tissue-bound or absorbed fecal fractions). MITC appeared to be even more readily taken up; by 24 hours 88-96% of the dose was absorbed, increasing to 94-100% by 168 hours.

2.   Distribution

For metam sodium, tissue content, expressed on a Fg/g basis, was highest in the thyroid at 168 hours after oral exposure. Kidneys and liver were among the sites with the highest retention of radioactivity and, along with the thyroid, were thought to be the tissues responsible for metabolism and excretion. Lung (particularly in females), adrenals and ovaries were also sites of relatively high accumulation. Whole blood accumulated a relatively high proportion of label at the high dose. The investigators concluded that the absorption was similar at both doses, but exhibited a somewhat different pattern of metabolism and excretion.

Tissue content following MITC administration was highest in the thyroid at 168 hours, with liver,

kidneys, whole blood and adrenals comprising relatively high secondary sites of accumulation. Female lungs at the high dose were also important sites.

Total tissue levels at 168 hours did not exceed 2.3% of the administered dose of metam sodium or MITC at either the low or high doses.

3. Biotransformation

The same urinary metabolites were identified for both compounds, although there were some differences in the relative proportions. Neither parent compound was present in the urine. A single major metabolite, M5, represented 16-25% of the dose for metam sodium and 56-66% of the dose for MITC. There was only one other metabolite, M4, that was formed in appreciable amounts from both compounds, representing 5-10% of the dose. There was no evidence for the presence of glucuronides or sulfate conjugates. M5 was identified as N-acetyl-S-(N-methylthiocarbamoyl)-L-cysteine. M4 was shown to chromatographically match to the corresponding cysteine conjugate. It was suggested that the metam sodium underwent acid hydrolysis in the stomach to form MITC and CS_2, but that a portion of the metam sodium may have been absorbed intact. That would explain the slower excretion compared with MITC (Wagner, 1989).

No toxicity data were available for the cysteine conjugates.

4. Excretion

Following doses of MITC the radioactivity was principally eliminated in the urine (see below) and in the expired air (as CO_2). With metam sodium, 52-58% of the low dose and 37-42% of the high dose was recovered in the urine. Expired air accounted for 33-38% of the low dose and 47-53% of the high dose. Much of the increase at the high dose was accounted for by a marked increase in the excretion of MITC. Other expired metabolites included CO_2, carbonyl sulfide and carbon disulfide (see Figure 1) (Jowa, 1992).

Urinary elimination occurred mainly during the first 8 hours following MITC administration and over the first 24 hours following metam administration. The difference in excretion rate was mirrored by a slower initial rate of elimination of radioactivity from the plasma of metam sodium-dosed animals.

The Hawkins study did not completely satisfy FIFRA guidelines because no multiple dosing regime was conducted. However, the study did provide useful information on the pharmacokinetics of metam / MITC in rats.

C. Dermal Route

The extent of absorption of metam sodium after dermal exposure was examined in the rat in a separate study (Stewart, 1992). A detailed analysis of this study is provided in the Exposure Assessment section, Part B, of the current document (DPR, 2002b). Briefly, rats were subjected to dermal

applications of ^{14}C-metam sodium and sacrificed times up to 72 hours post dose. Absorption, indicated by the radioactivity in urine, feces, blood, carcass and skin, was essentially complete by 1 hour. The corrected dermal absorption value of 2.5% for the lowest dose (8.6 Ƒg/cm) was considered appropriate for use in the worker exposure estimates for metam sodium because this dose should be representative of exposure experienced by agricultural workers.

D.   Intraperitoneal Route

A study from the open literature (Lam *et al*, 1993) determined the fate of radiolabeled metam and MITC following intraperitoneal injection of mice (Swiss-Webster, male) and rats (Sprague-Dawley, male). A mean value of 58% of the metam and 80% of the MITC was excreted in mouse urine by 48 hours. Feces, expired CO_2 and carcass accounted for 6, 5 and 7.5% of the total metam dose, respectively, and 5, 4 and 6% of the total MITC dose in mice. Radioactive label was widely distributed among tissues. Liver, kidney and, interestingly, hair, accounted for the largest proportions of the tissue fraction for both metam and MITC. Metabolite studies identified the conversion to the GSH conjugate as common to both compounds, resulting in mercapturates in the urine. Methylamine and other unidentified metabolites were also detected in urine. Quantitative differences in the relative proportions of mercapturate, methylamine, unidentified polar metabolites and other unidentified metabolites were observed between mice and rats.

E.   Dazomet (Oral Route)

The kinetics of dazomet metabolism mirrored those of metam sodium with one exception: metam appeared to produce MITC in the expired air at greater efficiency than dazomet at the high dose of 100 mg/kg (Hawkins, *et al*, 1987). Thus while 23-25% of the metam dose was detected as MITC in the expired air both at 24 and 72 hr, only 1-3% of the dazomet dose appeared as MITC at those times. Much of this difference was made up in the more efficient excretion of dazomet in the urine.

The formation of metabolites was similar for dazomet, metam sodium and MITC, though there was evidence for one dazomet-specific metabolite, referred to only as M9.

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Table 1. Absorption, excretion and retention of radioactivity (% of dose)
following gavage exposure to metam sodium or MITC

	Metam sodium				MITC			
	10 mg/kg		100 mg/kg		4.4 mg/kg		33 mg/kg	
	M	F	M	F	M	F	M	F
Urine								
0 - 8 hr	24.19	26.04	17.83	19.17	71.43	73.65	58.76	54.62
0 - 24 hr	46.49	53.34	33.83	38.34	80.68	82.45	81.69	80.13
0 - 168 hr	52.02	58.09	37.34	42.42	84.43	86.36	87.09	85.57
Expired air (Trap 1: MITC)								
0 - 24 hr	0.37	1.12	23.91	23.39	0.69	1.24	0.49	1.20
0 - 168 hr	0.45	1.26	24.53	24.04	0.95	1.51	0.72	1.67
Expired air (Trap 2: CO_2)								
0 - 24 hr	18.44	17.03	6.68	5.00	15.24	14.09	6.78	6.53
0 - 168 hr	19.56	18.13	7.20	5.53	16.08	14.88	7.32	7.23
Expired air (Trap 3: $COS + CS_2$)								
0 - 24 hr	17.99	13.55	20.41	17.00	0.04	0.04	0.29	0.33
0 - 168 hr	18.35	13.80	21.34	17.63	0.05	0.04	0.43	0.48
Amount absorbed (minimal estimate*)								
0 - 24 hr	83.29	85.04	84.83	83.73	96.65	97.82	89.25	88.19
0 - 168 hr	90.38	91.28	90.41	89.62	101.5	102.8	95.56	94.95
Feces								
0 - 24 hr	2.98	0.83	0.96	0.66	1.99	0.66	1.13	0.93
0 - 168 hr	4.48	2.88	1.87	1.57	2.74	1.45	1.93	1.83
Cage washings (total)	0.10	0.05	0.06	0.04	0.15	0.07	0.18	0.15
Tissues (168 hr)	2.01	1.75	1.17	1.32	2.20	1.86	1.71	2.29
Total recovery	96.96	95.95	93.50	92.55	106.6	106.2	99.37	99.22

*These values are considered minimal estimates of absorption because they take into account only urinary and expired air. Tissue levels are not included because no 24-hr data were available. Fecal levels are not included because there was no attempt to discern which fraction of the fecal radioactivity was excreted into the gut from circulating (*i.e.*, absorbed) pools.

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Figure 1. Proposed degradation / metabolic pathways for metam sodium and MITC (redrawn from Jowa, 1992).

$CH_3NHC \overset{S}{\underset{SNa}{}}$ (Stomach)

Metam sodium

(H^+)

(H^+)

CH_3NCS (Stomach)
MITC

(H^+)

$CH_3NH_2 + CS_2$

$COS + NH_2CH_3 + CO_2$

CH_3NCS (Blood)

COS

Glutathione transferase

Exhaled products

CO_2

$CH_3NHC\text{-}SCH_2CHCO_2H$
$\overset{\|}{S}$ $\underset{NHCOCH_3}{|}$

Tissues
thyroid
liver
kidney
lung

Primary metabolite M5
(urine)

LEGEND

Significant pathway

Less significant pathway

14
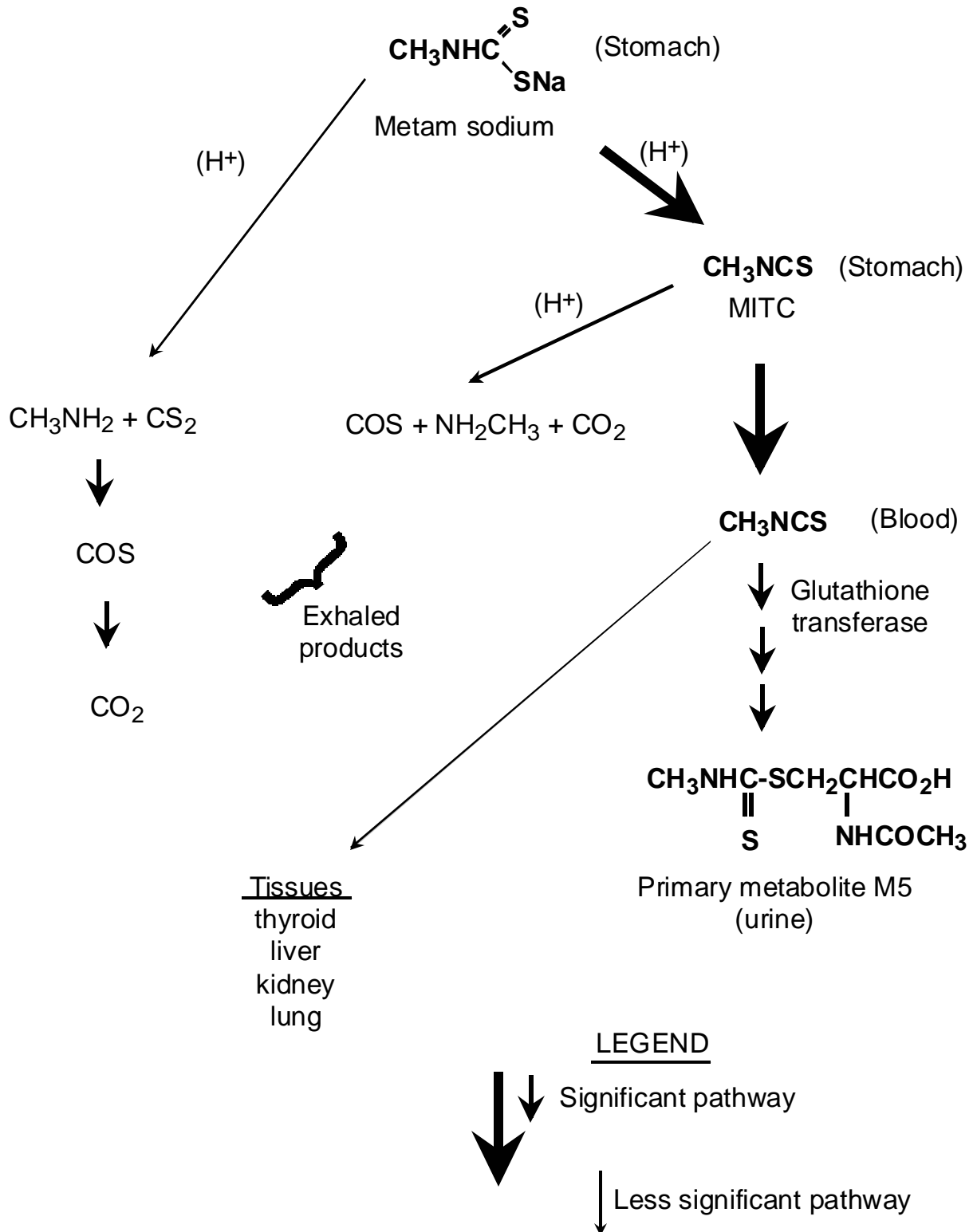
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IV.   ACUTE TOXICITY

A.   Human Health Effects

1.   California illness surveillance program (*The following section is taken largely from Part B [Exposure Assessment] of this document (DPR, 2002b.*)

In California, there was no separate classification of illnesses/injuries resulting from exposure to MITC. There were 390 illnesses/injuries attributed to exposure to metam sodium/MITC alone and 2 further illnesses / injuries attributed to exposure to metam sodium / MITC in combination with other pesticides (DPR, 2000). According to the data shown in Table 2a, annual illness/injury cases classified as definitely, probably or possibly related to metam sodium/MITC exposure totaled 6.9, 25.2, and 6.9, respectively, for each category. The majority of illness/injury cases from 1990 to 1999 occurred to residents / bystanders, and were classified as non-occupational exposure (Table 2b).The large number of illness / injury reports in 1999 may be due to the Earlimart incident, discussed in section IV.A.1. Workers exposed during loading, applying, and field fumigation operations were also subject to illnesses and injury. A pronounced increase in illness / injury reports from non-occupational sources occurred in 1995 and 1996 compared to 1990 - 1994. This effect may be attributable to increased metam use rates over those years, though it is noted that non-occupational incidence rates declined to negligible levels in 1997 and 1998 when metam sodium use remained high (DPR, 2002b).

Illness/injury cases were also grouped according to symptoms experienced by affected persons (Table 3). These cases excluded illnesses / injuries as a result of the 1991 Sacramento River spill. It was assumed that the majority of illnesses / injuries were caused by exposure to MITC because it is the major degradation product of metam sodium after application to soil. The extent of the contribution of other degradation products such as H2S and MIC to this illness picture is currently unknown. As noted in previous drafts of the Exposure Assessment covering years up to 1996, a majority of cases referred to as "systemic" include not only systemic symptoms, but also irritative symptoms to the respiratory tract, eyes, or skin (these "systemic plus" reports were discontinued at that time). This is very likely also to be the case after 1996. For example, in 1999, when most of the reports stemmed from the Earlimart incident, irritative symptoms were a very prominent component of the total reports (see section IV.A.1.).

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Table 2a. Case reports received by the CA Pesticide Illness Surveillance Program (PISP) in which health effects were attributed to exposure to metam sodium/MITC (1990-1999)

Year	Illness / injury attributable to metam / MITC			
	Definite	Probable	Possible	Total
1990	6	6	8	**20**
1991	2	2	9	**13**
1992	1	9	8	**18**
1993	14	4	0	**18**
1994	4	5	1	**10**
1995	27	20	1	**48**
1996	9	43	4	**56**
1997	5	12	3	**20**
1998	0	2	2	**4**
1999	1	149	33	**183**
Average	**6.9**	**25.2**	**6.9**	**Avg.: 39.0** **Total: 390**

Table 2b. Case reports received by the CA PISP in which health effects were attributed to exposure to metam sodium/MITC (1990-1999): Classified according to activities

Activity	Number of case reports										
	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	Tot.
Loader	0	1	1	3	3	3	3	5	0	0	19
Applicator	1	0	0	2	0	1	3	2	2	0	11
Fumigation, field	14	7	1	1	3	0	0	5	1	2	34
Drift, Occupational	2	0	0	0	0	0	2	0	0	8	12
Non-occupational	0	0	11	11	0	40	48	0	1	167	278
All others	3	5	5	1	4	4	0	8	0	6	36
Total	**20**	**13**	**18**	**18**	**10**	**48**	**56**	**20**	**40**	**183**	**390**

Notes: In 1993, there were two illnesses/injuries attributed to exposure to metam sodium/MITC in combination with other pesticides. Thus, there were a total of 392 illness/injury cases from 1990 to 1999. Case reports resulting from the 1991 Sacramento River were not included in this tally.

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Table 3. Case reports received by the CA PISP in which health effects were attribute to exposure to metam sodium/MITC (1990-1999): Classified according to symptoms[a]

Year	Systemic	Skin	Eye & eye/skin	Respiratory & resp./eye	Total
1990	8	11	1	0	20
1991	4	6	1	2	13
1992	8	4	5	1	18
1993	10	6	0	2	18
1994	3	6	1	0	10
1995	40	2	6	0	48
1996	22	6	28	0	56
1997	10	9	0	1	20
1998	2	1	1	0	4
1999	162	18	4	0	184
Average	26.9	6.9	5.0	3.0	39.1

[a]Examples of reported symptoms were: eye - watery, burning, itchy, blurred vision; skin - rash, burns, redness, swelling; systemic - nausea, chest pain, scratchy throat, diarrhea, weakness, dizziness, headache, malaise, salivation, vomiting; respiratory - cough, shortness of breath.

2. Human subject study

To date only one study on human responses to MITC exposure has been conducted in a laboratory setting. This study, an assessment of human eye irritation and odor thresholds, is reviewed here.

In order to determine the NOEL for human eye irritation produced by MITC vapors, as well as its odor threshold, human volunteers were exposed to air concentrations of MITC in a laboratory setting (Russell and Rush, 1996). The study specifically focused on assessing these parameters at different times of exposure. An olfactometer was used which permitted the operator to dispense the test material through a manifold system. The test material could thus be diluted over a 100-fold concentration range. The material was dispensed by diffusion from a glass vessel which could be maintained at any temperature ±0.1EC over a range of 30EC to 70EC. As stated in the study, "The concentrations of irritants in the [eye] masks were calculated from the rate of evaporation in the source chamber and the dilution factors inferred from the flow rates of the gas streams in the olfactometer. These values were checked at the beginning and end of the active study by carbon-tube sampling and gas chromatographic

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analysis (CTGC)". A Total Hydrocarbon Analyzer (THA) was used to monitor the flow of test material during the exposure period. Every effort was undertaken to minimize the reaction of the test material with the tubing and other equipment used in the delivery system.

In the olfactory threshold study, 33 individuals (16 males, 17 females) with a mean age of 25 years (range, 18 to 34 years) were tested. They were exposed to three positive control odorants, pyridine, acetic acid, and n-butyl alcohol, as well as to MITC. The technician chose the odorant and concentration level. The odorant was dispensed in double blind fashion from one of three presentation ports. The subject was responsible for identifying from which of the presentation ports the odorant was dispersed. A 30-second rest period between exposures was permitted in order to allow the subject to recover prior to the next exposure. The operator tested each subject over the range of concentrations for each odorant until he was assured that the threshold had been adequately ascertained. A standard procedure was employed in order to make this determination. The observed odor threshold for MITC ranged from 0.2 to 8 ppm with a geometric mean of 1.7 ppm.

In the NOEL determination for eye irritation, the olfactometer was modified by attaching goggles to the presentation line. This permitted the test material to be directed only to the eyes. Five parameters were used to ascertain an irritation response: 1. the subjects' subjective estimation of irritation (using the "Likert" scale); 2. photographs of the subjects' eyes prior to and after exposure; 3. blink rate as measured by electromyography; 4. effect upon visual acuity; 5. tear production. Both a positive control (acetic acid) and a negative control (air) were employed. Baseline responses for each of the assessment parameters were determined under pre-exposure conditions and upon exposure to the negative control for the prescribed period. A positive irritation response was based on three criteria: 1. the average response must be quantitatively greater than the pre-exposure response; 2. the average response must be greater than the pre-exposure response by more than would be expected statistically from individual to individual differences within the group; 3. the average treated response must be greater than the air-only group's response by more than would be expected statistically from individual differences observed within the group.

Seventy individuals (38 males, 32 females) with a mean age of 32 years (range, 18-67 years; median age, 28 years) were exposed to air, MITC, and/or acetic acid. Between 9 and 16 subjects were examined under each dose/time period combination. Three exposure periods, 14 minutes, 4 hours and 8 hours were used. In the eight hour test, subjective responses, blink rates and tearing were assessed at 0, 1.5, 3, 3.5, 6 and 8 hours (tearing was not measured at 3.5 hours). Two 15-minute rest breaks and a 30-minute lunch break were permitted during the 8-hour period. In the four hour test, these same parameters were assessed at 0, 1, 2, 3 and 4 hours (tearing was not measured at 0, 2 and 3 hours). In the 14-minute exposure protocol, subjective responses and blink rates were measured at 0, 1, 4 and 14 minutes after the start of exposure. Tearing was measured at 14 minutes only. Visual acuity and ocular morphology were assessed at the beginning and end of each exposure period. All analyses were

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performed in a double-blind manner.

In the 4- and 8-hour tests, subjects exposed to 0.22 ppm (220 ppb) MITC did not mount a statistically significant irritation response to the test material. A 4-hour exposure to 0.8 ppm (800 ppb) MITC resulted in a statistically significant positive response based on averaging the subjective assessments by the subjects using the Likert scale methodology. In that test, as many as 8 out of 9 subjects exposed under those particular conditions showed a positive response at 1 and 2 hours, the first two time points examined. (*Note*: Judgement of a positive response is itself somewhat subjective in light of the variability observed among control subjects.) Mean responses at those times, expressed as the percentage of the full Likert scale indicated by the subject, were 25%±14% and 26%±14%, respectively, compared to 2%±2% in zero-time untreated controls (a judgement of 50% was stated to be equivalent to the irritation one might expect from the cutting of a single mild onion). One-hour and 2-hour air-only controls exhibited responses of 6%±9% and 5%±8%, respectively. By 3 and 4 hours, all 9 subjects appeared to respond positively, with mean responses of 39%±19% and 39%±26%, respectively. Air-only controls at the latter 2 times were 5%±6% and 4%±6%, respectively. Shorter exposures to 0.6 ppm did not result in statistically significant increases, though 1 of 9 individuals appeared to respond at 4 and 14 minutes. Exposure to 1.9 ppm or 3.3 ppm MITC resulted in positive subjective responses at 4 and 14 minutes. At 1 minute of exposure, levels as high as 3.3 ppm did not evoke a statistically significant positive response.

Blink rate measurements at 0.8 ppm were statistically significantly increased at the 2- and 3-hour time points, with 7 of 9 subjects responding positively. Mean blinks per minute (minus the zero-time rate) were 16±11 and 14±13 at those times. Air-only control rates at 2 and 3 hours were 3±9 and 3±8 blinks per minute, respectively. Statistical significance was not achieved at 1 and 4 hours, though a positive response was suggested in several individuals. The blink response to 0.6 ppm and 1.9 ppm at 1, 4, and 14 minutes did not indicate positivity. At 3.3 ppm, statistical significance was achieved at 4 and 14 minutes. A strong suggestion of a response was also present at 1 minute, though it was not statistically significant.

No statistically positive tearing responses were observed. However, 2 of 9 individuals exposed to 3.3 ppm MITC showed apparently positive responses at 14 minutes (longer exposures were not evaluated at this concentration).

With respect to the possibility that there were changes in ocular morphology or visual acuity, the following passage is quoted from the study report (page 39):

> Preliminary analysis of the photographs of test subjects' eyes indicated that no notable, exposure related changes were observable in the large majority of tests. In a few tests in which minimal increases in redness and swelling were observed, it appeared that they were more likely to occur in exposures to air than in exposures to MITC. A few individuals evinced a degree of mild edema at the highest level of MITC exposure, but

this tended to be canceled out by other subjects who evinced some native edema and redness, pre-exposure in the early morning. Changes in subjects' visual acuity were also few and apparently random. Accordingly the results of the photographic and acuity tests were not considered to provide any meaningful information on chemical exposure. Results from these tests are retained in study records.

Rates of recovery from irritating MITC exposures were not evaluated directly. The comments of the test subjects indicated that recovery began immediately upon removal of the masks, and was complete within 20 minutes at the highest concentration tested, and sooner at lower concentrations.

Based on the 8-hour eye irritation responses described in this study at 0.8 ppm, a NOEL of 0.22 ppm (220 ppb) was considered the critical NOEL for evaluation of potential human short-term exposure to airborne MITC.

3.   Dermal sensitization

Two separate reports of dermal sensitization attributed to MITC exposure have appeared in the clinical literature. Schubert (1978) reported his general experience that older solutions of Nematin, a metam sodium-containing product, "always produce stronger allergic patch test reactions than fresh ones." This implies that MITC in the product may have been the effector. Schubert also felt that MITC would be a strong effector candidate due to the allegedly high protein binding capacity of the isothiocyanate group. Richter (1980) recounted a number of dermatitis patients that showed positive patch test reactions to Vapam (metam sodium), concluding that the initial effector must have been MITC. One patient, exposed to dazomet through rubber boots, developed burns and a bullous eruption over 5% of her body surface. Positive Vapam patch tests were obtained at the original presentation and again one year later. A further complication, "hypersensitivity-hepatitis of nonspecific type" was diagnosed after liver biopsy. While conditioning exposure to oral contraceptives was implicated in this response, Richter concluded that MITC absorption through the skin was also involved. Indeed, "such side effects must be considered if large areas of skin have been affected."

4.   Other specific syndromes / sequelae
a.   The Sacramento River Spill

The most detailed picture of the health effects resulting from short term MITC exposure in humans emerged in the aftermath of the July 14, 1991 derailment of a railroad tank car on the Cantara Loop, a tight bridge curve spanning the Sacramento River 45 miles north of Shasta Lake and 6 miles north of the town of Dunsmuir, California. The tanker, part of a 97-car / 4-locomotive train, ended up in the river under the bridge, disgorging nearly all of its 19,500 gallons of 32.7% metam sodium within the hour.

Measurements of MITC air levels in the area were not available for the first three days after

the spill. Consequently, the potential maximum concentrations on those days were estimated using Gaussian plume dispersion modeling. There was substantial uncertainty associated with the parameters required to estimate peak MITC air concentrations using such an approach. These parameters included, but were not limited to, the metam sodium-to-MITC conversion rate, the flux of MITC from the water surface, and the meteorologic conditions (wind speed and air dispersion factors). Because the magnitude of the uncertainties was unknown, any estimates of peak MITC air concentrations should be interpreted with great caution. In the US EPA model employed by Alexeeff *et. al.* (1994) of Cal-EPA's Office of Environmental Health Hazard Assessment (OEHHA), MITC was assumed to emanate from a point source on the river. The flux and meteorologic conditions were approximated based on knowledge of the physicochemical characteristics of metam sodium and default meteorologic assumptions. A peak 1-hour time weighted average concentration of 1300 ppb was calculated for a distance of up to 100 meters *from the river*[1], dipping to 340 ppb at 500 meters using the point source approach. In a more recent analysis, DPR used the line source form of the Gaussian plume model and updated assumptions about the flux and meteorological conditions (Barry, 2001a, b). The resultant maximal 1-hour time weighted average estimates were 4500 ppb at 100 meters and 1240 ppb at 500 meters *downwind* from the river. The Metam Sodium Task Force conducted a screening level sensitivity analysis, estimating maximum 1-hour time weighted average air concentrations of MITC *at the river's edge* to be between 3 ppm and 650 ppm. This range was dependent on assumptions concerning the emission rate of MITC from the river and the effect of local canyon topography on MITC air dispersion (Sullivan, 2001).

By post-spill day 4, reliable measurements of MITC air concentrations became available. Citing data from a California Air Resources Board report, Alexeeff *et. al.* (1994) reported, "...air levels along the river on the fourth day (12-hr integrated samples) ranged from 0.0007-0.124 mg/m^3 (0.2-37 ppb). The 12- to 24-hr average levels reported by ARB on the fifth through the tenth day ranged from below the detection limit to 0.0085 mg/m^3 (<0.1-2.6 ppb). The highest reported levels of MITC in air occurred in the Dunsmuir area, 4 days after the spill. The levels reported were 0.41 and 0.39 mg/m^3 (125 and 119 ppb), based on a 1-hr sample taken near the water/air interface. Thus, they represent potential peak exposures, but not breathing level samples."

Records from the temporary evacuation and triage shelter in Dunsmuir and from area physicians and hospitals were evaluated in a detailed epidemiologic study conducted by the California Department of Health Services (Kreutzer *et. al.*, 1994) and discussed by OEHHA in a risk assessment

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[1]Italics are utilized in the following few sentences to call the reader's attention to the different distance scenarios used to calculate MITC air concentrations in the various estimates. OEHHA's concentration estimates were based on a perpendicular distance from the river, while DPR's estimates assumed a downwind distance. This translated functionally as the concentration calculated at a 45E angle from the river. Finally, as stated, the MSTF estimate was for the concentration at the river's edge.

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performed after the spill (OEHHA, 1992). Of the 848 spill-related hospital visits (705 separate individuals) in the month following the accident, 64% reported headache, 49% eye irritation, 42% throat irritation, 46% nausea, 30% dizziness, 27% shortness of breath, 25% diarrhea, 23% nasal irritation and 22% chest tightness. Gender appeared to be irrelevant. There were 7 hospitilizations, with respiratory problems accounting for 4 (of which 3 had preexisting respiratory disease), fainting for 2 and disorientation / irregular heartbeat for 1 (the latter individual may have received a particularly intense exposure). The occurrence of eye irritation suggests that there were exposures above the eye irritation NOEL of 220 ppb (Russell and Rush, 1996). Of eight women exposed during pregnancy, 2 exposed during the first trimester opted for an abortion. Four exposed during the second trimester were advised that their pregnancies were progressing normally. Two women could not be contacted. Emergency response personnel also experienced respiratory symptoms in the week following the spill.

Interestingly, complaints of abdominal pain, weakness, diarrhea, rash and cough continued after one week, when ambient levels had dipped below the published reference exposure level of 0.4 ppb (recalculated to 0.5 ppb in Alexeeff *et. al.,* 1994) based on eye irritation in cats (Nesterova, 1969). These symptoms were attributed to unknown toxicologic properties of MITC or other metam sodium breakdown products, sensitization to lower MITC concentrations caused by earlier exposure to higher concentrations, slow resolution of symptoms related to the earlier exposure, greater than anticipated range of human sensitivity, desire to document symptoms for legal purposes, anxiety-related effects similar to post traumatic stress disorder, or to causes unrelated to exposure (Kreutzer *et. al.*, 1994).

When the Dunsmuir data were stratified based on distance of the impacted person's home from the Sacramento River, no clear pattern could be discerned (Kreutzer *et. al.*, 1994). For those living between 0 and 300 feet from the river, 86 people out of a total of 406 (21.2%) reported symptoms. At 301-600 feet, the ratio was 87/637 (13.6%); at 601-900 feet, 99/689 (14.4%); at 901-1200 feet, 76/590 (12.9%); and at 1201-1500 feet, 43/217 (19.8%). In total, 391/2539 (15.4%) of Dunsmuir residents reported symptoms. The reasons for the lack of clear correlation between symptoms and distance from the river are not clear, particularly as it is expected that those closer to the river were exposed to higher concentrations (Barry, 2001; see discussion above). At least two factors could have influenced this result. First, the stratification was based on the home location of the impacted individuals, which may not accurately reflect their location at the time of peak exposure. And second, concentration differences manifesting in successive 300-foot areas out to 1500 feet (the outer edge of the town) may not have existed, or at least may not have been sufficient to show quantitative epidemiologic differences in a small population sample.

In a further study, 30 of 197 adults referred to health practitioners for evaluation of potentially spill-related health problems were considered positive for persistent effects (Cone *et al.*, 1994). Twenty of these were cases of persistent irritant-induced asthma while 10 were cases of persistent exacerbation of asthma. Exposure duration ranged between 4 and 166 hours, with distance from the

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river being 0.5 miles or less (among those who reported a distance). The sampling technique did not permit an assessment of the incidence of such symptoms in the total exposed population following the spill. However, the study did establish a strong likelihood of longer-term pathology in some individuals. Both the persistent irritant-induced asthma and the persistent exacerbation of asthma met the criteria for a condition known as "reactive airways dysfunction syndrome" (RADS), first characterized in 1985 (Brooks *et al.*, 1985) in a number of patients acutely or subacutely exposed to irritant airborne contaminants. The most relevant of these criteria were: (1) onset of symptoms within 24 hours of a single exposure to an airborne irritant, (2) persistence of symptoms (mainly asthma with cough, wheezing and dyspnea) for at least 3 months, (3) airflow obstruction (evident with pulmonary function tests) and (4) a positive methacholine challenge test. An additional potential problem, unevaluated in the Cone study, is that RADS sufferers may be sensitized to much lower subsequent doses of irritant airborne contaminants. This possibility has been raised with respect to the general category of isocyanates, which are recognized not only as powerful occupational inducers of asthma, but are considered sensitizers as well (Wheeler *et al.*, 1998). In one case, a worker exposed to gaseous diphenylmethane diisocyante from a spill developed asthmatic sensitivity to non-irritating concentrations of, presumably, the same compound (LeRoyer *et al.*, 1998).

A newspaper report dated June 15, 1997 (Bowman, 1997) described serious long-term sequelae in 3 railroad workers dispatched in the hours after the spill to salvage the damaged train. Immediately upon reaching the scene they experienced a series of symptoms including burning eyes, dry mouth, difficulty swallowing, ill feeling, shortness of breath, nasty metallic taste and lightheadedness. Symptoms manifesting themselves in the years after the spill included permanent neuropsychological damage, RADS, irregular heartbeat, low blood oxygen, depression, coughing fits, back-to-back colds, loss of drive and peeling away of the mucous membranes in the mouth. The latter symptoms are sufficiently serious to warrant follow-up investigation with these patients by trained personnel to assess the possibility of long term effects resulting from acute or subacute MITC exposures. Unfortunately, no further report of these particular patients can be found in the medical or toxicologic literature. However, higher levels of anxiety and depression, with related physiological sequelae were detected in a study carried out on 350 exposed residents (114 non-exposed controls) 3 to 4 months after the spill (Bowler *et al*, 1994).

In conclusion, the air concentrations of MITC in the Dunsmuir area during the first three days after the spill were not directly measured. However, Gaussian plume modeling estimates of the peak concentrations were calculated. These estimates were subject to an unknown, but likely substantial, degree of uncertainty. One early report, which used a point source representation of MITC emission from the river, suggested that the peak 1-hour level was 1300 ppb within 100 meters and 340 ppb at 500 meters (Alexeeff *et. al.,* 1994) from the river. A later analysis using a line source representation, set 4500 ppb as the maximum 1-hour air concentration at 100 meters and 1240 ppb at 500 meters

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downwind from the river (Barry, 2001a, b). The Metam Sodium Task Force estimated maximum 1-hour time weighted average air concentrations of MITC at the river's edge to be between 3 ppm and 650 ppm, depending upon the assumptions that were made concerning the emission rate of MITC from the river and the effect of local canyon topography on MITC air dispersion (Sullivan, 2001). In any case, short term and, possibly, persistent adverse effects were produced in exposed individuals.

b. The Earlimart Incident

On the evening of November 13, 1999, an MITC drift incident occurred in the town of Earlimart, California, located in the Central Valley approximately 75 miles south of Fresno. It resulted primarily from the last of a series of six sprinkler applications ("sets") of metam sodium to a field just south of the town between 3:00 p.m. and 7:30 p.m. (sunset occurred at 4:51 p.m.). (The applicator was later cited by DPR for several violations [DPR, 2001].) Sets 4 and 5, applied earlier in the day, were also considered to have contributed to the plume, though their contribution was less than that of set 6. Movement of the gaseous plume into the neighborhood was likely the result of a wind directional shift toward the town during the application, coupled with the development of an inversion layer after sunset. By 5:00 p.m. local emergency services were receiving odor complaints. Reports of symptoms (see below) provoked a general evacuation order for residents of a neighborhood located 0.45-0.6 miles from the northern end of the treated field. A decontamination center was designated at a school in the northern part of the town. Some residents were directed to local hospitals for evaluation.

There were no direct measurements of the MITC air concentrations during the Earlimart incident. Estimates of MITC were made by DPR using the US EPA's Industrial Source Complex Short Term Version 3 (ISCST3) air dispersion model (Barry, 2000). ISCST3 is a Gaussian plume model similar to those used to estimate the MITC concentrations after the Cantara Loop spill. Data from two prior metam sodium field studies, as well as weather data for November 13, 5:00-7:00 p.m., collected at the Formoso CIMIS station, were also used in generating the estimation. Certain simplifying assumptions were made, including the lack of contribution from sets 1, 2, and 3, the lack of effect of varying water seal depths during autumn temperature applications, and the homogeneity of the downwind distribution of the plume. In addition, the concentrations of other metam sodium degradation products were not calculated. Because of the substantial uncertainties regarding these assumptions, the final estimates are likely to be lower bound estimates. Actual air concentrations may have been higher than those determined by this method.

The MITC concentrations in the populated area of zone A (up to 0.6 miles from the field) were estimated to fall, for the most part, between 0.5 and 1 ppm (modeling results; 1-hour time weighted averages, 6:00 to 7:00 p.m.). The concentration in part of the southern boundary of this neighborhood nearest the field may have risen to 1-1.5 ppm. The 0.5-1 ppm concentration range extended considerably north of zone A, into zones B (0.6-0.82 miles) and, possibly, C (0.82-1.08 miles) and D

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(>1.08 miles). The estimated concentrations immediately adjacent to the treated field rose above 3 ppm.

To ascertain the nature of the resultant symptoms, interviews were conducted with some impacted individuals at a local church six days after the incident (November 19) (DPR, 2001). Symptom data were also garnered from complaints submitted to the Tulare County Department of Agriculture and local emergency services, as well as from pesticide illness reports and medical records collected during the investigation.

A total of 171 individuals were evaluated. Of these, 136 (78.6%) were exposed in zone A. One hundred and thirty three people from zone A were exposed in the neighborhood that was eventually evacuated. Three zone A individuals were exposed at the edge of the field. Another 18 individuals (10.4%) were exposed in zone B, 10 in zone C (5.8%), and 5 (2.9%) in zone D. Four individuals were exposed at non-specified sites, though it was considered likely that they were in zone A.

As noted in the DPR report on this incident (DPR, 2001), "symptoms of eye or upper respiratory irritation (typically, burning of the eyes, nose, or throat) were present in the majority of reported cases in all 4 zones. These included 81% of the 136 cases from zone A, 61% of the 18 cases from zone B, 50% of the 10 cases from zone C, and 60% of the 5 cases reported from zone D. Non-specific systemic symptoms (including complaints of headache, nausea, dizziness, shortness of breath, abdominal pain, vomiting, and weakness) were also present in 61.3% of the 173 individuals evaluated. Twenty-eight (16%) had respiratory complaints, including 5 (2.9%) with asthma or other lower airway problems..." The asthma cases appeared to be exacerbations of previously existing conditions.

Of the 23 cases of lower airways problems that were not considered to constitute asthma, there were 16 individuals with dyspnea / chest pain, 6 with cough, and 1 with both cough and dyspnea. Two cases may have reflected non-respiratory symptoms, including a man with a peptic ulcer treated for vomiting and coughing blood and one with hypertension possibly aggravated by anxiety related to the incident. Of the 8 cases of skin rash, one may have been due to chicken pox. The presence or absence of longer-term complications such as RADS is currently unknown.

In conclusion, a series of eye and/or respiratory irritation symptoms, possible asthmatic exacerbations, and non-specific systemic effects were induced in residents of Earlimart, California, by exposure to airborne MITC and other metam sodium by-products. These exposures resulted from a sprinkler application of metam sodium to a field bordering the town to the south. Off-site movement of the gaseous plume may have been caused by a wind directional shift toward the town during the application, as well as the development of an inversion layer after sunset. MITC air concentrations were estimated to range from 0.5 to 3 ppm, depending upon the distance from the application, during the 1-hour period of highest exposure. The range estimate was 0.5 to 1 ppm in the neighborhood closest to the application. These were likely to be lower bound estimates.

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B. Animal Studies

1. Studies with the technical material

The acute toxicity of MITC *via* inhalation, dermal, oral gavage, or ocular administration was studied in rats, mice, rabbits, dogs, cats, guinea pigs and monkeys. Clinical signs and/or tissue changes were reflective of damage at the site of contact with MITC. The acute toxicity of MITC is summarized in Table 4.

The studies of Ullman (1985a-f) laid out the basic acute toxicity and primary irritation characteristics of MITC. Acute oral exposure in rats between 25 and 300 mg/kg led to sedation, dyspnea, altered body positions, ruffled fur, vocalization, spasms and exophthalmos. The LD_{50} in that study was 55 mg/kg in females and 82 mg/kg in males. Similar clinical signs were noted upon dermal exposure of rats at a dose range of 60-600 mg/kg (LD_{50} = 181 [F] and 225 [M] mg/kg) and rabbits at a dose range of 50-300 mg/kg (LD_{50} = 202 [F] and 145 [M] mg/kg). MITC was also shown to be a powerful irritant to both skin and eyes.

Studies of acute inhalation exposure to MITC in animals generate a conflicting picture of the resultant toxicity, particularly in rats. Ullman (1985b), using Wistar rats, showed that all animals died within 30 minutes of nose-only exposure to 29.6 mg/m³ (~10 ppm) MITC. However, there was no description of how this value was obtained, nor was there an indication as to whether it was nominal or analytically derived. Associated clinical signs included restlessness and excitement. In contrast, 1900 mg/m³ (~633 ppm) was the LC_{50} value generated in the 1-hour whole-body exposure study of Clark and Jackson (1977) using Sprague-Dawley rats. No animals died at 630 mg/m³, a dose 21-fold higher than the dose causing 100% lethality in the Ullman study. Clinical signs included initial hyperactivity followed by hypoactivity, eye irritation, dyspnea and, at 2200 mg/m³ and above, convulsions. The same group reported a 4-hour LC_{50} of 540 mg/m³ (~180 ppm) in Sprague-Dawley rats (Jackson *et al.*, 1981), 18-fold higher than the dose required to kill all animals in the Ullman study, but similar (considering the increased exposure time) to the finding of Clark and Jackson (1977). Finally, Nesterova (1969) claimed that no rats died at MITC doses of at least 79.1 mg/m³ (~26.4 ppm). Again, the strain was undefined. Nesterova also showed that 80-100% of mice died at 75-79 mg/m³ (~25-26 ppm) and that no cats died at 0.5 mg/m³ (~0.167 ppm). Unfortunately, because the Nesterova study was bereft of experimental and methodologic detail, its quantitative aspects are considered of little use for regulatory purposes.

There was no clear explanation for the disparity in the rat inhalation data. Two subchronic inhalation studies are described in section V.A. in which Wistar rats (the strain used in the Ullmann acute inhalation study) were exposed on a daily basis to MITC air concentrations as high as 45 ppm. No deaths were observed in those studies, even at the highest concentrations and even after as long as 13 weeks of daily exposure (Klimisch *et al.*, 1987; Rosskamp, 1979). The Ullmann study is therefore viewed as an outlier that, for unexplained reasons, may be anomalous. The studies of Clark and Jackson (1977) and Jackson *et al.* (1981) in Sprague-Dawley rats provide full methodologic and toxicologic

accounts, and are consistent with each other and with the later subchronic studies on the Wistar rat. For the present they are deemed the most reliable acute lethality studies. (For a more complete discussion of this issue, see DPR [2002e].)

The most sensitive acute laboratory animal endpoint reported in the literature was irritation of the ocular mucosa in cats with a 4-hour NOEL of 33 ppb (Nesterova, 1969). Unfortunately, as mentioned above, important details of that study were not reported, rendering the quantitative aspects of the study useless. Nonetheless, the irritating effect of MITC on ocular and respiratory tissues was confirmed in other animal studies, some of which are discussed above. The same effects have been reported in people exposed to MITC after the Sacramento River spill (see above), or after agricultural use (DPR, 1993; DPR, 2002b).

There is one report of dermal sensitization in guinea pigs in which an initiating dose of metam sodium was followed by challenge doses with either metam sodium or MITC (Mutter, 1987). The author considered it an open question whether the initiating effector was the parent compound or the degradation product.

     2.   **Studies with specific product formulations applicable to ambient air exposure**

LD_{50} and LC_{50} values from acute oral, dermal and inhalation toxicity studies on Trapex 40, a formulation containing 40% MITC, were listed in a review published in 1990 (Nihon Schering K.K. and Shionogi & Co. K.K. and Shionogi & Co., 1990). These appear in Table 4. No description of the actual studies was provided. No further acute toxicologic studies on formulations are currently available. That review, however, furnished a short description of a study in guinea pigs showing MITC, administered as Trapex 40, was a mild dermal sensitizer.

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Table 4. The acute toxicity of MITC

Endpoint/Species	Dose/Score	References[a]
Technical Grade		
Eye Irritation		
Human volunteers	0.22 ppm (220 ppb) 1, 4 & 8 hours	1
Inhalation LC_{100}		
Rat (Wistar)	$\leq$29.6 mg/m^3 ($\leq$~10ppm)[c] 0.5 hr, nose-only	2[b]
Inhalation LC_{50}		
Rat (Sprague-Dawley)	540 mg/m^3 (~180 ppm) 4 hours	3[b]
Rat (Sprague-Dawley)	1900 mg/m^3 (~633 ppm) 1 hr, whole body	4[b]
Mouse	LC_{80-100} = 75-79 mg/m^3 (~25-26 ppm) 4 hour	5[b]
Oral LD_{50}		
Rat	55-82 mg/kg	6[b]
Rat	72-175 mg/kg	7[b],8[b]
Rat	50 mg/kg	8[b]
Rat	305 mg/kg	9[b]
Rat	220 mg/kg	9[b]
Mouse	90-104 mg/kg	6[b], 10[b]
Mouse	110 mg/kg	9[b]
Dog	LD_{50} not reported (deaths at 21.5 & 100 mg/kg).	8[b]
Rabbit	21.5 mg/kg	8[b]
Monkey	>46.4 mg/kg but <100 mg/kg	8[b]
Dermal LD_{50}		
Rat	181-225 mg/kg (24-hr exposure)	11[b]
Rat	2780 mg/kg	7[b]
Mouse	1870 mg/kg	7[b]

[a] References: 1. Russell and Rush, 1996 2. Ullmann, 1985b; 3. Jackson *et al*., 1981; 4. Clark and Jackson, 1977; 5. Nesterova, 1969; 6 Ullmann, 1985a; 7 Nihon Schering K.K. and Shionogi & Co., 1990; 8 Nor-Am Ag, Inc., 1982; 9 Vernot, 1977; 10. Schering AG, 1980?; 11. Ullmann, 1985c
 [b] Purity not reported. [c] Lowest dose tested.

Table 4. The acute toxicity of MITC (continued)

Endpoint/Species	Dose/Score	References[a]
Technical Grade (continued)		
Dermal LD$_{50}$		
Rabbit	33-202 mg/kg	1,7[b]
Subcutaneous LD$_{50}$		
Rat	59-60 mg/kg	2[b]
Mouse	75-89 mg/kg	2[b]
Intraperitoneal LD$_{50}$		
Rat	45-56 mg/kg	2[b]
Mice	82-89 mg/kg	2[b]
Ocular Irritation		
Rabbit	Corrosive-"Powerful Irritant"	3,4[b]
Dermal Irritation		
Rabbit	Corrosive-"Powerful Irritant"	4,5[b]
Dermal Sensitization		
Guinea pig	Possible sensitizer	6
Liquid Formulation (40%)		
Inhalation LD$_{50}$		
Rat	1500 mg/m^3 (~500 ppm) 4 hours	2
Oral LD$_{50}$		
Rat	166 mg/kg	2
Dermal LD$_{50}$		
Rabbit	~120 mg/kg	2
Ocular Irritation		
Rabbit	Severe inflammation, corneal opacity, iritis	2
Dermal Irritation		
Rabbit	Corrosive	2
Dermal Sensitization		
Guinea pig	Positive	2

[a] References: 1. Vernot, 1977; 2. Nihon Schering K.K. and Shionogi & Co., 1990; 3. Ullmann, 1985e; 4. ICI Americas, 1991; 5. Ullmann, 1985f; 6. Mutter, 1987; 7. Ullmann, 1985d
[b] Purity not reported.

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V. SUBCHRONIC TOXICITY

Summary. An estimated NOEL of 100 ppb based on nasal epithelial atrophy at 300 ppb was established in the 4-week rat inhalation study of Klimisch *et al.* (1987). This value was used as the critical subchronic NOEL. Nesterova (1969) noted increases in salivation and nasal discharge, and degenerative changes in various internal organs in rats exposed to MITC vapors for 4 hours a day for 4 months at 370 ppb, with vascular disturbances occurring at 153 ppb. Cats exposed to 33 ppb for that time had no toxic effects. However, due both to the summary nature of this report and to the apparent inability by others to reproduce the results, these data were not considered sufficient to provide endpoints with regulatory significance. A NOEL of 1 ppm based on decreased body weight, decreased serum total protein and increased water consumption was established in the 12-13 week rat inhalation study (Rosskamp, 1978). The data in this study were also summary in nature and not considered suitable for regulatory purposes. The results of several subchronic oral studies in mice and rats identified a NOEL of 0.7 mg/kg/day based on retarded weight gain, increased liver weight and increased ovary weights at 1 mg/kg/day. A LOEL of 1 mg/kg/day based on skin damage, decreased serum albumin and plasma cholinesterase, and altered hematopoietic activity in rats exposed by the dermal route was also established. The NOELs determined from the subchronic studies evaluated in this section are summarized in Table 7.

A. Inhalation Studies

Rats. In a 4-week, whole-body inhalation study, 5 Wistar rats/sex/dose group were exposed to vapors of MITC (96.9% pure) at doses of 0, 5.1, 19.9 or 100.0 mg/m³ (Klimisch *et al.*, 1987). These were equivalent to concentrations of 0, 1.7, 6.8 and 34 ppm (no estimate was provided of the amount of MITC that may have been ingested by grooming). Exposure was for 6 hours/day, 5 days/week. MITC daily mean concentrations in each chamber were determined by gas chromatography using six measurements made during each 6-hour exposure period. The doses listed above were the grand means of the daily determinations.

No deaths occurred during the study. High dose males suffered a substantial weight loss during the first week (-19.2 grams, compared to a gain of 23.5 grams in control males, p<0.01). High dose females gained less weight than controls during the first week (1.1 grams *vs.* 13.1 grams in controls), though statistical significance was not established. These differences were maintained throughout the study in both sexes. High dose male body weights on days 7, 14, 21, and 27 were lower than controls by statistically significant margins. No convincing weight differences were observed at either of the two lower doses in either sex. There were no measurements of food consumption, making it difficult to determine the reasons for the body weight effects at the high dose.

Clinical observations at the high dose revealed marked mucous membrane and respiratory tract irritation (reddish nasal discharge, salivation, eye discharge) resulting in a change in breathing pattern

and whooping respiration. Intensified cleaning and stretched posture were also noted. As the study progressed, certain signs (ruffled fur and respiratory sounds) ceased being reversible. Less severe signs (eyelid closure, somnolence, and ruffled fur) were noted at the mid dose from day 3 onward. However, unlike the high dose animals, mid dose animals began to recover following the completion of each day's dosing. Incidence rates for these clinical signs were not reported. This deficiency drove the current document to the health conservative assumption that all of the animals were affected in the dose groups in which they were reported.

Clinical chemistry revealed statistically significant decreases in serum urea (22%), glucose (17%), triglyceride (58%), and albumin (9%) in high dose males. The urea and glucose concentrations in high dose females were likewise statistically depressed (30% and 14%, respectively). These changes were ascribed by the authors to "the retarded body weight change observed and, in connection with this, [to] a catabolic metabolic condition." Total bilirubin concentrations and thromboplastin time (a measure of blood clotting ability) were markedly increased in high dose males (92% and 16%, respectively), though not in females. These changes were difficult to ascribe to a particular clinical picture. Smaller changes in urea (-17%), glucose (-5%), bilirubin (+41%), and thromboplastin time (+14%) were also present in mid dose males, though these did not achieve statistical significance.

Mid and high dose males exhibited increased numbers of neutrophilic polymorphonuclear granulocytes. The mean concentration in giga/L at increasing doses was 0.43 ± 0.05, 0.48 ± 0.07, $0.69 \pm 0.07^*$, and $1.42 \pm 0.18^{**}$ ($^*p < 0.05$, $^{**}p < 0.01$, Student's t test on log-transformed data [Dunnett's parametric t test was positive at the high dose only]). Females showed this increase only at the high dose, with mean concentrations in giga/L at increasing doses of 0.52 ± 0.09, 0.52 ± 0.10, 0.55 ± 0.08, and $1.44 \pm 0.28^{**}$. Overall leukocyte counts were also increased in high dose females (mean concentration in giga/L: 5.19, 4.72, 4.54, 6.73). These changes were considered related to inflammatory processes occurring in the respiratory tract.

Organ weight determinations showed statistically significant decreases in liver (18%) and kidney (17%) weights and an increase in lung (70%) weight among high dose males. Females showed only the increase in lung weight (83%). Gross pathology showed the lungs of all high dose males and three females to be pale, rigid, and of a puffy consistency.

Histopathology revealed an increase in incidence and severity of rhinitis in the nasal cavity at the high dose in both sexes (incidence in males: 2/5, 2/5, 2/5, 5/5; females: 0/5, 3/5, 1/5, 5/5). Other histopathologic findings at the high dose included: metaplasia of the nasal epithelium (3 males in section plane 1 only, 5 females in section planes 1 and, to a lesser extent, section plane 2), tracheal epithelial proliferation and single cell necrosis (all high dose animals), bronchopneumonia and bronchial and bronchiolar epithelial proliferation (5 males, 2 females), and emphysema (3 males, 2 females). One finding, nasal epithelial atrophy, may have been induced at the low dose. This was based on increases in focal atrophy at that dose, as noted in section plane 2 in both sexes (Table 6a) (the four nasal

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histological section planes from each animal were assumed to represent an outside-to-inside order of sectioning), and increases in total atrophy. The latter was evident both in the number of animals exhibiting focal + non-focal atrophy (Table 6b, 3rd row) and in the fraction of section planes, particularly among females, showing atrophy (Table 6b, 4th row). The absence of a further increase at the mid dose may be due in part to a process by which focal atrophy at lower doses was replaced by "non-focal" atrophy, considered to be a more general (*i.e.*, diffuse) and potentially more serious lesion, at higher doses. Such a process was very clear at the high dose, where virtually all reported atrophy (28 of 30 sections representing planes 2-4) was non-focal. Another possible contributor to the apparent lack of a dose response between the low and mid doses was the small number of animals used in the study, which decreased the statistical power of the experiment. In fact, the per-rat incidence rates at the low and mid doses were not statistically distinguishable from controls by a Fisher exact comparison (to achieve statistical significance in the Fisher exact test using approximately the observed incidence rates, it would have been necessary to test 18-22 animals per dose group). The conclusion that MITC caused nasal epithelial atrophy at the low dose was therefore dependent on five considerations: 1) such an effect was plausible in view of the known irritant properties of the compound, 2) the most clearly affected section plane (#2) was closest to the outside air and thus likely received a higher *effective* MITC dose (as section plane 1 did not show any nasal atrophy even at the high dose, it was considered insensitive to this effect; see footnote #4, section V.A.), 3) females showed a statistically significant increase in total atrophy at the low dose when incidence was expressed as the fraction of all the section planes exhibiting this character, 4) focal and non-focal atrophy represented a progression in the severity of a single pathologic process; thus it was legitimate to consider their incidence rates together as representative of total atrophy, and 5) total atrophy, when expressed on a per-rat incidence basis, was increased in both sexes at the low dose. For a more detailed discussion of the strengths and weaknesses of this analysis, see section V.A.

The LOEL for this study was set at the lowest dose of 5.1 mg/m^3 (1.7 ppm), based on increased nasal epithelial atrophy in both sexes. At the mid dose of 19.9 mg/m^3 (6.8ppm), nasal epithelial atrophy was seen in addition to clinical signs in both sexes and an increase in neutrophilic polymorphonuclear granulocytes in males. The latter sign, which also occurred in females at the high dose, was considered to reflect an inflammatory process in the lungs. Such a process was clearly evident at the high dose, where increased lung weight and severe histopathologic signs were observed. The clinical signs at the mid dose (eye closure, somnolence, and ruffled fur), suggested a failure to thrive at that dose, though it was not known if the signs were reflective of pulmonary irritation or systemic effects. Nonetheless, the most prominent effects of MITC appeared to be irritative in nature. Haber's Law, which assumes that the longer the exposure time, the less MITC would be required to elicit a particular response, was invoked to convert the 6-hours/day, 5-days/week exposure regimen to 24-hours/day, 7- days/week. This resulted in an estimated LOEL of 300 ppb. A default uncertainty

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factor of 3 was then used to calculate an estimated critical subchronic NOEL of 100 ppb.

Table 5. Incidence of focal and non-focal nasal epithelial atrophy; enumeration by section plane

MITC (ppm):	Males				Females			
	0	1.7	6.8	34	0	1.7	6.8	34
S2[1], focal[2]	1/5	2/5	1/5	0/5	1/5	3/5	1/5	0/5
S2, non-focal[2]	1/5	1/5	2/5	5/5*	0/5	0/5	2/5	5/5**
S2, total	2/5	3/5	3/5	5/5	1/5	3/5	3/5	5/5*
S3, focal	0/5	1/5	2/5	0/5	0/5	0/5	0/5	0/5
S3, non-focal	2/5	1/5	1/5	5/5	0/5	2/5	1/5	5/5**
S3, total	2/5	2/5	3/5	5/5	0/5	2/5	1/5	5/5**
S4, focal	1/5	0/5	1/5	1/5	0/5	1/5	0/5	1/5
S4, non-focal	0/5	0/5	0/5	4/5*	0/5	0/5	0/5	4/5*
S4, total	1/5	0/5	1/5	5/5*	0/5	1/5	0/5	5/5**

*p<0.05; **p<0.01, Fischer exact test, comparison with control values.
[1]"S" refers to the section plane.
[2]The terms "focal" and "non-focal" atrophy were not defined in the study report. "Focal" atrophy was therefore considered by the reviewers as comprising nasal tissue containing discrete areas of damage bounded by larger areas of undamaged tissue. "Non-focal" atrophy was considered to represent nasal tissues containing more general, diffuse tissue damage and less histologically undamaged tissue.

Table 6. Incidence of combined nasal atrophy; enumeration on a per-rat basis, excluding double-counts

	MITC, ppm							
	0 (Controls)		1.7		6.8		34	
	M	F	M	F	M	F	M	F
rats w/focal atrophy	2/5	1/5	2/5	3/5	3/5	1/5	1/5	0/5
total	**3/10**		**5/10**		**4/10**		**1/10**	
rats w/non-focal atrophy	2/5	0/5	1/5	2/5	2/5	2/5	5/5	5/5**
total	**2/10**		**3/10**		**4/10**		**10/10*****	
rats w/focal + non-focal atrophy	2/5	1/5	3/5	3/5	3/5	3/5	5/5	5/5*
total	**3/10**		**6/10**		**6/10**		**10/10****	
section planes showing atrophy (excludes S1)	5/15	1/15	5/15	6/15*	7/15	4/15	15/15 ***	15/15 ***
total	**6/30**		**11/30**		**11/30**		**30/30*****	

*p<0.05; **p<0.01; ***p<0.001, Fischer exact test, comparison with control values.

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In a 12-13-week nose-only inhalation study, 10 Wistar rats/sex/dose were exposed to 0 (2 control groups: untreated, sham dosed), 1, 10, or 45 ppm MITC (95.7%) for 4 hours/day (Rosskamp, 1978). Individual study data were not submitted to DPR; therefore, only summary information was available for evaluation. MITC-related clinical signs were present in all animals only at 45 ppm. These included mild apathy (10M/10F, days 27-85), moderate apathy (1M, day 72 only), weight loss (1M, days 73-84), vocalization (4M/2F, days 58-78), dyspnea (1M, days 71-72), stiff-legged walk (1M, day 85 only), enlarged abdomen (1M, day 85 only), and increased salivation and nasal discharge (10M/10F, days 1-85). Body weight gain in males and females over the entire test period was 37% and 53% of sham controls, respectively, at the high dose (p<0.01). At 10 ppm, body weight gain was 89% and 85% of sham controls. This suppression was probably due to MITC exposure, though statistical significance was not attained at that dose. Water consumption over the same period was 111% and 116% of sham controls at the high dose in males and females (significant in females only at p<0.05). At 10 ppm, water consumption was 114% and 116% of sham controls (p<0.05 in both sexes). Food consumption was decreased at the high dose only: 94% and 92% of sham controls in males and females (p<0.05 in males, p<0.01 in females). A "slight" statistically significant reduction in serum total protein was present at 45 ppm for females, and at 10 and 45 ppm for males, though actual figures were not included in the report. No compound-related histological effects were reported. However, examination of the nasal cavity was not undertaken. Based on decreased body weight, decreased serum total protein, and increased water consumption the NOEL was 1 ppm.

In a 24-day inhalation toxicity study, adult E ("young growing Sprague-Dawley") rats of both sexes were exposed to 0, 250, 500, 900, or 1400 mg/m^3 (0, 84, 167, 300 or 468 ppm) MITC (purity unstated) for 2 hours/day (Schering AG, *c*. 1980). At 1400 mg/m^3 (468 ppm), 3 of 16 rats died after the first day's exposure. A total of 11 of 16 rats died during the first 5 days at that exposure level. Reduced weight gain and albuminuria were present at all dose levels, producing a LOEL of 250 mg/m^3 (84 ppm). Individual study data were not submitted to DPR; therefore, only summary information was available for evaluation.

Rats and cats. In a 4-month inhalation toxicity study in rats and cats, metam sodium was placed in flasks containing wet soil and heated to produce MITC vapors (Nesterova, 1969) . Rats exposed to 1.1 mg/m^3 (370 ppb) for 4 hours/day had lung damage characterized by "vascular disturbances, emphysematous areas in the lungs, and infiltrates of plasma cells and lymphoid cells", and decreased relative weight. Increased sulfhydryl groups were noted in blood serum, and numerous bi- and trinucleate cells were present in the liver, kidneys, and heart. Rats exposed to 0.46 mg/m^3 (154 ppb) 4 hours/day had "vascular disturbances" of the lungs. Based on "vascular disturbances" of the lungs, the LOEL was 0.46 mg/m^3 (154 ppb) (lower doses do not appear to have been examined in rats).

Cats exposed to 0.1 mg/m^3 (33 ppb) for 4 hours/day had no evidence of toxicity. Based on this information, the NOEL was 0.1 mg/m^3 (33 ppb). Individual study data were not submitted to DPR for either species; therefore, only summary information was available for evaluation.

B.   Oral Studies

Mice. In a 3-week toxicity study, groups of 5 JCL-ICR mice/sex/dose received MITC (97%) in the drinking water at nominal concentrations of 0, 0.5, 2.0, 5.0, or 50 ppm (males~ 0, 0.135, 0.452, 1.115 or 10.754 mg/kg/day; females~ 0, 0.1, 0.5,1.1, or 10.0 mg/kg/day, based on the stability of MITC and water consumption) (Sato and Sato, 1977a). No treatment-related effects were reported in any dose group. Based on no effects seen, the NOEL was $ 10.0 mg/kg/day. Individual study data were not submitted to DPR; therefore, only summary information was available for evaluation.

In a another 3-week toxicity study, groups of 5 JCL-ICR mice/sex/dose received MITC (97%) in the drinking water at nominal concentrations of 0, 100, or 200 ppm (males~ 0, 22.9, or 46.3 mg/kg/day; females~ 0, 23.3, or 44.3 mg/kg/day, based on the stability of MITC and water consumption) (Sato and Sato, 1977b). Clinical signs of toxicity were evident only at 200 ppm and included dull hair coat, raised hair, and a decrease in food consumption and body weight gain. Necropsy findings included thickening of the stomach wall in several mice at 200 ppm. Based on the above findings, the NOEL was 22.9 mg/kg/day (100 ppm). Individual study data were not submitted to DPR; therefore, only summary information were available for evaluation.

In a 3-month oral toxicity study, male and female dd-strain mice were administered 1, 5, or 20 mg/kg/day MITC (purity unstated) by gavage (Nor-Am Agricultural Products, Inc., 1982, and Nihon Schering K.K. and Shionogi & Co., 1990). At 20 mg/kg/day toxic effects included thickening of the forestomach lining, inflammation of the liver, and slight disturbance of spermatogenesis with edema of the interstitial area of the testis. These effects were noted occasionally in the 5 mg/kg/day group, and slight changes were present at 1 mg/kg/day. Other changes in the 1 mg/kg/day group included alterations in adrenal and ovary weights. Details on the adrenal gland were lacking, but both absolute and relative ovary weights were significantly decreased at 1 mg/kg/day, although no histologic changes were reported. Based on the changes in the stomach, liver, testis, adrenals, and ovary, the LOEL was 1 mg/kg/day. Individual study data were not submitted to DPR; therefore, only summary information was available for evaluation.

In a subsequent 3-month oral toxicity conducted to investigate the ovarian effects, Slc:ddy strain mice were gavaged with 0.35, 0.5, 0.7, or 1.0 mg/kg/day MITC (purity unstated) (NOR-AM Agricultural Products, Inc., 1982; and Nihon Schering K.K. and Shionogi & Co., 1990). The only

treatment-related effects reported were retarded body weight gain and increased liver weight in the 1.0 mg/kg/day group. Based on those effects, the NOEL was 0.7 mg/kg/day. Individual study data were not submitted to DPR; therefore, only summary information was available for evaluation.

In another 3-month toxicity study, MITC (purity unstated) was administered by oral gavage to dd strain mice at 2.5, 5, or 10 mg/kg/day (Nihon Schering K.K. and Shionogi & Co., 1990). The only significant changes reported were at 10 mg/kg/day, and consisted of an increase in the total white blood cell count. That effect was characterized by an increased proportion of neutrophils and a decreased proportion of lymphocytes. Based on the changes in white blood cell numbers, the NOEL was 5 mg/kg/day. Individual study data were not submitted to DPR; therefore, only summary information were available for evaluation.

Rats. In a 10-day oral toxicity study, male and female Wistar rats received repeated doses of 25, 50, or 100 mg/kg MITC (purity unstated) (Nor-Am Agricultural Products, Inc., 1982). The clinical signs noted after repeated administration were inactivity, soft or black feces, and weight loss. Two females in the 50 mg/kg group died before the end of the study. After two doses of 100 mg/kg, 4 males and 6 females died, and dosing was discontinued at that level. Gross lesions recorded at necropsy included ulceration of the stomach and duodenum, with adhesions to adjacent organs. Based on the information available to DPR, it was not possible to determine a no effect level. Individual study data were not submitted to DPR; therefore only summary information was available for evaluation.

In a 3-month oral gavage toxicity study, male and female Wistar rats received 2, 10, or 40 mg/kg/day MITC (purity unstated) (Nor-Am Agricultural Products, Inc., 1982; and Nihon Schering K.K. and Shionogi & Co., 1990). At 40 mg/kg/day toxic effects consisted of undefined stomach lesions, inflammation of the liver, and a slight spermatogenic disorder. The changes were also occasionally noted at 10 mg/kg/day, with slight effects reported at 2 mg/kg/day. Alterations in adrenal and ovary weight were present at 2 mg/kg/day. Details on the adrenal were lacking, but the absolute and relative ovary weights were increased. No histologic adrenal or ovarian changes were reported. Based on the stomach, liver, testes, adrenal, and ovarian abnormalities, the LOEL was 2 mg/kg/day. Individual study data were not submitted to DPR; therefore, only summary information was available for evaluation.

In an 8-month oral-gavage toxicity study of MITC (purity unstated), Charles River rats were dosed at levels of 3, 10, or 30 mg/kg/day (Nor-Am Agricultural Products, Inc., 1982). The 8-month treatment was followed by a 6-month recovery period. Interim sacrifices occurred after 5 and 8 months during the treatment period, and at monthly intervals during the 6-month recovery period.

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Clinical signs of toxicity were apparently noted only at 30 mg/kg/day, and consisted primarily of excessive salivation prior to dosing accompanied by rapid and unexpected aggressive movements after dosing. The signs reportedly decreased in incidence by the end of the treatment period. Males at 30 mg/kg/day had "significantly" reduced body weight gain during treatment which did not completely reverse during the 6-month recovery period. "Significant" reductions in absolute and relative liver and thymus weights were noted for 10 and 30 mg/kg/day animals at the 5-month sacrifice. Treatment-related necropsy findings during the treatment and recovery phases were apparently limited to thickening of the lining of the forestomach in animals at 10 and 30 mg/kg/day. The incidence of this lesion decreased during the recovery period and was present only in 1 animal at termination. Histologic changes present during the treatment and recovery periods included dose-related acanthosis, hyperkeratosis, and sub-mucosal cyst formation in the forestomach. Based upon the information available to DPR, the LOEL was 3 mg/kg/day for degenerative changes in the forestomach. Individual study data were not submitted to DPR; therefore only summary information was available for evaluation.

C. Dermal Studies

Rats. In a one-month dermal toxicity study involving Sprague-Dawley rats, 120, 240, or 480 mg/kg/day of MITC (purity unstated) was applied to shaved areas of the skin (Nor-Am Agricultural Products, Inc., 1982; Nihon Schering K.K. and Shionogi & Co., 1990). The report indicated that, depending upon the dose administered, damage to the skin consisted of ulceration, crust formation, and neutrophil infiltration. MITC-induced enlargement of the peri-bronchial lymph nodes was also present. Changes in the genital organs, or liver were sporadic and not regarded by the investigators as significant. There were no histologic changes in the nervous system or cornea. Based upon the summary information provided to DPR, it was not possible to determine either a NOEL or a LOEL. Individual study data were not submitted to DPR; therefore, only summary information were available for evaluation.

In a 31-day dermal toxicity study, male and female Wistar rats received 1, 10, or 100 mg/kg/day MITC (purity unstated) on shaved areas of the skin (Nor-Am Agricultural Products, Inc., 1982). At 100 mg/kg/day severe necrosis of the skin was noted. Desquamation and erythema of the skin were present at 1 and 10 mg/kg/day. Decreased food consumption and weight gain were present in females, while weight loss and decreased eosinophil production by the bone marrow occurred in males. Also present in males, at all dose levels, was a dose-dependent decrease in serum albumin. Plasma cholinesterase inhibition (percent not stated) was present at 100 mg/kg/day in males, and at all dose levels in females (this was the only report of cholinesterase inhibition in response to MITC). Increased erythropoietic activity occurred at 10 and 100 mg/kg/day in males, and at all dose levels in females. Based on skin

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damage, decreased serum albumin and plasma cholinesterase, and altered hematopoietic activity, the LOEL was 1 mg/kg/day. Individual study data were not submitted to DPR; therefore, only summary information were available for evaluation.

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Table 7. No-Observed-Effect-Level (NOEL)/Lowest-Observed-Effect-Level (LOEL) for subchronic and chronic toxicity of MITC

Species/Sex	Exposure Regimen	Effects at LOEL	NOEL	LOEL	Reference
			(ppm)		
Rat (M/F)	4-wk inhalation	nasal epithelial atrophy	0.1[a]	0.3[a]	Klimisch *et al.*, 1987
Rat (M/F)	12-13 wk inhal.	\ bw gain, \ serum protein, [water consumption	1[b,c]	10[b,c]	Rosskamp, 1978
Rat (M/F)	4-day inhalation (1 hr/day)	Apathy, wet noses & mouths.	-	100[c,d]	Rosskamp, 1978
Rat (M/F)	4-day inhalation (4 hr/day)	80% mortality, bloody noses & mouths.	-	100[c,d]	Rosskamp, 1978
Rat (NR)	4-month inhal.	"vascular disturbances" of the lungs.	-	0.153[d]	Nesterova, 1969
Cat	4-month inhal.	No effects reported	0.033[e,f]	-	Nesterova, 1969
Rat (M/F)	24-day inhal.	\ bw gain	-	84[g]	Schering AG, 1980
			(mg/kg/day)		
Rat (M/F)	10-day gavage	Inactivity, weight loss, abnormal feces	-	25[d]	Nor-Am Ag Products, 1982
Rat (M/F) Nihon	3-mo. gavage	Liver inflammation, undefined stomach lesions, altered ovary and adrenal weight, spermato-genic disorder	-	2[d]	Nor-Am Ag Products, 1982; Nihon Schering, 1990
Rat (M/F)	8-mo. gavage with 6-mo. recovery	Forestomach acanthosis, hyperkeratosis, & submucosal cyst formation.	-	**3[d]**	Nor-Am Ag Products, 1982
Mouse (M/F)	3-month gavage	Liver inflammation, thickened forestomach, altered ovary & adrenal weight, spermatogenic disorder.	-	**1[d]**	Nor-Am Ag Products, 1982; Nihon Schering, 1990

[a]24 hr/day, 7 days/wk LOEL and NOEL values were calculated using Haber's Law. The NOEL is equal to the LOEL divided by an uncertainty factor of 3.
[b]4 hr/day, 5 days/wk values. [c]Nasal cavity not histologically examined. [d]Lowest dose tested. [e]Highest dose tested. [f]Calculated by dividing the air concentration (0.1 mg/m^3) by the quotient of the molecular weight (73.3 g/M) and the molar volume (24.45 L). [g]4 hr/day value.

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Table 7. No-Observed-Effect Level (NOEL)/Lowest-Observed-Effect Level (LOEL) for subchronic and chronic toxicity of MITC (continued)

Species/Sex	Exposure Regimen	Effects at LOEL	NOEL	LOEL	Reference
			(mg/kg/day)		
Mouse (M/F)	3-month gavage	[liver weight, decreased body weight gain	0.7	1	Nihon Schering, 1990
Mouse (M/F)	3-month gavage	[total WBC count, [neutrophils, \ lymphs.	5	10	Nihon Schering, 1990
Mouse (M/F)	3-wk drinking water	No effects seen.	≥10.0[a]	-	Sato and Sato., 1977a
Mouse (M/F)	3-wk drinking water	\ food consumption & body weight, dull hair coat, thickened stomach wall.	22.9	44.3[a]	Sato and Sato., 1977b
Rat (M/F)	1-month dermal	Skin ulceration, crust formation, neutrophil infiltration, enlarged peribronchial hymph nodes.	-	120[b]	Nor-Am Ag Products, 1982; Nihon Schering, 1990
Rat (M/F)	31-day dermal	Skin desquamation & erythema, \ serum albumin & plasma ChE.	-	1[b]	Nor-Am Ag Products, 1982
Guinea Pig	1-month inhalation	No "allergization"	0.21[a, c]	-	Nesterova, 1969
Rat (M/F)	2-year drinking H_2O	\ water consumption & body wt.	0.46	2.075	Brown, 1981
Mouse (M/F)	2-year drinking H_2O	\ water consumption & body wt., raised hair coat.	2.74	9.82	Sato, 1980
Dog (M/F)	1-year gavage	Decreased body weight, food consumption, general condition.	0.4	2.0	Harling et al., 1989

[a] Highest dose tested [b] Lowest dose tested. [c] Calculated by multiplying the given ambient air concentration (0.46 F/L) by the default respiratory rate for guinea pigs (450 L/kg/day). The length of daily exposure was not provided.

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VI. CHRONIC TOXICITY AND ONCOGENICITY

Summary. The oncogenicity and/or chronic toxicity of MITC via oral gavage or drinking water administration was studied in rats, mice, and dogs. The 2-year rat drinking water study yielded weak evidence for a possible MITC-induced increase in mammary fibroadenomas and carcinomas. A possible MITC-induced increase in subcutaneous fibromas was also noted. The 2-year mouse drinking water study yielded evidence for a possible MITC-induced increase in cutaneous fibrosarcomas in both sexes. The NOELs determined from the chronic studies evaluated are summarized (along with the subchronic studies) in Table 7. They were based primarily on decreased water consumption and body weight in rats (LOEL = 2.075 mg/kg/day) and mice (LOEL = 9.82 mg/kg/day), and decreased food consumption and body weight along with poor general condition in dogs (LOEL = 2 mg/kg/day). Some blood and liver effects were seen in mice and dogs at higher doses.

A. Inhalation Studies

No chronic toxicity or oncogenicity studies utilizing the inhalation route of exposure have been published in the open literature or submitted to the Department of Pesticide Regulation for evaluation.

B. Oral Studies

Rats. In a 2-year combined chronic toxicity/oncogenicity study, groups of 60 CD rats/sex/group received MITC (95.36-96.06%) in the drinking water at nominal concentrations of 0, 2, 10, or 50 ppm (Uncorrected TWA [range], males: 0, 0.095 [0.066-0.312], 0.463 [0.306-1.366] and 2.075 [1.413-6.010] mg/kg/day; females: 0, 0.140 [0.092-0.312], 0.692 [0.463-1.375] and 3.189 [2.196-6.478] mg/kg/day) (Brown, 1981). These values do not take into account MITC lability in the water bottles. On the day of preparation, MITC concentrations ranged between 72 and 89% of nominal. Three days later the range was 58-80%.). Males at 50 ppm (2.075 mg/kg/day) had a 9-12% decrease in water consumption and body weight. The investigators considered the decreased body weight to be secondary to the decrease in water consumption which, they believed, was probably due to a loss of palatability following the addition of MITC.

Histopatholgy revealed an increased incidence of female survivors with multiple fibroadenomas in all treatment groups compared to controls (Table 8). A marginal level of statistical significance (p=0.054, Fisher exact test) was attained at the high dose, which exhibited a 2-fold higher incidence than controls. The incidence of terminal survivors with single fibroadenomas showed no indication of a MITC-dependent effect, nor was an effect discerned in decedent animals (animals dying before 104 weeks) bearing either multiple or single fibroadenomas. (While the validity of data on decedents may be questioned due to possible problems with tissue preservation, examination of the survival curves [Figure 2] shows that virtually all of the deaths occurred during the 2nd year of the study, mostly after week 70. This was likely sufficient time for fibroadenomas to become visible as subcutaneous masses. In addition,

there was no effect of MITC on the death rate.) Fibroadenoma incidence was extremely high in all groups of terminal survivors, including controls, with a combined rate exceeding the upper end of the historical control range for this strain established in contemporary data from IRDC (% incidence, mean [range] = 33.5 [3.3-47.0], 16 two-year studies, 1975-1979; data from Hazleton Laboratories, which conducted the present study, were not available in the report). Even so, fibroadenoma incidence may have been underestimated because tumors not appearing as visible subcutaneous masses were likely not to have been counted. Mammary carcinomas were detected only in dosed females (not in controls), though dose responsiveness was not noted. Carcinoma incidence did not exceed the boundaries of the contemporary historical control range at IRDC (% incidence, mean [range] = 9.5 [1.5-21.4]). Statistically significant trends were not detected either for carcinomas or fibroadenomas. Totaling the fibroadenomas and carcinomas from these various groups did not clarify the question of whether or not MITC exposure was implicated in their formation. No histopathologic changes within the mammary gland could be correlated with any effect of exposure. Nonetheless, it remains possible that MITC played a role in mammary tumor induction. Finally, incidence of males with subcutaneous fibromas appeared to increase at the high dose. Among all males, the incidence rate at ascending doses was 3/60, 3/60, 2/60 and 8/60. Among terminal survivors, the male incidence rate was 2/37, 1/33, 2/30 and 5/38. Statistical significance compared to controls was not achieved at any dose.

Based on the decreased water consumption and body weight in males at 50 ppm (2.075 mg/kg/day), the NOEL was 10 ppm (0.463 mg/kg/day). The study was acceptable under FIFRA.

Figure 2. Female survival curves for the rat 104-week drinking water study
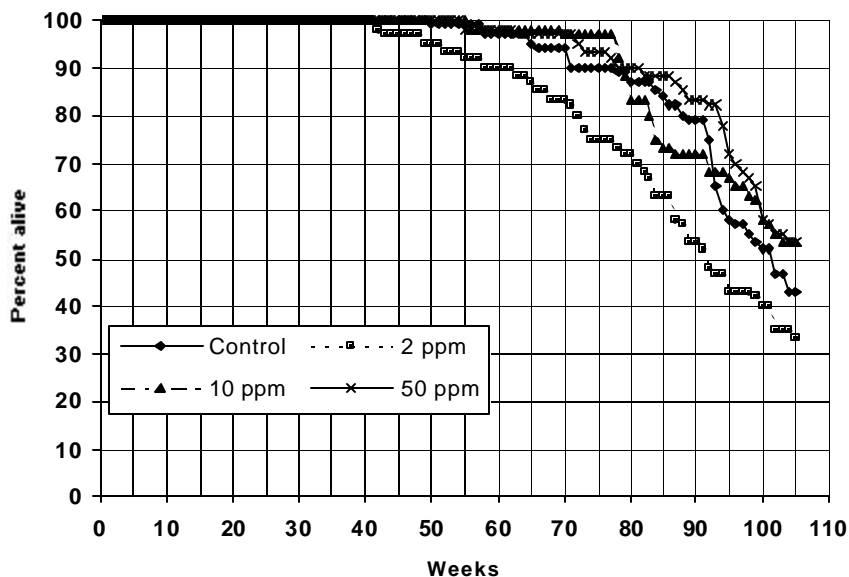
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<u>Table 8. Mammary tumor incidence as a function of MITC concentration in</u>
<u>drinking water - 104-week study in CD rats</u>

	0	**2**	**10**	**50**
		MITC (ppm)		
<u>**Terminal survivors**</u>				
multiple fibroadenoma[1]	6/25	8/20	14/32	15/31*
	24%	40%	44%	48%
single fibroadenoma	8/25	6/20	9/32	8/31
	32%	30%	28%	26%
multiple + single	14/25	14/20	23/32	23/31
fibroadenoma	56%	70%	72%	74%
carcinoma	0/25	1/20	2/32	0/31
	0%	5%	6%	0%
<u>**Decedents**</u>				
multiple fibroadenoma[1]	5/35	8/40	8/28	5/29
	14%	20%	29%	17%
single fibroadenoma	10/35	8/40	9/28	8/29
	29%	20%	32%	28%
multiple + single	15/35	16/40	17/28	13/29
fibroadenoma	43%	40%	61%	45%
carcinoma	0/35	4/40	3/28	2/29
	0%	10%	11%	7%
total fibroadenoma	29/60	30/60	40/60*	36/60
	48%	50%	67%	60%
total carcinoma	0/60	5/60*	5/60*	2/60
	0%	8%	8%	3%
total animals	30/60	36/60	44/60**	36/60
bearing tumors	50%	60%	73%	60%

* $p \leq 0.05$, Fisher exact test. For high dose incidence of multiple fibroadenomas in terminal survivors, $p = 0.054$. If the terminal survivor dose groups are combined, such that the incidence of animals with multiple fibroadenomas in controls was 6/25 *vs.* 37/83 in dosed animals, a Fisher exact test yields a p-value of 0.052. Similarly, for total carcinomas (survivors + decedents), control incidence was 0/60 *vs.* 12/180 in dosed animals, with a resultant Fisher p-value of 0.029

** $p \leq 0.01$, Fisher exact test.

[1]Fibroadenomas were detected mostly as comprising visible subcutaneous masses. Because quantitative sectioning was not done, *in situ* fibroadenomas were largely undetected. Multiple fibroadenomas were counted either as animals registering more than one mammary fibroadenoma or as animals registering one mammary fibroadenoma plus one other mammary tumor type (fibroma, adenoma, carcinoma).

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Mice. In a 2-year oncogenicity study, 70 ICI-JCR mice/sex/group received MITC (93.14%) in the drinking water at nominal concentrations of 0, 5, 20, 80, or 200 ppm (males~ 0, 0.68, 2.74, 9.82, or 21.34 mg/kg/day; females~ 0, 0.76, 3.04,10.81, or 24.09 mg/kg/day, based on drinking water analysis and water consumption) (Sato, 1980). Interim sacrifices, 6/sex/dose, were done at 26 and 52 weeks. Virtually all of the deaths occurred during the 2nd year of the study. Male deaths commenced at around week 75, female deaths around week 55. Survival at 106 weeks was, at increasing doses, 40%, 57%, 50%, 52% and 37% in males, and 36%, 34%, 36%, 34% and 36% in females, with no apparent effect of dosing. Indications of toxicity, as evidenced by dull coat, raised hair, and decreased body weight (92-95% of control), were present in 80 and 200 ppm males and 200 ppm females. Water consumption was decreased (67-87% of control) in both sexes at 80 and 200 ppm. At the 26-week sacrifice, blood platelets were increased in number (131% of control) in 200 ppm females, and total serum protein was decreased (90-92% of control) at 80 and 200 ppm for both sexes. At the 52-week sacrifice, males at 80 and 200 ppm had decreased hematocrit and RBC counts (79-87% of control), along with an inverse ratio of lymphocytes and neutrophils. Urine potassium was increased (148-162% of control) in 80 ppm males and 80 and 200 ppm females. At the 106-week terminal sacrifice, SGOT (serum glutamic-oxaloacetic transaminase) was increased (125% of control) in 200 ppm females. Histologically, various non-neoplastic lesions had increased incidences in MITC treated groups. In females, the lesions included small round cell infiltration of the kidney at 80 and 200 ppm, and cellular infiltration of the spleen at 200 ppm, in mice that died prior to the terminal sacrifice. Ovarian cysts were increased in 200 ppm females at terminal sacrifice, with incidence rates at ascending doses of 2/21, 4/20, 3/21, 5/19 and 10/21** (**p<0.01). In males, the incidence of amyloid degeneration of the kidney was increased in all dose groups at terminal sacrifice, with incidence rates at ascending doses of 4/23, 10/33, 14/29*, 11/30 and 12/21** (*p<0.05; **p<0.01). Cutaneous fibrosarcomas were elevated in both sexes at the high dose. Fibrosarcoma incidence rates in animals that survived into the 2nd year (also excluding interim sacrifices) were 0/58, 0/58, 0/58, 0/58 and 2/57 in males, and 0/58, 0/58, 0/58, 0/57 and 3/58 in females. These tumors were detected only in animals that perished, though in all 5 cases the deaths occurred after week 89. It was not reported whether or not the fibrosarcomas were the apparent cause of death. Statistical significance by Fisher exact test was not attained when the sexes were considered separately. However, significance was attained when the data for both sexes was combined, yielding an incidence rate of 0/116, 0/116, 0/116, 0/115 and 5/115* (*p<0.05). MITC exposure is considered the likely cause of this increase. There was no other evidence for oncogenicity in this study. Information on historical control incidences of ovarian cysts, amyloid degeneration or cutaneous fibrosarcomas was not available. Based on decreased body weight and water intake, and clinical observations of raised hair coat, the NOEL was 2.74 mg/kg/day (20 ppm).The study was considered acceptable under FIFRA guidelines.

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Dogs. The 1-year dog oral toxicity study (Harling *et al.*, 1989) was only made available to DPR for a short period in 1991 and subsequently withdrawn by the registrant. Consequently, only a summary written by a DPR toxicologist, not the complete report (including the individual animal data), were available in-house for review purposes as the current document was being prepared. MITC (95.64%) was administered by gavage to 6 Beagle dogs/sex/dose at 0 (corn oil vehicle), 0.04, 0.4, or 2.0 mg/kg/day. The required volumes were given as two daily doses, with 4-5 hours between subdoses. For the first 15 weeks of the study the volume administered was 5 ml/kg followed by a flush with 20 ml of tap water. The vehicle volume was reduced to 0.25 ml/kg, probably because the initial high volume had led to inappetance in at least some dogs. Vomiting, excessive salivation, and liquid feces were reported in both sexes at 2.0 mg MITC/kg/day, with at least an appearance of dose relatedness. The first two of these signs may be related to irritation caused by MITC as it is broken down in the acidic environment of the stomach. Alternatively, it could have been due to the toxic effects of thiocyanate, which include vomiting and excessive salivation. The signs increased in severity between weeks 15-52, *after* the vehicle volume had been reduced, as compared to the initial part of the study. At the 0.4 mg/kg/day level, 2 females were removed from MITC treatment during week 14 due to loss of body weight, poor food consumption and poor general condition. They were restarted on treatment at week 17 and continued though the end of the study without obvious need to again discontinue the treatment. As this degree of disability was not observed at the high dose, it seems likely that the effect on these dogs was related to the initial administration of vehicle at high volumes. By week 26, there was a statistically significant decrease in hematocrit, hemoglobin, and RBC numbers, as well as increased platelet numbers and activated partial thromboplastin time in the 2.0 mg/kg/day males. Male liver weights were also increased at 2.0 mg/kg/day. Based on decreased body weight gain, poor food consumption, loose stool, increased liver weights, decreased serum protein/albumin and hematologic effects, an apparent NOEL was established at 0.4 mg/kg/day. The study was not acceptable to DPR under FIFRA testing guidelines due to the removal of some dogs from treatment during the study.

VII.   GENOTOXICITY

Summary. Genotoxicity studies were evaluated under Toxic Substances Control Act (TSCA) guidelines promulgated in 1983. There was no evidence for mutagenicity in a mammalian cell assay, and no acceptable microbial cell assays were submitted. Cytogenetic investigations of possible chromosome effects indicated no evidence of chromosomal aberrations in human lymphocytes and a weakly positive response in Chinese hamster V79 cells. Tests for DNA damage, and sister chromatid exchange were negative. MITC is not considered to be genotoxic.

A.   Gene Mutation
The results from gene mutation assays using two different microorganisms (*Salmonella*

typhimurium, *Escherichia coli*) were negative for MITC mutagenicity (Glass et al., 1976; Moriya et al.,1983; Nor-Am Agricultural Products, 1982; Shirasu et al., 1978; and Shirasu et al., 1981). However, none of the studies were acceptable to DPR due to various deviations from TSCA guidelines.

In a mammalian cell assay (Miltenburger, 1985a), Chinese hamster V79 cells exposed to 0, 0.1, 0.25, 0.5, or 1.0 µg/ml (without S9 activation) or 0, 0.25, 0.5, 1.0, or 2.5 µg/ml (with S9 activation) MITC (purity unstated) for 4 hours, showed no increase in mutation frequency in two trials. The study was acceptable to DPR under TSCA.

B.   Chromosomal Aberrations

In an *in vitro* chromosome aberration test (Miltenburger, 1985b), Chinese hamster V79 cells were exposed to MITC (purity unstated) for 4 hours at 0, 0.25, 0.75, or 2.5 µg/ml with S-9 activation, and 0, 0.1, 0.5 or 1.0 µg/ml without S-9. Cells were harvested at 6, 12, and 28 hours after treatment (the highest concentration was harvested only at 6 and 28 hours). MITC was weakly positive for aberrations at 12 and 28 hours (primarily breaks and some exchanges). The study was not acceptable to DPR under TSCA due to unstated purity of test article, no concurrent cytotoxicity information, and no definitions of aberrations as scored.

In a chromosome aberration assay (Heidemann, 1988a), phytohemagglutinin stimulated human peripheral lymphocytes were exposed, after 48 hours culture, to MITC (95.6%) at 3.0 or 5.0 µg/ml for 4 hours. Cells were harvested at 24 and 48 hours after treatment. There were no significant increase in chromosomal aberrations at any dose or time point. The study was acceptable to DPR under TSCA.

In a mouse micronucleus test (Nihon Schering K.K. and Shionogi & Co., 1990), MITC (purity unstated) was administered to CD-1 mice by gavage at a single dose of 110 mg/kg. The dose of 110 mg/kg was stated by the investigators to be expected to be lethal to approximately 10% of the animals within 72 hours after dosing. Bone marrow smears were obtained 24, 48, and 72 hours after dosing. It was reported that no significant increase in the frequency of micronucleated polychromatic erythrocytes was seen at any of the sampling times. At 24 hours, the ratio of polychromatic erythrocytes to normochromatic erythrocytes was comparable with controls. At 48 and 72 hours the ratio was significantly lower. The lowered ratios were considered to be indicative of cytotoxicity to the bone marrow cells. Individual study data were not submitted to DPR; therefore, only summary information were available for evaluation.

C.   Other Genotoxic Effects

In a sister chromatid exchange assay (Heidemann, 1988b), Chinese hamster V79 cells were exposed to MITC (95.6%) for 4 hours at 0, 0.1, 2.5, or 5.0 µg/ml with S-9 activation, or 0, 0.1, 2.0, or

3.5 µg/ml without S-9 activation. The cells were then incubated for an additional 24 hours with BrdU (5-bromo-2'-deoxyuridine) No increase in sister chromatid exchange was observed. The study was acceptable to DPR under TSCA.

In a rat primary hepatocyte unscheduled DNA synthesis assay (Cifone, 1985), rat hepatocytes were exposed to technical MITC (purity unstated) for 18 hours at 0, 0.253, 0.505, 1.01, 2.53,5.05, 10.1, 15.2, or 30.3 µg/ml with H-TdR. No significant increase in net grains/nucleus was reported. The study was acceptable to DPR under TSCA.

Three *Bacillus subtilis* assays (Shirasu, 1978; Nor-Am Agricultural Products, 1982; and Nihon Schering K.K. and Shionogi & Co., 1990) were not evaluated due to technical difficulties during the assays and/or insufficient submission of data.

VIII.   REPRODUCTIVE TOXICITY

Summary.   A 2-generation drinking-water study, and a 3-generation oral gavage study were conducted in rats. While a decrease in pre-weaning viability was noted in the F_1 pups at all doses (2-generation study), the lack of dose-response and of statistical significance makes it unlikely that MITC exposure was responsible. Dams exhibited decreased water consumption and occasional body weight decrements at 10 and 50 ppm (~0.9 and 4.5 mg/kg/day). No clear effects on pups were noted in either study. Neither study was considered acceptable by FIFRA standards.

A.   Inhalation Studies

No reproductive toxicity studies utilizing the inhalation route of exposure have been published in the open literature or submitted to the Department of Pesticide Regulation for evaluation.

B.   Oral Studies

Rats. In a two-generation reproductive toxicity study, MITC (95.86-96.51%) was administered in the drinking water to 25-30 Sprague-Dawley rats/sex/group at concentrations of 0, 2, 10, or 50 ppm (males: controls and calculated equivalent doses of 0.16, 0.7, or 3.49 mg/kg/day; females: controls and calculated equivalent doses of 0.2, 0.94, or 4.49 mg/kg/day), for two generations, one litter per generation (Barker, 1987; and Nihon Schering K.K. and Shionogi & Co., 1990). Pre-weaning viability was decreased in F_1 pups at each of the treatment levels (pre-weaning loss at ascending doses was 6.6%, 17.8%, 17.1% and 14.4%). However, for the following reasons, this was not considered to be due to MITC exposure: 1. a clear dose-relationship was lacking, 2. statistical significance (p≤0.05) was not achieved, 3. pup weights indicated that they were growing normally, 4. pup deaths did not occur within a discrete window, but appeared to occur randomly, and 5. the pattern of pre-weaning loss

was not repeated among the F_2 pups (pre-weaning loss, F_2 pups: 8.3%, 9.3%, 16.7% and 11.8%). Unfortunately, historical control data, which may have permitted a clearer evaluation of whether or not these incidence rates were within the control range, were not available in this study. At 10 and 50 ppm, parental water consumption was significantly decreased in both generations, and decreased body weight gains were also reported during various time periods of the study. Based on decreased water consumption at 10 ppm (~0.94 mg/kg/day), the parental NOEL was set at 2 ppm (~0.16 mg/kg/day). The study was not acceptable to DPR under FIFRA guidelines, due to inadequate histological examination of adult animals.

In a 3-generation reproductive toxicity study, albino CD rats received MITC (purity unstated) at 0, 1, 3, or 10 mg/kg/day (Nor-Am Agricultural Products, Inc., 1982). Body weights of F_0 males at 3 and 10 mg/kg/day were less than controls. Also, females given 3 mg/kg/day weaned fewer F_{3a} progeny than controls. It was stated that no substance-related reproductive effects were detected in the study. The only treatment-related histological findings in adult animals were undefined lesions of the non-glandular stomach. Individual study data were not submitted to DPR; therefore, only summary information was available for evaluation.

IX.   DEVELOPMENTAL TOXICITY

Summary.   The developmental toxicity of MITC was studied in rats and rabbits. Maternal toxicity was expressed as decrements in body weight and food consumption at as low as 5 mg/kg in both species. Thickened maternal stomach lining was evident at 25 mg/kg in rats. Decreases in fetal size and weight were noted in rats at 25 mg/kg and in rabbits at 5 mg/kg, with a separate study showing embryo toxicity and reduction in 24-hour survival noted at 10 mg/kg. MITC was not a unique developmental toxicant as doses which affected fetal parameters also produced maternal toxicity.

A.   Inhalation Studies

No developmental toxicity studies utilizing the inhalation route of exposure have been published in the open literature or submitted to the Department of Pesticide Regulation for evaluation.

B.   Oral Studies

Rats.   MITC (95%) was administered by oral gavage to 24-28 Sprague-Dawley female rats/dose at 0 (corn oil vehicle), 1, 5, or 25 mg/kg/day on days 6-15 of gestation (Irvine, 1983; Nihon Schering K.K. and Shionogi & Co., 1990). At 25 mg/kg/day, there was a decrease in mean fetal body weight (91% of control) and mean size (96% of control). Maternal toxicity at 25 mg/kg/day was evident in the significant decrease in food consumption (79% of control) and body weight gain (67% of control) during the treatment period, and thickening of the stomach lining at necropsy. At 5 mg/kg/day, maternal toxicity was limited to decreased body weight gain (93% of control). Based on decreased body weight

gain, the maternal NOEL was 1 mg/kg/day. Based on decreased fetal body weight and size, the developmental NOEL was 5 mg/kg/day. The study was acceptable to DPR based on FIFRA guidelines.

Rabbits. MITC (96%) was administered via oral gavage to 16 New Zealand White female rabbits/dose at 0 (corn oil control), 1, 3, or 5 mg/kg/day on days 7-19 of gestation (Irvine, 1984; Nihon Schering K.K. and Shionogi & Co., 1990). Reductions in mean fetal weight (88% of control) and mean crown-rump length (96% of control) were observed in fetuses at 5 mg/kg/day. The finding was considered to most likely be secondary to larger litter sizes present in that dose group (Hafez, 1968 and 1970), since associated evidence suggestive of developmental toxicity was lacking. Maternal toxicity, evidenced by a marginal decrease in food consumption and weight gain during the early stages of treatment, was also present at 5 mg/kg/day. Based on the listed changes, the maternal and development NOELs were established at 3 mg/kg/day. This study was acceptable to DPR based on FIFRA guidelines.

MITC (purity unstated) was administered via gelatin capsules to female albino rabbits at 1, 3, or 10 mg/kg/day on days 6-18 of gestation (Nor-Am Agricultural Products, Inc., 1982). Maternal toxicity, embryo toxicity, reduced fetal body weights, and a reduction in fetal 24-hour survival were noted at 10 mg/kg/day. Possible maternal toxicity was reported at 3 mg/kg/day. Examination for fetal external and skeletal development did not reveal any MITC-related effects. However, it was stated that prenatal exposure to MITC appeared to have increased the incidence of incidental skeletal findings. Based on the summary information available, the maternal NOEL was 1 mg/kg/day, and the developmental NOEL was apparently 3 mg/kg/day. Individual study data were not submitted to DPR; therefore, only summary information were available for evaluation.

X. NEUROTOXICITY

FIFRA guideline studies are not required for MITC because it is not an organophosphate. Plasma cholinesterase inhibition was reportedly present in the 31-day dermal toxicity study at as low as 1 mg/kg/day in female Wistar rats and as low as 100 mg/kg/day in males (Nor-Am Agricultural Products, Inc., 1982).

There was, however, one study that related specifically to effects on the central nervous system. Mice or rabbits (strain unstated) were administered 10, 30, or 100 mg/kg MITC (purity unstated) by oral gavage (Nihon Schering K.K. and Shionogi & Co., 1990). Mice were observed following an "Irwin procedure" and rabbits were observed for clinical signs. In mice, marked excitation was reported at the lethal dose of 100 mg/kg, and slight excitation was present at 30 mg/kg. In rabbits no excitation was reported, but marked intoxication characterized by hypopnea and flaccid muscles was

observed at 100 mg/kg prior to death. The symptoms were mild at 30 mg/kg, and absent at 10 mg/kg. At necropsy, congestion and hemorrhage were found in the stomach and intestines of dead mice and rabbits. Individual study data were not submitted to DPR; therefore, only summary information were available for evaluation.

XI.   SPECIAL TOXICITY OR PHARMACOLOGY STUDIES

Various non FIFRA-guideline studies were reported in the published literature for MITC.

Effects on the Immune System. In a published study of the immunotoxicity of MITC, groups of five 8-10 week old female B6C3F1 mice/dose were given 0, 15, 30, or 45 mg/kg/day MITC (purity unstated) in water for 5 days by oral gavage (Kiel et al., 1996). Hematological determinations were evaluated on the day after the last dose. Thymus and spleen were removed for cellular and flow cytometric analysis of control and 45 mg/kg/day animals. Body weight decreased by less than 10% in all MITC-treated groups. Significant changes in thymus weight (~50% of control) and thymus cell subpopulations were reported in mice given 45 mg/kg/day. There were no significant changes in spleen weight or natural killer (NK) activity. Total WBC numbers were increased (~175% of control) at 30 mg/kg/day. The percentage of blood neutrophils was increased (~200% of control), and the percentage of blood lymphocytes was decreased (~80-90% of control) at 15 and 45 mg/kg/day. Flow cytometric analysis of thymus cells indicated a decrease (~90% of control) of $CD4^+CD8^+$ thymocytes, and an increase (~140% of control) in the percentage (but not absolute number) of $CD4^-CD8^-$ thymocytes. Individual study data were not submitted to DPR; therefore, only summary information were available for evaluation.

Effects on the Cardiovascular System. One hundred mg/kg of MITC (purity unstated) was administered orally to anesthetized cats, and respiration, blood pressure, heart rate, and electrocardiograms (ECG) were monitored (Nihon Schering K.K. and Shionogi & Co. Ltd., 1990). The oral administration of 100 mg/kg MITC increased blood pressure immediately after administration, and then blood pressure gradually decreased in direct relation to a decrease in pulse pressure. Heart rate increased from 60 minutes to 90 minutes after (Nihon Schering K.K. and Shionogi & Co. Ltd., 1990). The oral administration of 100 mg/kg MITC increased blood pressure immediately after administration, and then blood pressure gradually decreased in direct relation to a decrease in pulse pressure. Heart rate increased from 60 minutes to 90 minutes after administration of MITC, and ECG showed a decrease in the voltage of QRS complex 10-15 minutes after administration. There were no marked changes in the voltage of P and T waves, but a slight changes in PR and QT intervals were observed in proportion to the changes in heart rate. Respiration did not show any marked changes until 90 minutes after administration, when it suddenly became slow and stopped by 120 minutes. Cardiac arrest

followed. Necropsy did not show any "serious" toxic effects of MITC on the gastro-intestinal tract. The investigators considered the possibility that direct cardiac toxicity was the lethal factor in MITC poisoning. However, the cardio-toxic effects were seen only at lethal or near-lethal doses, so it was stated that cardiac toxicity may not have been a specific compound-related effect. Individual study data were not submitted to DPR; therefore, only summary information were available for evaluation.

Noradrenaline, which enhances cardiac functions, glutathione, which is implicated in the metabolism of MITC, and sodium thiosulfate a cyanide antidote, were also used to investigate the mechanisms of any detoxifying action on MITC toxicity. The drugs were administered continuously following oral treatment with MITC, in order to investigate any direct MITC effects on cardiac function. Noradrenaline slightly delayed the time when respiration stopped, but did not correct the abnormal changes in blood pressure and ECG caused by MITC. The toxicity of MITC was not affected by glutathione or sodium thiosulfate. Individual study data were not submitted to DPR; therefore, only summary information were available for evaluation.

Effect On Blood Coagulation. Blood samples were collected from the abdominal aorta of anesthetized rats 1 hour after the oral administration of 10, 30, or 100 mg/kg MITC (purity unstated). Prothrombin time (PT) and activated partial thromboplastin time (APPT) were determined using the plasma samples (Nihon Schering K.K. and Shionogi & Co. Ltd., 1990). No effects of MITC was observed on PT or APPT at any dose level. No abnormality was seen in the color of venous blood. Individual study data were not submitted to DPR; therefore, only summary information were available for evaluation.

Effect On Hemolysis. Blood samples were collected from the auricular vein of rabbits and a suspension of erythrocytes was prepared (Nihon Schering K.K. and Shionogi & Co. Ltd., 1990). Hemolysis was checked by addition of the suspension to MITC solutions. No hemolysis was observed at MITC concentrations of 0.0076% or 0.076%. Slight hemolysis was observed at 0.76%. Individual study data were not submitted to DPR; therefore, only summary information were available for evaluation.

Effects on the Central Nervous System. Mice or rabbits (strain unstated) were administered 10, 30, or 100 mg/kg MITC (purity unstated) by oral gavage (Nihon Schering K.K. and Shionogi & Co. Ltd., 1990). Mice were observed following an "Irwin procedure" and rabbits were observed for clinical signs. In mice, marked excitation was reported at the lethal dose of 100 mg/kg, and slight excitation was present at 30 mg/kg. In rabbits no excitation was reported, but marked intoxication characterized by hypopnea and flaccid muscles was observed at 100 mg/kg prior to death. The symptoms were mild at 30 mg/kg, and absent at 10 mg/kg. At necropsy, congestion and hemorrhage were found in the stomach

and intestines of dead mice and rabbits. Individual study data were not submitted to DPR; therefore, only summary information were available for evaluation.

XII. TOXICITY OF THE PARENT COMPOUND AND BREAKDOWN PRODUCTS / METABOLITES OF MITC

A. **Metam Sodium** (The complete draft DPR toxicologic risk characterization for metam sodium may be found in DPR [2002c]. This document is currently under review and thus has not yet been finalized.)

Exposure. Direct human exposure to metam sodium is expected only under occupational settings and only *via* the dermal route.

Acute toxicity. Acute oral exposure to high doses of metam sodium resulted in what appeared to be cholinergic signs, with LD_{50}s as low as 781 mg/kg. Exposure *via* the skin yielded LD_{50}s as low as 1050 mg/kg and clear local and systemic pathology and clinical signs. LC_{50} values as low as 2.20 mg/L were obtained in acute inhalation studies, again accompanied by local and systemic pathology and clinical signs. Metam sodium had mild eye irritating properties in some studies (corneal involvement or irritation for 1-7 days using 0.1 ml/eye), and was a dermal sensitizer.

The critical acute NOEL was 5 mg/kg/day, established in a Wistar rat developmental toxicity study. It was based on acute maternal and developmental effects at the LOEL dose of 20 mg/kg/day. The maternal effects included clinical signs (salivation, vaginal bleeding, and oral staining) and decrements in body weight gain and food consumption. The acute nature of these signs was inferred by their rapid development after the onset of dosing on gestation day 6. The developmental effects included suppression fetal body weights and numerous skeletal developmental delays. As these growth effects were considered to be functions of the acute maternal growth effects, they too were considered likely to be acute in nature.

The NOEL of 5 mg/kg/day was supported by the maternal acute NOEL 5 mg/kg/day established in a developmental toxicity study in New Zealand White rabbits. This was based on reductions in food consumption and body weight losses at 20 mg/kg/day recorded within the first 3 dosing days. (The developmental NOEL in that study was also 5 mg/kg/day, based on an increase in skeletal variants at 20 mg/kg/day. However, it was not clear that this effect could be considered acute.)

Developmental toxicity. Six developmental toxicity studies, 4 in rats and 2 in rabbits, were examined for the metam sodium risk characterization document. All utilized the oral route of exposure. In both rabbit studies, metam sodium induced early resorptions. In Himalayan rabbits, resorptions were induced at the sub-maternally toxic dose of 30 mg/kg/day. Fetal malformations (meningocele + spina bifida) occurred at the slightly maternally toxic dose of 100 mg/kg/day. In New Zealand White rabbits, resorption incidence was overwhelming at the high dose of 60 mg/kg/day, which saw only slight maternal toxicity. In the Wistar rat, malformations (meningocele, microphthalmia, anophthalmia, skull

malformation, hydrocephaly and abnormal zygomatic arch) were noted at the maternally toxic dose of 60 mg/kg/day.

Dermal irritation. The risk of occupational dermal irritation was assessed because primary dermal irritation studies in animals as well as incident reports in the California Pesticide Illness Surveillance Program indicated that metam sodium is corrosive and may have caused irritation in the field. A rabbit 21-day dermal toxicity study produced a local irritation NOEL of 31.25 mg/kg/day, based on a finding of erythema, edema and dermatitis in a majority of animals at 62.5 mg/kg/day. Conversion to units of local concentration produced critical NOEL and LOEL values of 360 and 720 Fg/cm²/day, respectively.

Subchronic toxicity. The critical subchronic NOEL of 0.3 mg/kg/day was established in a dog 90-day oral gavage study. It was estimated from the low dose LOEL of 1 mg/kg/day, using a default uncertainty factor of 3. The LOEL was based on an observation of bile duct proliferation / inflammatory cell infiltration and elevated plasma alanine aminotransferase activity in 1/4 females at that dose. These signs were considered progenitors of the severe hepatitis (hepatocyte degeneration and necrosis, inflammation, and increased pigmentation, collapse of hepatic cords with an influx of blood, and biliary proliferation) present in all animals at the high dose of 10 mg/kg/day, and the similar, if less severe, hepatitis present at the mid dose of 5 mg/kg/day. The primary effects observed at necropsy were also related to the liver. These included the accentuated lobular pattern, pale coloration, and red depressed areas indicative of collapsed hepatic cords with an influx of blood at 10 mg/kg. The latter sign was also noted at 5 mg/kg. Designation of 0.3 mg/kg/day and 1 mg/kg/day as the critical NOEL and LOEL, respectively, was directly supported by similar findings in a 1-year dog oral gavage study conducted by the same laboratory (see below). It seems clear, therefore, that occasional beagle dogs exhibit great sensitivity in hepatic responsiveness to relatively low doses of metam sodium.

A 90-day mouse drinking water study established a NOEL of 0.79 mg/kg/day. This was calculated from a nominal concentration of 0.018 mg/ml using measurements of the maximum metam degradation rate in water bottles. The NOEL determination was based on a reduction in hemoglobin, hematocrit, and red blood cell numbers (an anemic effect), increased liver weights, and eosinophilic granules in transitional epithelial cells of the urinary bladder, all at the mid-low dose of 4.48 mg/kg/day. The effects on liver weight may have been related to necropsy findings of livers with pale or accentuated lobular patterns noted at the top two doses (36.05 and 60.36 mg/kg/day). The appearance of eosinophilic granules in the urinary bladder was predictive of clearer bladder toxicity (cystitis and mucosal hyperplasia) at the top two doses. The hematologic evidence for anemia at the top 3 doses may have underestimated the actual effect because the suppression of water consumption noted at those doses, which probably was due to decreased palatability, may have resulted in dehydration.

A 90-day rat inhalation toxicity study established a NOEL of 1.11 mg/kg/day (6.5 mg/m³) based on apparent liver effects at 7.71 mg/kg/day (45 mg/m³) . Irritation of the nasal passages, stomach, and

lungs was apparent at the high dose of 27.43 mg/kg/day (160 mg/m^3), with some nasal irritation evident at the mid dose (45 mg/m^3). Mean air concentrations of MITC, which due to its corrosive properties could be implicated in some of the irritative effects, were also determined. These were 0, 0.7, 2.2 or 5.7 mg/m^3 (0, 0.23, 0.74, and 1.90 ppm).

Chronic toxicity. A critical NOEL of 0.1 mg/kg/day for chronic toxicity was obtained in a 1-year dog study in which 4 beagles/sex/dose were administered 0, 0.05, 0.1 or 1 mg/kg/day by gelatin capsules. Mean plasma alanine aminotransferase activities were elevated in high dose females. This was due to an ~800% rise in one individual recorded in weeks 26, 32, 39, 45, and 52. The same individual exhibited signs of hepatotoxicity (a slight increase in hepatocyte and macrophage/Kupffer cell pigmentation, slight mononuclear cell infiltration, slight telangiectasis) that, in view of similar results in the dog 90-day study, was probably test article-related. Other observations, including an increase in mean alkaline phosphatase activity (up to 47%, both sexes, mostly high dose) and a reduction in mean plasma triglycerides (up to 34%, females, high dose) may also indicate liver dysfunction. An increase in kaolin-cephalin time (also known as activated partial thromboplastin time) of up to 15% in both sexes at the high dose indicates some interference with blood clotting.

Genotoxicity. Metam sodium is a clastogen both *in vivo* (hamsters) and *in vitro* (human lymphocytes). Other assays for genotoxicity, including gene mutation, DNA damage and micronucleus induction, were either negative or equivocal.

Oncogenic effects. Incidence of angiosarcoma, a malignant vascular tumor, following exposure of male mice to metam sodium in the drinking water for 2 years was 7/53, 12/53, 12/55, and 27/53 at internal doses of 0, 1.9, 7.2, and 28.9 mg/kg/day, respectively. Incidence at the high dose was highly significant compared to controls (p<0.001, Fisher Exact Test). The incidence curve was also positive for trend (p<0.001, Cochran-Armitage trend test) Incidence in females was 4/55, 2/55, 6/46, and 10/52 at internal doses of 0, 2,6, 9.6, and 31.2 mg/kg/day, respectively. While Fisher Exact tests in females were not significant at any dose (p>0.05), the incidence curve was significant in a Cochran-Armitage trend test (p<0.01).

Angiosarcoma was the major contributor to death among male mice that had these tumors. However, there was no statistically significant evidence it decreased the overall survival time or that death from angiosarcoma among high dose animals occurred sooner than death from angiosarcoma among control animals. For these reasons, the oncogenic potency was calculated using the linearized multistage model of tumor development, GLOBAL 86, instead of the Weibul time-to-tumor method. Extrapolation of the mouse doses to humans was done by multiplying the doses by the relative body weights using an interspecies scaling factor: $(BWt_A / BWt_H)^{0.25} = (0.03 \text{ kg} / 70 \text{ kg})^{0.25} = 0.144$. The estimated oncogenic potency using the incidence rate for all "at risk" male mice (that is, all mice surviving for at least 1 year of exposure) ranged from a $Q_1 = 8.56 \times 10^{-2}$ (mg/kg/day)$^{-1}$ (the maximum likelihood estimate or MLE) to a $Q_1^* = 1.85 \times 10^{-1}$ (mg/kg/day)$^{-1}$ (the 95% upper bound or 95% UB).

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As a vascular tissue disease, it is not unexpected that angiosarcomas were found in several different mouse organs including liver (where they may have been responsible for the increase in palpable masses), subcutaneous tissues and, especially, spleen. Interestingly, non-neoplastic histopathology included findings of increased hepatocytic fat vacuolization and splenic hemosiderosis at the high dose.

Support for a role for metam sodium in the induction of angiosarcomas in male mice came from a 2-year drinking water study in rats. Incidence of hemangiosarcoma, a subcategory of angiosarcoma, was 0/50, 3/50, 8/51, and 3/51 at internal doses of 0, 1.5, 4.3, and 12.5 mg/kg/day, respectively. Incidence at the mid dose was statistically significant compared to controls ($p<0.01$, Fisher Exact test), though neither the low or high dose incidences achieved statistical significance. These data were considered ambiguous with regard to a role for metam sodium in the induction of hemangiosarcoma in rats. However, in recognition of the similarity of this tumor to that induced in mice, along with the observation that incidence rates were higher at all doses compared to controls, the rat data were considered to enhance the level of concern over metam sodium as a potential vascular oncogen.

Metam sodium risk calculations. As noted, based on current use scenarios, occupational dermal exposure was the sole projected exposure route for metam sodium. A single occupational dermal exposure study utilizing sodium tetrathiocarbonate as a surrogate was used to estimate acute and seasonal exposures (the weaknesses inherent in estimating human exposures by this method are explicitly detailed in DPR's draft risk characterization document on metam sodium [DPR, 2002c]). The acute exposure estimates resulted in acute systemic margins of exposure (MOEs) between 1724 and 3333. Using estimated local dermal exposure rates from the same surrogate acute dermal study, dermal irritation MOEs fell between 679 and 1333. Seasonal systemic MOEs fell between 909 and 2500. Chronic, non-oncogenic MOEs were not estimated because chronic exposure was not expected. Oncogenic risk values were calculated from Lifetime Average Daily Doses (LADDs), which in turn were estimated from the seasonal exposure data. The risk to workers for development of angiosarcoma fell between 1.71×10^{-6} and 5.14×10^{-6} when expressed as the maximum likelihood estimate and between 3.70×10^{-6} and 1.11×10^{-5} when expressed as the 95% upper bound estimate. These estimates were based on the assumption that metam sodium acted through a linearized, multistage, non-threshold-dependent mechanism. However, experimental support for this assumption was considered to be incomplete.

B. Methyl Isocyanate

Human exposure to methyl isocyanate (MIC) may occur following metam sodium applications due to photolysis of the metam sodium breakdown product MITC. Methyl isocyanate is not a registered pesticide in California. Therefore, detailed reports of human and animal experimental toxicity studies were not submitted to DPR for review. Information was obtained from toxicity reviews of MIC

supplied by the American Conference of Governmental Industrial Hygienists (ACGIH), the Hazardous Substance Data Bank (HSDB) of the National Library of Medicine, the University of California at Davis (UCD), and the US EPA, as well as from reports published in the open literature in the wake of the Bhopal MIC disaster of December, 1984, which killed 2500-5000 people and injured up to 200,000 (Mehta *et al.*, 1990).

Acute toxicity, including data from Bhopal. Methyl isocyanate is highly acutely toxic to humans and animals, causing tissue damage by reacting with sulfhydryl, carboxyl and hydroxyl groups (Bajaj *et al.*, 1993). The rate of MIC hydrolysis to methylamine and dimethylurea is thought to be much slower in moist air than in water (US EPA, 1986), making it likely in the case of an environmental release that human exposure will be to the parent compound. Symptoms following acute exposure to high air concentrations of MIC include pulmonary edema, dyspnea, respiratory failure, asthma, chest pain, skin and eye injuries, and death. The 6-hr LC_{50} is 6100 ppb (14 mg/m^3) in rats, 12,200 ppb (28 mg/m^3) in mice and 5400 ppb (12 mg/m^3) in guinea pigs (Dodd *et al*, 1986). There were no deaths in any species at 2400 ppb, indicating that the LC_{50} curve is steep. No direct evidence was found to indicate that MIC can cause pulmonary sensitization. However, a Mellon Institute report (1970) indicated that not only is MIC a strong dermal sensitizer in guinea pigs, but that inhalation exposure may elicit dermal sensitization upon subsequent dermal exposure (though the possibility remained that the inhalation protocol was not well-controlled for dermal exposure). Interestingly, conventional pulmonary sensitization in guinea pigs was not observed in the Mellon study. The report concluded that the data did not permit exclusion of MIC as a possible pulmonary sensitizer, particularly in view of the technical difficulties in measuring this response and the well-known ability of other isocyanates to induce pulmonary sensitization.

Three acute human studies were reviewed by the American Conference of Governmental Industrial Hygienists (ACGIH, 1986). The following is quoted from that source:

> In the first study, "acute experiments of one- to five- minute duration were performed on four human volunteers. At 0.4 ppm MIC the subjects could not perceive odor and experienced no irritation of the eye, nose, or throat. At 2 ppm no odor was detected, but the subjects experienced irritation and lachrymation. At 4 ppm the symptoms of irritation were more marked. Exposure was unbearable at 21 ppm. In another study, eight human volunteers in a ceramic lined chamber were exposed for one minute at 1.75 ppm MIC. None perceived an odor, all experienced eye irritation, seven had tearing, and three had nose and/or throat irritation. At the end of exposure all effects disappeared within 10 minutes, except that one woman reported of having something in her eye for 45 minutes. Six of the same persons were exposed for 10 minutes at 0.5 ppm. Eye irritation was evident earliest and was experienced by all. Tearing and nose / throat irritation were less evident. One person perceived an odor. In a third study, seven male volunteers were exposed to various concentrations of MIC, usually for one minute. Airborne concentrations studied were 0, 0.3, 1.0, 2.5, or 5.0 ppm. Only three of the seven subjects could detect 5 ppm MIC by its odor. There was no consistent relationship between odor detection and vapor concentration. All persons who

perceived an odor reported olfactory fatigue. The only unanimous responses consisted of eye irritation and tear formation at 5 ppm in 50 seconds or less. All responses disappeared within 3 minutes after exposure."

There is no consensus with respect to the concentration of airborne MIC attained at Bhopal, though levels between 13 (Dave, 1985) and 100 ppm (Varma, 1986) have been estimated. Nonetheless, much insight on the toxicity of the compound after acute exposure has been gained by studying the survivors, as well as in investigations on laboratory animals in which the experimental design was informed by the clinical data from Bhopal. Much of the following discussion of this subject is distilled from a comprehensive review by Mehta *et al.* (1990).

The main symptom resulting from the acute exposure at Bhopal, and the main cause of death, was pulmonary distress, expressed in many facets of lung function and pathological examination. Adverse impacts were also noted on the circulatory, gastrointestinal and central nervous systems. Studies on survivors suggested that the pulmonary effects were due to the corrosivity of MIC. Clinical signs reported within the first 3 days of the accident included breathlessness, cough, throat irritation / choking, chest pain and hemoptysis (expectoration of blood) (Misra *et al.*, 1987). Radiological signs included interstitial and alveolar edema and destructive lesions with cavitation, pneumomediastinum (presence of air in the mediastinum which may interfere with respiration and circulation) and emphysema (Sharma and Gaur, 1987). Lung function tests revealed chronic respiratory impairment indicative of restrictive lung disease with alveolitis (Patel *et al.*, 1987), as well as pulmonary hypertension in some victims. Biopsies performed 6-8 months post exposure revealed alveolar wall thickening, interstitial fibrosis and bronchiolar exudates (Kamat *et al.*, 1985). Fibrosing bronchiolitis obliterans appears to be a long term result of injury. Animal studies corroborate the field observations, with a 50% decrease in respiratory rate noted at 1.3 ppm in mice during a 90-minute exposure, suggesting that MIC is a potent respiratory irritant (Alarie *et al,* 1987*)*. Mice exposed to 30 ppm MIC for 2 hours exhibited extensive tracheal / bronchial necrosis followed by rapid epithelial regeneration, but with persistent bronchial fibrosis in some animals for at least the 3-month period of the experiment (Boorman *et al.*, 1987). Pulmonary hypertension was evident in rats 4-6 months after a 2-hour exposure to 10 ppm MIC (Tepper *et al.*, 1987).

A host of ophthalmic effects were noted at Bhopal. These included severe tearing, photophobia,, profuse lid edema, corneal ulcerations, ocular pain, diminished vision, corneal opacity, cataracts and night blindness. Some of these symptoms were persistent, though permanent serious eye damage was not reported (Mehta *et al*., 1990).

Fetal loss rose precipitously after the accident, from an estimated background incidence of 6-10% to 43% in the exposed population, with a disproportionate rise in first trimester spontaneous abortions (Varma, 1987). Fourteen percent of live-born infants exposed *in utero* died within 30 days of birth, an increase of over 4-fold compared to background rates (Varma, 1987). Gynecological effects

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(increased leukorrhea, irregular menses, menorrhagia [excessive menstrual bleeding at menstruation] and excessive vaginal discharge with, in many cases, inflammation) were common at 15 weeks post exposure (Shilotri *et al.*, 1986). Exposure of pregnant mice to MIC for 6 hours per day on gestation days 14-17 led to increased mortalities over controls in fetuses at 1 and 3 ppm (dead fetuses at increasing doses: 0.4%, 3.3%* and 6.4%* , *p<0.05), and in neonates at 3 ppm (neonatal deaths between days 0-4: 2.0%, 0.8% and 11.3%*, *p<0.05) (Schwetz *et al*, 1987). Exposure of pregnant mice to 9 and 15 ppm MIC for 3 hours on gestation day 8 resulted in greater than 80% resorptions, suppressed fetal skeletal growth, induced persistent diestrus, decreased female fertility and male reproductive performance (Varma *et al.*, 1987).

MIC is clastogenic and cytotoxic. Statistically higher frequencies of damaged peripheral lymphocytes and chromosomal aberrations per damaged lymphocyte were detected in MIC-exposed Bhopal females than in their non-exposed counterparts (Ghosh *et al.*, 1990). Shelby *et al.* (1987) demonstrated an increase in trifluorothymidine-resistant clones in response to MIC in cultured L5178Y mouse lymphoma cells. Increased frequencies of sister chromatid exchanges and chomosomal aberrations in Chinese hamster ovary cells (methylamine also showed positivity in the L5178Y assay, but at several hundred-fold higher concentrations) were also observed. Chromosomal aberrations in bone marrow cells, sister chromatid exchanges in lung cells and micronucleus formation in peripheral erythrocytes were noted in mice exposed to 1, 3 or 6 ppm MIC by inhalation on 4 succeeding days. A negative response in the *Salmonella* mutagenicity and *Drosophila* sex-linked recessive lethal tests prompted the authors to hypothesize that the positive L5178Y cell response, conventionally regarded as indicative of mutagenicity, may actually reflect loss of thymidine kinase activity in positive clones due to chromosomal rearrangements. This is supported by the evidence for chromosomal impacts delineated in the same study (see above). However, some evidence for positivity in the *Salmonella* assay was noted in two base pair substitution strains when care is taken to stabilize the MIC by lowering the temperature and increasing the time during the preincubation step (Meshram *et al.*, 1988).

Immunologic effects were also detected in Bhopal survivors. Cell-mediated immunity was suppressed, as indicated by a suppression of phagocytosis and T-cell population, and an increase in chromosomal aberrations in peripheral lymphocytes were observed in exposed victims 2.5 months after the event (Saxena *et al.*, 1988). Some animal experiments suggest immunotoxicity. For example, Hong *et al.* (1987) reported myelotoxicity (hypocellularity, suppression of pluripotent stem cells, granulocyte-macrophage progenitors and erythrocyte precursors) in female mice exposed to 1 and 3 ppm MIC for 6 hours per day on 4 succeeding days.

Neurologic effects of MIC at Bhopal were indicated by the reports of loss of consciousness, muscle weakness, tremors, vertigo, ataxia and fatigue (Bharucha and Bharucha, 1987). Nonetheless, these authors were impressed by the apparently low incidence of neurologic dysfunction considering the magnitude of the exposure.

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Subchronic / chronic toxicity. Little information on the toxic effects of longer-term low level exposure to MIC in humans or animals is available. Two studies examined the effects of an 8-day, 6 hours/day, inhalation exposure in rats to MIC at 0, 0.15, 0.6 and 3.1 ppm (Dodd *et al.*, 1987; Fowler and Dodd, 1987). Effects were observed only at the high dose. These included decreased body weights detected after the first day's exposure and maintained throughout the exposure period, decreased food consumption following days 3 and 8, and increased hemoglobin concentration and decreased blood oxygen saturation in males and increased lung weights in both sexes at the conclusion of the exposure period. In addition, there was histological evidence for pulmonary inflammation, epithelial degeneration, squamous metaplasia, regenerative hyperplasia, rhinitis, fibroplasia and bronchiolitis. Hypoactivity and increased respiratory rates were detected in the first 15 days post-exposure. Male mortality, likely due to respiratory impacts, was greatly increased over controls and over females during the recovery period. Some recovery from the cytotoxic effects of MIC was indicated in survivors. Interestingly, an epidemiological study of workers from a facility that produced and used MIC did not reveal functional pulmonary deficits, though the levels of exposure could not be specified (Avashia *et al.*, 1996).

Oncogenicity. The potential oncogenicity of MIC was examined in a special carcinogenicity bioassay designed to mimic the conditions of exposure at the Bhopal disaster. At the National Toxicology Program (NTP), groups of 50 or 100 six to eight-week-old F344 rats and B6C3F1 mice were exposed once to MIC by inhalation at 0, 3, or 10 ppm for 2 hours. After 2 years, animals were sacrificed and tissues and organs were examined microscopically. No differences in survival rates or body weight gains were found in MIC exposed animals versus controls. Male and female rats exposed to 10 ppm MIC had 42% and 36% incidence, respectively, of intraluminal fibrosis of lung secondary bronchi; no evidence of this lesion was seen in controls or animals exposed to lower concentrations. For male and female mice and female rats, no neoplastic lesions were significantly associated with MIC exposure. Male rats exposed to MIC had marginally increased rates of pheochromocytomas of the adrenal medulla, and adenoma of pancreatic acinar cell (HSDB, 1997). No information was available on the carcinogenic effects in humans. Nonetheless, MIC's clastogenic and cytotoxic properties may have some bearing on this question. The US EPA has classified MIC as in Group D, "not classifiable as to human carcinogenicity" (US EPA, 1994a).

Conditional acute REL calculation. The lowest air concentration for which adverse effects were noted was 0.5 ppm, associated with eye irritation in humans exposed for 10 minutes in ceramic chambers (ACGIH, 1986; Mellon Institute, 1970). An eye irritation NOEL was not determined in that study. An estimated NOEL (ENOEL) for the 10-minute exposure was calculated by dividing the LOEL (500 ppb) by an uncertainty factor of 10, to yield 50 ppb. A one-hour ENOEL was then calculated from the 10-minute ENOEL using Haber's Law:

$$C^n \times T = K$$

C = concentration, n = 1.1 [a chemical-specific value established in OEHHA (1999b)], T = time

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To generate the acute REL, the 1-hour ENOEL value is divided by an uncertainty factor of 10 to account for intra-human variation. These calculations follow:

1-hr acute ENOEL
$(50 \text{ ppb})^{1.1} \times 0.167 \text{ hr} = 12.35 \text{ ppb·hr}$
$(y)^{1.1} \times 1 \text{ hr} = 12.35 \text{ ppb·hr}$
$y = 9.8 \text{ ppb}$

1-hr acute REL
$9.8 \text{ ppb} \div 10 = 0.98 \text{ ppb}$

Calculation of a subchronic or chronic REL for MIC was deferred until adequate toxicological data on repeated exposure become available.

        <u>MIC levels after metam applications; regulatory limits</u>. The yield of MIC from MITC has been reported to be about 7% in laboratory experiments (Geddes et al., 1995). Inspection of the laboratory by the California Department of Toxic Substance Control confirmed the validity of the findings (DTSC, 1993; OEHHA, 1993). Preliminary measurement of MIC after agricultural use of metam sodium in Kern County revealed MIC levels between 0.09 and 2.5 ppb (ARB, 1995; DPR, 2002a). The potential highest level of MIC to be found under conditions similar to those in this study, estimated by correcting for maximum metam application rate and recovery similarly to MITC (DPR, 2002b), would be 5.4 ppb. This exceeds by more than 5-fold the 1-hour acute REL calculated above. Additional field measurements will be necessary before quantitative estimation of potential human risk from MIC following agricultural use of metam sodium can be undertaken. In any case, this study would not have conformed to current Technical Information Bulletin requirements for the application of metam sodium.

        On the basis of the high reactivity, marked corrosive, irritating properties to mucus membranes, and to prevent possible sensitizing doses to the pulmonary tract, an 8-hour Threshold Limit Value - Time Weighted Average (TLV-TWA) standard of 0.02 ppm (20 ppb) was recommended by the American Conference of Governmental Industrial Hygienists (ACGIH). The TLV-TWA is a time-weighted average concentration for a normal 8-hour workday and a 40-hour work week. The United States Occupational Safety and Health Administration (OSHA) 8-hour Permissible Exposure Limit (PEL), as well as the Cal OSHA PEL, is also 0.02 ppm. The US EPA has not established a Reference Dose (RfD), or Reference Concentration (RfC) for MIC, nor are ambient air standards available.

        C.   **Hydrogen Sulfide**

        H_2S is formed as part of the same monomolecular cleavage reaction that produces MITC from metam sodium under dilute aqueous conditions (Kreutzer *et al*, 1994). While it was not detected in official air monitoring studies after the Cantara Loop spill, its characteristic "rotten egg" smell was reported by individuals in the area; it was thus assumed to be present, at least for a short period. The low water solubility and high vapor pressure of H_2S would have favored rapid off-gassing from the river and

subsequent rapid breakdown (OEHHA, 1992). H_2S would presumably be produced upon application of metam sodium in an agricultural setting as an adjunct to MITC evolution. Sulfur dioxide and sulfuric acid may be formed when atmospheric H_2S is oxidized.

Like cyanide, H_2S disrupts intracellular electron transport by inhibiting cytochrome oxidase. Metabolic acidosis results when the shift from aerobic to anaerobic metabolism occurs, provoking a build-up of lactate. H_2S is also a mucus membrane and respiratory irritant. Death results from respiratory arrest and hypoxia (Ellenhorn, 1997).

Acute toxicity. The geometric mean human odor threshold for H_2S is 0.008 ppm, with a range of 0.00007 to 1.4 ppm, based on a review of 26 studies (Amoore, 1985; cited in OEHHA, 1999). H_2S levels between 2.5 and 5 ppm were associated with coughing and throat irritation after 15 minutes (Bhambani and Singh, 1985; cited in OEHHA, 1999). Exposure to 7 ppm H_2S during moderate physical exercise led to impaired oxygen and lactate uptake in the blood (Bhambani and Singh, 1991). Olfactory fatigue occurs at 50-100 ppm, with respiratory tract and eye irritation developing at 150-300 ppm (though some ocular effects may be present at 10 ppm), severe systemic toxicity at 500 ppm and cardiovascular arrest and death at 700 ppm and above (Ellenhorn, 1997). Symptoms commonly reported after accidental human exposures include dyspnea, sore throat, coughing, chest pain and signs of pulmonary obstruction. Less common symptoms include pulmonary edema, cyanosis and pneumonia. Severe neurologic and cardiovascular effects can be present in those recovering from high level exposures. Mortality resulting from severe acute exposure situations reportedly ranges between 2.8% and 6 % (Arnold *et al.*, 1985).

The mean 4-hour LC_{50} in rats was determined to be 440 ppm (Tansy *et al.*, 1981). The 1-hour lethal concentration in mice was 673 ppm (RTECS, 1994). Respiratory arrest and death occurred in dogs at 1000 ppm (Haggard and Henderson, 1922). A NOAEL of 10 ppm, based on depression of lung mitochondrial cytochrome c oxidase at 50 ppm was established in a study with Fischer 344 rats (Khan *et al.*, 1990).

Subchronic and chronic toxicity; other effects CIIT conducted companion studies of the effects on rats and mice of H_2S exposure 6 hours/day, 5 days/week, for 90 days at 0, 10.1, 30.5 or 80 ppm (CIIT, 1983). Weight decrements were observed at the high dose in both sexes. The only other effect was inflammation of the nasal mucosa of the anterior segment of the nose in mice, also at the high dose. The NOAEL was set at 30.5 ppm based on the latter effect

Hulbert *et al.* (1989; cited in OEHHA, 1992) noted moderate to severe dose-related proliferation of ciliated and basal cells and decreased non-ciliated Clara cell numbers in Fischer 344 rats following exposure to 10 and 100 ppm H_2S for 8 hours/day, 5 days/week, 5 weeks. Other pulmonary effects, including lymphocytic infiltrates, pulmonary edema and tracheitis, were also observed. Mild acute suppurative tracheitis, laryngitis and mild chronic nephritis were noted in guinea pigs exposed to 220 ppm H_2S for 7 days (Renne *et al.*, 1980; cited in OEHHA, 1992).

H_2S appeared weakly mutagenic in one *Salmonella* study. No long-term carcinogenicity studies

have been conducted. Two reproduction studies in rats did not indicate adverse effects, though there was a dose-dependent increase in delivery time (cited in Alexeeff *et al.*, 1992).

H_2S exposure may be associated with a risk of spontaneous abortion. A retrospective epidemiologic study in China conducted in a large petrochemical complex identified 106 reproductive-aged married women exposed only to H_2S. The overall odds ratio for spontaneous abortion was 2.3 (95% confidence interval, 1.2-4.4) (Xu *et al.*, 1998). H_2S levels were not identified.

H_2S levels after metam applications; regulatory limits. Measurements of H_2S after applications of metam sodium showed levels reaching 76 ppb at 1-4 hours post application, becoming non-detectable at 5-7 hours and rising again to 8 ppb at 21-24 hours (DPR, 2002a). As no recoveries for H_2S were reported in that study, the potential for H_2S exposure in those fields is not known. The ACGIH TLV (TWA) and the Cal OSHA PEL for H_2S is 10,000 ppb. The short term exposure limit (STEL) is15,000 ppb. The Agency for Toxic Substances and Disease Registry (ATSDR) lists an acute minimum risk level (MRL) of 70 ppb (ATSDR, 1999). It is based on a LOAEL of 2 ppm for respiratory effects (bronchial obstruction) in humans, incorporating uncertainty factors of 10 for the LOAEL to NOAEL extrapolation and 3 for intra-human variability. The California Ambient Air Quality Standard is 30 ppb for a 1-hour average (CCR, Title 17, Section 70200). This is based on an odor threshold study conducted by the California State Dept. of Public Health (1969; cited in OEHHA, 1999) which established a geometric mean threshold for 16 individuals of 0.029 ppm (rounded to 0.03 ppm [or 30 ppb]; range, 0.012-0.069 ppm). OEHHA (1999a), which also designated 30 ppb as the acute REL, argued that this concentration may be inappropriately high for 2 reasons: 1. there are reports of lower odor thresholds (see above) and 2. other symptoms, *eg.,* headaches, may occur in some people at 30 ppb.

An intermediate duration MRL of 30 ppb was established by ATSDR based on a NOAEL of 30.5 ppm in mice for respiratory effects. Adjustments were made for intermittent exposure, species-to-species extrapolation and intrahuman variability. OEHHA (199b) based their determination of the chronic REL of 7 ppb on this study. A human equivalent exposure concentration was calculated, and uncertainty factors of 3 for subchronic-to-chronic extrapolation, 3 for interspecies extrapolation, and 10 for intraspecies extrapolation.

D. Carbon Disulfide (*Note: Except where noted, this section was extracted from US EPA, 1994b.)*

Human exposure to carbon disulfide (CS_2) may follow metam applications because CS_2 is a degradation product of metam sodium, particularly under acidic conditions (pH<5).

Pharmacokinetics. CS_2 is readily absorbed in humans after inhalation exposure. Human and animal studies also indicate absorption of the vapor through skin, and animal studies indicate absorption through the gastrointestinal tract. Following inhalation exposure in mice, CS_2 was found in body fat, blood, lungs, liver and kidneys, whereas in rats and rabbits the highest levels appeared in lipid-rich tissues,

brain and liver. CS_2 crosses the mouse placenta and is found in the milk of occupationally exposed human mothers. The following metabolites have been detected in the urine of exposed workers: thiocarbamide, 2-thio-5-thiazolidinone, 2-thioazolidine-4-carboxylic acid and thiourea. 70-90% of the absorbed dose is metabolized and excreted by the kidneys, with 10-30% excreted unchanged by the lungs (decreasing rapidly once exposure stops). Sulfur excretion in rat urine is complete by 12-14 hours after an 8-hr inhalation exposure.

Acute toxicity. Acute human exposure to CS_2 *via* inhalation leads to local irritation, pharyngitis and CNS toxicity. Such exposure becomes life-threatening after 30 minutes at 3210-3850 ppm and fatal after 30 minutes at 4815 ppm. Oral exposure to 15 ml is also fatal. Dermal and ocular exposure causes severe burns. Oral LD_{50} values: rat, 3188 mg/kg; mouse, 2780 mg/kg; rabbit, 2550 mg/kg; guinea pig, 2125 mg/kg. LC_{50} (2-hr) values: rat, 25 g/m^3 (8 ppm); mouse, 10 g/m^3 (3.2 ppm).

Subchronic / chronic toxicity. Central nervous system, cardiovascular, gastrointestinal and immune toxicity result from exposures in humans in the range of 3-320 ppm for periods of months to years. Subchronic and chronic exposures in animals impact the nervous system, cardiovascular system and kidneys. There is no definitive evidence for CS_2-mediated carcinogenicity in humans or animals, though some possibility remains from epidemiologic studies of exposed populations. Genotoxicity data were not reported.

Epidemiology. The following impacts have been noted in epidemiologic studies of potential reproductive / developmental effects (length of exposures not indicated): inhalation exposure at 13-77 ppm: changes in sperm morphology, decreased hormone levels, decreased male libido, menstrual irregularities; 12-18 ppm: menstrual disorders and higher incidence of toxemia in pregnancy; 9 ppm: increased spontaneous abortions; fetal malformations do not appear elevated. Animal studies: increased fetal resorption in rabbits at 25 mg/kg/day (oral) during gestation. CS_2 is listed as a developmental toxin and a male and female reproductive toxin under Proposition 65.

Adverse impacts on the nervous system follow inhalation exposure in both humans and animals. Acute effects in humans include dizziness, fatigue, headache, mood change, lethargy, blurred vision, agitation, delirium, hallucinations, convulsions and coma. Symptomology is evident at as low as 320-390 ppm for several hours. Chronic exposures in humans can result in polyneuritis, encephalopathy, tremors, vertigo, psychosis, myopathy and reductions in nerve fiber conduction velocity. The latter sign was evident after exposure to 1 - 7.6 ppm for an average of ~12 years. Degenerative nervous system changes are also evident in animal studies.

CS_2 levels after metam applications; regulatory limits. Measurements of CS_2 after applications of metam sodium showed levels at or below the detection level of 4 ppb (Wofford *et al*, 1994). The TLV (TWA) for CS_2 is 10 ppm. The Cal OSHA PEL is 4 ppm, with a STEL (short term exposure limit) of 12 ppm. No ambient air exposure values are available. OEHHA (1999a) set an acute REL of 2 ppm. This was based on a developmental toxicity study in which pregnant rats were exposed to 0, 100, 200, 400 or

800 ppm CS_2 for 6 hours/day on gestation days 6-20. Significant reductions in fetal body weight were observed at the LOAEL dose of 400 ppm, establishing the NOAEL at 200 ppm. Uncertainty factors of 10 each for inter- and intra-species extrapolations were used to determine the acute REL. A chronic REL is not currently available.

E. Methylamine (Proctor *et al*, 1988)

Like CS_2, methylamine is produced upon cleavage of metam sodium under acidic conditions. Acidic cleavage of MITC will also produce methylamine. This compound is known for its irritant properties to eyes, nose and throat upon brief exposures to 20 - 100 ppm. Severe methylamine exposure may lead to pulmonary edema. The oral LD_{50} in rats is 100 - 200 mg/kg. As noted above, Shelby *et al*. (1987) demonstrated positivity in the L5178Y mutagenicity assay, but at several hundred-fold higher concentrations than MIC.

Regulatory limits. The OSHA PEL for methylamine is 10 ppm. The ACGIH TLV (TWA) is 5 ppm, with a STEL of 15 ppm.

F. Carbonyl sulfide (OPPT [1994] except where noted)

Like methylamine, carbonyl sulfide is produced upon cleavage of MITC in the gut. Acute inhalation exposure at levels >1000 ppm can result in fatality due to respiratory paralysis with little warning *via* local irritation or olfaction. Clinical signs following sublethal inhalation include giddiness, headache, vertigo, amnesia, confusion, unconsciousness, salivation, nausea, vomiting, diarrhea, cardiac arrhythmia, albuminuria, weakness and cramps. Rat studies showed deaths in 0/6 animals subjected to inhalation exposure at 1000 ppm for 75 minutes, 3/6 at 3000 ppm for 9 minutes and 3/6 at 1000 ppm for 90 minutes. COS administered by intraperitoneal injection as a gas to male Sprague-Dawley rats resulted in an acute LD_{50} of 22.5 mg/kg. Pretreatment with the carbonic anhydrase inhibitor acetazolamide considerably reduced mortality, suggesting that conversion to hydrogen sulfide was an important mediating step in COS toxicity (Chengelis and Neal, 1980). No information on subchronic/chronic effects in humans was available. A 12-week inhalation study in rabbits exposed to 50 ppm COS did not result in clear adverse effects. No information is available on the carcinogenicity, genotoxicity or developmental / reproductive toxicity of COS. In addition, regulatory limits have apparently not been established.

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XIII. RISK ANALYSIS
A. Hazard Identification and Dose-Response Assessment
1. Acute toxicity

The most relevant and supportable acute toxicity endpoint was eye irritation, identified in an experimental study conducted with human volunteers (Russell and Rush, 1996). The air concentration NOEL was 220 ppb for exposure only to the eye region via special goggles. A NOEL for an absorbed dose was not calculated for this study as MITC was not inhaled by the subjects. The irritating effect of MITC on ocular and respiratory tissues was also present in animal studies (Nesterova, 1969; Ullmann, 1985e) and in humans after agricultural use (DPR, 1993; DPR, 2002b; DPR, 2001) and after the 1991 Sacramento River metam sodium spill (Alexeeff *et al.*, 1994; Kreutzer *et al.*, 1994). Attempts to estimate peak MITC levels during the first 3 days after the spill were based on Gaussian plume modeling, using estimates of the amount of metam sodium spilled and the meteorologic conditions, as well as knowledge of the physicochemical properties of metam sodium and MITC. The splay in the resultant values, which ranged between 1300 and 4500 ppb at 100 meters in analyses conducted by the California Environmental Protection Agency (Alexeeff *et. al.*, 1994; Barry, 2001a) and between 3 and 650 ppm at the river's edge in a screening level sensitivity analysis conducted by the Metam Sodium Task Force (Sullivan, 2001), evidenced the considerable uncertainty involved in these calculations. For example, the assumption of worst-case meteorological conditions, where wind speed and direction would favor the build-up of MITC air concentrations in Dunsmuir, may have resulted in overestimates of the actual concentration. Therefore, whether or not the adverse effects that arose in the wake of the spill were relevant at air concentrations as low or lower than the eye irritation LOEL of 800 ppb is unknown. Nonetheless, it should be emphasized that short term MITC exposure, at concentrations that were not directly measured and thus were not known with certainty but were nonetheless estimated as detailed in this document, sent 705 residents of the Dunsmuir area into the local health care system (hospitals, private doctors, and the triage center) complaining of irritation to the eyes and upper respiratory tract, headache, nausea and other symptoms. In addition, serious long-term sequelae, including most prominently the reactive airways dysfunction syndrome, but possibly other effects as well, may have occurred in some members of the exposed population.

An MITC exposure incident in Earlimart, California also resulted in eye and upper respiratory complaints. These originated from residents living predominantly in the neighborhood closest to a field undergoing an illegally conducted sprinkler treatment with metam sodium (DPR, 2001). Due both to a wind shift and to the development of an inversion layer after sunset, a plume containing MITC and other metam sodium by-products moved over that neighborhood and adjoining neighborhoods. Barry (2000) used a Gaussian plume model similar to that used at Cantara Loop to estimate the 1-hour time weighted average MITC concentrations. Lower bound estimates ranged between 0.5-1 ppm over most of the neighborhood during the period of peak exposure.

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Despite the uncertainties inherent in *ex post facto* modeling of MITC air concentrations, the concentration estimates and clinical sequelae of both the Sacramento River spill and the Earlimart incident support designation of the acute eye irritation NOEL of 220 ppb as the critical acute NOEL. This value will be used in the assessment of the risks of potential short-term human exposure to airborne MITC in residents and bystanders (adults and children). The use of a NOEL based on eye irritation is considered a prudent step to protect the population not only from MITC's ocular effects, but also from potentially more serious impacts, notably to the respiratory system. Note, however, that while data exist to estimate an eye irritation threshold, DPR investigators, in their report on the Earlimart incident, have cautioned that "The corresponding average thresholds for upper and lower respiratory irritation are not known. It is also uncertain to what degree the irritation threshold is lowered in patients with asthma and other lower respiratory conditions, or to what degree the presence of pterygia, allergic conjunctivitis or other ocular conditions increase susceptibility to ocular irritation from MITC." (DPR, 2001). It is, notwithstanding, also reasonable to assert that eye irritation itself is an effect worthy of regulatory concern.

2. Subchronic toxicity

The animal study selected for evaluation of potential risk due to seasonal exposure to airborne MITC was the 4-week Wistar rat whole-body inhalation study of Klimisch *et al.* (1987). The endpoint used to establish the LOEL was an increase in nasal epithelial atrophy at the low dose of 1.7 ppm. A NOEL was not identified. At the mid dose of 6.8 ppm, in addition to nasal epithelial atrophy, clinical signs (somnolence, eye closure, and ruffled fur) were noted along with an increase in neutrophilic polymorphonuclear granulocytes. The latter sign was considered by the study authors to be a likely response to low-level lung irritation. The irritative nature of this sign was supported not only by its co-appearance with nasal epithelial atrophy, but also by the evidence from animals exposed to the high dose. This evidence included marked mucous membrane and respiratory tract irritation (reddish nasal discharge, salivation, eye discharge), resulting in a change in breathing pattern and whooping respiration. Ruffled fur, respiratory sounds, intensified cleaning behavior, and stretched posture were also noted at the high dose. The report provided no sense of the timing of the clinical signs, nor did it provide speculation as to their source. Neutrophilic polymorphonuclear granulocyte counts were even more elevated at the high dose than at the mid dose, with the effect at that dose clearly visible in females as well as males. Lung weights were massively increased at the high dose (70% and 83% in males and females, respectively), an effect that probably resulted from pulmonary inflammation and edema. Histopathology at the high dose revealed an increased incidence and severity of rhinitis in the nasal cavity, nasal epithelial atrophy, metaplasia of the nasal epithelium, tracheal epithelial proliferation and single cell necrosis, bronchopneumonia and bronchial and bronchiolar epithelial proliferation, and emphysema. The histopathological findings applied to both sexes. Careful examination of the nasal

epithelial atrophy data by DPR toxicologists revealed that those lesions described as focal at the low dose may have been displaced by more serious non-focal lesions at the mid dose. As noted in the description of the study in section V.A., this process then became very clear at the high dose, where virtually all of the atrophy noted was non-focal (see further discussion in section XIII.D.1.).

It was thus concluded by DPR that the major impact of inhalation exposure in these animals was respiratory irritation, with irritative effects also possibly noted in the eye as evidenced by the ocular discharge (on the other hand, eye closure, noted as a clinical sign at the high dose, could not clearly be ascribed to irritation). As was the case for acute toxicity, it was not necessary to estimate absorbed doses in calculating the MOE. Only the external analytical concentration was required. As a corollary, no distinction between males, females, and children was necessary, since there was no *a priori* reason to believe that the severity of, or sensitivity to, irritation effects in humans would be affected by sex or age (though it is noted that male rats appeared to be more sensitive in the Klimisch inhalation study).

A 6-hour/day, 5-days/week LOEL of 1700 ppb (1.7 ppm) based on nasal epithelial atrophy was identified in the 4-week rat inhalation study of Klimisch *et al.* (1987). To convert this LOEL to the more useful 24 hour/day, 7 days/week value, an extrapolation utilizing Haber's Law was invoked (for further discussion of the use of Haber's Law in this instance, see section XIII.D.1.):

1. An equivalent human 6-hour subchronic inhalation LOEL was estimated by multiplying the rat LOEL (1700 ppb) by 5/7 to correct for the 5 days/week exposure regimen,. Thus,

$$(1700 \text{ ppb}) (5 \text{ days} /7 \text{ days}) = 1214 \text{ ppb}$$

2. A 24-hour subchronic inhalation LOEL was calculated from the 6-hour value by invoking Haber's Law, with n set to a default value of 1,

$C^n \times T = K$, Haber's Law
C = concentration, n = chemical-specific adjustment factor set arbitrarily at 1
    [an empirical value is not known], T = exposure time, K = constant)
1214 ppb x 6 hr = 7284
C^1 ppm x 24 hr = 7284
C = 304 ppb . 300 ppb = LOEL

A default uncertainty factor of 10 is usually applied when estimating a NOEL from a LOEL value. However, in the present case, where there is considerable question about the interpretation of the determining endpoint (nasal epithelial atrophy), an uncertainty factor of 3 was considered appropriate. Thus 100 ppb (*i.e.*, 300 ppb ÷ 3) was designated as the critical estimated NOEL for subchronic toxicity. It was used in the evaluation of risks inherent in seasonal exposure to MITC in residents and bystanders.

As an alternative to conventional NOEL/LOEL determinations, regulatory air concentrations can

be established using benchmark dose (BMD) methodologies. The BMD is "a statistical lower confidence limit on the dose producing a predetermined level of change in adverse response compared with the response in untreated animals" (US EPA, 1995c). BMD values are established by modeling the dose-response relationship using the entire dataset from a toxicologic study (as opposed to the single determining LOEL dose relied upon in the conventional approach). Depending on the characteristics of the toxic responses involved, a 95% lower bound estimate (LED) of the 1%, 5%, or 10% effect level (ED) may be selected as the BMD. In general terms, the more serious the toxicologic effect, the lower the effect level that may be chosen to generate the BMD.

In the current case, the per-rat nasal epithelial atrophy incidence data - 3/10, 6/10, 6/10, 10/10 at approximate Haber-converted air concentrations of 0, 300, 1200, and 6000 ppb, respectively - were best approximated by a probit curve model. Using this model, the LED_{05} was 75 ppb (ED_{05} = 145 ppb) and the LED_{10} was 148 ppb (ED_{10} = 286 ppb). These values effectively bracketed, and supported, the conventionally-derived NOEL of 100 ppb (which was established by dividing the LOEL by an uncertainty factor of three, as discussed above). Using the same probit curve model, 100 ppb defined a lower bound estimate at the 6.7% effect level. It can thus be concluded that the BMD approach, using a probit approximation at a 5% - 10% effect level, generates similar values to the conventional NOEL/LOEL approach in this case. As the latter approach is the current default used by the Department of Pesticide Regulation, it will be relied upon for regulatory purposes in this assessment, though in full recognition of the support afforded by the alternate BMD approach.

It may be asked why the 12-13-week rat nose-only inhalation study of Rosskamp (1978) did not provide the critical subchronic NOEL. In this study, the endpoints used to establish the NOEL of 1 ppm were decrements in weight gain, increased water consumption and decreased serum protein levels, all occurring at the LOEL concentration of 10 ppm. Overt toxicity in the form of salivation / nasal discharge, mild and moderate apathy, vocalization and a much more severe weight gain decrement were detected at the high dose of 45 ppm. However, there was massive uncertainty inherent in the study report, which made it very difficult to rely upon it for risk assessment purposes, particularly as another more adequate study using the same strain of rat was available. The uncertainties are delineated as follows:

1.   The toxicologic significance of the three endpoints used to establish the NOEL was not clear. Statistically significant decreases in body weight gain with respect to sham-treated controls only occurred at the high dose of 45 ppm, while a much lower, non-statistically significant decrement was noted at the mid dose (10 ppm). Interestingly, a much larger suppression of body weight gain was evident in the sham-treated controls when compared to the untreated controls than occurred when comparing the sham-treated controls to the 10 ppm animals. This may indicate the presence of a stress effect imposed on the animals as they were fitted into the nose-only apparatus day after day. Individual animal data were not supplied, making it impossible to say with assurance what the effect was on individual animals. In the case

of water consumption, the increase that was observed compared to sham-treated controls was statistically significant at both 10 and 45 ppm (in females only at the latter dose), though the values at 45 ppm were not greater than those at 10 ppm. The significant decrease in serum protein in mid and high dose males and high dose females was conceivably a consequence of the increased water consumption. (However, a similar lowering of serum protein was detected in the chronic mouse drinking water study in conjunction with a *decrease* in water consumption [Sato, 1980], raising the possibility that protein levels were suppressed independent of an effect on water consumption, perhaps due to an effect of MITC on the liver.)

2.   Insufficient analytic data were provided in the study report to validate the reported chamber concentrations. According to the report, MITC levels were determined at hours 1 and 3 during each 4-hour exposure by withdrawing the chamber air for 10 minutes and routing it to an infra-red analyzer. The reported levels were thus mean values computed from hundreds of separate determinations. Without a report of the daily levels or, at the very least, standard deviations from the mean values, there is an implicit assumption that those mean values were in fact the levels that the animals were actually responding to, and were not in reality much lower or higher for significant periods.

3.   Rosskamp (1978) failed to provide a histological examination of the nasal cavity of the exposed rats. As MITC is known to cause irritation of mucus membranes, the lack of nasal examination in that study may have resulted in the assignment of an inappropriately high NOEL value or, at the very least, an appreciation of the importance of irritation to the overall toxic response.

3.   Chronic toxicity

   No chronic inhalation toxicity studies of MITC were submitted to the Department of Pesticide Regulation or were identified in the published literature. Chronic exposure of rats and mice through the drinking water resulted in decrements in body weight gain most likely secondary to decreased water consumption (Brown, 1981; Sato, 1980). The latter sign was, in turn, due to unpalatability. In mice, various serum chemical, hematological and histological alterations were also noted at the top 2 doses, with amyloid degeneration in the kidney possibly increased in low dose males. However, the significance of amyloid degeneration was obscured by the lack of a historical control data base. The chronic NOELs in rats and mice, 0.46 and 2.74 mg/kg/day, were based on signs largely, if not completely, dependent on the decreased water consumption.

   In dogs exposed to MITC by gavage, initial frank toxicity leading to the temporary discontinuance in the dosing regime of 2 mid dose females, may have been partially due to the high volume of corn oil vehicle (Harling *et al.*, 1989). Lowering the corn oil dose allowed determination of an apparent NOEL for MITC of 0.4 mg/kg/day based on a plethora of signs both irritative and systemic in nature. Unfortunately, a more precise evaluation of this study has not been possible because the study is

not available to DPR (a summary review was carried out in 1991 when the study was temporarily supplied to DPR by the registrant).

In the absence of a chronic inhalation toxicity study, an estimated chronic NOEL (ENOEL) may be calculated using a default approach of dividing the subchronic inhalation NOEL by an uncertainty factor of 10. Such a procedure was suggested by Dourson and Stara (1983) for calculating Acceptable Daily Intakes when only less-than-chronic toxicity data were available. This calculation would give a chronic ENOEL of 10 ppb.

4.   Oncogenic effects

The 2-year rat drinking water study provided weak evidence that MITC may have induced fibroadenomas and carcinomas in female mammary glands. In addition, the 2-year mouse drinking water study provided evidence that MITC may have induced cutaneous fibrosarcomas in both sexes. However, the statistical evidence from neither study was sufficient to trigger a quantitative oncogenic risk evaluation. The pros and cons of the question of MITC's possible mammary and cutaneous oncogenicity are discussed in section XIII.D.1.

B.   Exposure Assessment

This risk assessment considers acute and seasonal exposures of residents and bystanders occurring via ambient and application site air (for definitions, see discussion below). Chronic exposure estimates were not provided in the Exposure Assessment (DPR, 2002b), though it is expected that they will be presented in upcoming revisions of the full Risk Characterization Document for MITC currently being prepared under SB 950 (DPR, 2002d). Therefore, chronic MOEs were not calculated in this document. The acute exposures were divided into 1-, 8-, and 24-hour estimates. Procedures for estimating air levels relevant to these time periods are summarized in Tables 9 and 11. Occupational exposures were not considered in this document, but will be presented in the full SB 950 RCD (DPR, 2002d).

1.   Ambient air exposure

Ambient air monitoring is designed to measure pesticide concentrations in the air during the time and in the region of peak use. It is not, however, related to a specific application. For MITC this was accomplished in 3 separate studies, two of which (referred to as #B.2 and B.7 in the Exposure Assessment, part B [DPR, 2002b]) were conducted in Kern County, while the third (#B.8) was conducted in Lompoc.

With respect to Kern County study #B.2, Part A (Environmental Fate) of this report (pp. 52-53) states:

"The 1991 PUR (the most recent data available at the time the study was

conducted) was used to determine possible areas of high usage and peak periods of application within California. According to the PUR, in Kern County growers historically used the highest amounts of metam sodium in July; nearly 370,000 pounds of metam sodium were applied during July 1991... Therefore, the ARB selected four sites in Kern County, near anticipated application areas. Three of these sites were on the rooftops of schools, or school district offices, in the communities of Weed Patch, Lamont, and Shafter. The fourth site was established on the roof-top of the ARB's district office in Bakersfield.... At each location, the investigators collected eight primary samples and eight duplicate samples - sixty-four total samples...

"The 1993 PUR provides information regarding the amount of metam sodium that was reported used in Kern County during July 1993... Nearly 274,000 pounds of metam sodium were applied in Kern County during July 1993, consistent with use in previous years. However, it should be noted that metam sodium use patterns began to shift beginning in 1992. During 1992 through 1995 (the most recent available data) metam sodium use increased significantly during the months of March, April, August, and September of those years... Although the ambient study was not conducted during the period of absolute highest use in 1993, it was conducted during a period of high use in the location of highest use during that period."

With respect to Kern County study #B.7, Part A of this report (p. 68) states:

"Seiber *et al.* (1999; for reference, see Part A) monitored the airborne concentrations of MITC near Kern County applications of metam-sodium during two monitoring periods: the first period was during the summer of 1997 and the second period was during the winter of 1998. They collected samples of both outdoor and indoor air in three towns during the summer monitoring period - Shafter, Lamont, and Weedpatch. During the wintertime, they collected indoor and outdoor air samples in Lamont, Weedpatch, and Arvin. For the summer samples, the number of measurable residues was greatest during the months of May through July, with some of the highest residues occurring during June and July. For the winter samples, the greatest number of measurable levels and the greatest residue levels occurred in January. Detectable concentrations were measured in both indoor (residential) and outdoor air, with the highest concentrations occurring in outdoor air during the summer months, when warm dry temperatures, and the increased use of metam sodium occur. It is interesting to note that indoor residential air concentrations were similar in magnitude (and sometimes exceeded) outdoor concentrations, both during the summer and winter studies. However, the authors did not note if residents kept their windows opened or closed, or used heating or air conditioning during the study periods. Considering the climate in Kern County, it is reasonable to assume that the residents may have opened their windows during March, May, or June, while they may have relied on air conditioning during the mid-summer months and heat during January. Proximity to the treated fields and prevailing wind directions seemed to be the contributing factors with respect to detected ambient concentrations."

With respect to the Lompoc monitoring study, Part A of this Report (p. 63) states:

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The MITC ambient monitoring portion of this study was conducted from August 31 through September 13, 1998. Five sites were selected in Lompoc. All sites were located near the city limits near the ag-urban interface in a pattern that surrounded the city. Sampling sites were identified by their location around the city perimeter: Northwest, Northeast, West, Southwest, and Central. Samples were collected on August 31, 1998, and then continuously from September 9-13, 1998. At each location, two 12-hour samples were collected daily in duplicate samples. Twenty-three percent of the samples collected contained detectable levels of MITC.

Short term determinations. Table 9 provides the 1-hour, 8-hour and 24-hour acute exposure estimates from each of the studies cited above. Absorbed dosages were not calculated because eye irritation (the critical acute toxic endpoint) was considered solely a function of the air concentration. Interestingly, the summer measurements were comparable in the same area in 1993 and 1997, but declines from summer to winter were apparent in the 1997 / 1998 Kern County data.

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Table 9. Ambient 1-hour, 8-hour and 24-hour air concentrations of MITC

Kern County, summer 1993 (#B.2)[a]			
	MITC, ppb		
	1-hour	**8-hour**	**24-hour**
Shafter	1.1	1.1	1.1
Bakersfield	2.9	2.9	2.9
Lamont	8.3	8.3	8.3
Weedpatch	8.8	8.8	8.8
Kern County, summer 1997 (#B.7)[b]			
Lamont			
houses	9.7	9.7	5.9
environment	5.0	5.0	2.5
Weedpatch			
environment	9.4	9.4	4.7
Shafter			
houses	13.1	13.1	6.6
environment	14.6	14.6	7.7
Kern County, winter 1998 (#B.7)[b]			
Lamont			
houses	1.9	1.9	1.2
Weedpatch			
environment	1.7	1.7	1.6
Arvin			
houses	1.4	1.4	0.7
environment	0.3	0.3	0.1
Lompoc, summer 1999 (#B.8)[c]			
environment	0.3	0.3	0.3

Explanatory note: Because these were ambient determinations, *i.e.*, not associated with particular metam sodium applications, they were not corrected for application rate (see DPR, 2002b).
[a]For the Kern County, summer 1993 study (#B2), the 1-, 8- and 24-hour air concentrations are the same because they were all derived from the same samples and sampling times, none of which was less than 1365 minutes.
[b]For the Kern County, summer/winter 1997 study (#B7), the sampling times were ~11-12 hours. Hence, the 1- and 8-hour points are the same (*i.e.,* they used the highest 11-12 hour value), but the 24-hour point is a time-weighted average (TWA) of two consecutive sampling periods.
[c]For the Lompoc, summer 1999 study (#B8), only the 24-hour time-weighted average air concentration was indicated in the original report. The individual sampling times that were averaged to establish this value were not provided. Hence the 1-, 8- and 24-hour concentrations were set at the same value, *i.e.*, the 24-hour TWA.

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Seasonal determinations. As was the case for the acute exposure estimates, seasonal exposures were expressed solely as air concentration estimates (parts per billion, ppb; Table 10). The calculation of internal doses was not necessary because the endpoints which determined the critical subchronic NOEL (clinical signs and increased polymorphonuclear granulocytes) were considered most likely to derive from local (*i.e.*, non-systemic) irritation of the respiratory tree.

Table 10. Ambient seasonal MITC air concentrations for the general public

Kern County, summer 1993 (#B.2)

	MITC, ppb	**n**
Shafter	0.15 (.003-0.94)	8
Bakersfield	0.88 (.13-2.53)	8
Lamont	2.47 (.17-7.21)	8
Weedpatch	3.54 (.81-7.59)	8

Kern County, summer 1997 (#B.7)

Lamont		
houses	0.93 (.04-5.15)	43
environment	0.81 (.09-2.19)	14
Weedpatch		
environment	1.20 (.09-4.13)	12
Shafter		
houses	0.40 (.03-5.68)	45
environment	0.51 (.02-6.67)	45

Kern County, winter 1998 (#B.7)

Lamont		
houses	0.32 (.04-1.05)	16
Weedpatch		
environment	0.43 (.03-1.41)	8
Arvin		
houses	0.16 (.03-.64)	15
environment	0.11 (.03-.23)	6

Lompoc, summer 1999 (#B.8)

environment	0.0006	1

Explanatory note: Moderate term values were the time weighted averages (TWAs) of several MITC air concentration determinations established over several days of monitoring. For example, in study #B2-Shafter, 8 concentration determinations averaging ~1400 minutes each were carried out over a 187-hour period. The TWAs were then multiplied by the ratio of 78 days / 90 days (to account for the number of exposure days per ambient exposure period) to generate the mean moderate term air concentration. Because these were ambient determinations, *i.e.*, not associated with particular metam sodium applications, they were not corrected for application rate (DPR, 2002b).

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2. Application site exposure

"Application site" exposures are those which can potentially occur to individuals who are not on the field of application but are be adjacent or near to it during a specific pesticide application, usually at the highest label-approved rate, at a defined time and place. Therefore, these exposures have the potential to be the highest among all non-occupational human exposure scenarios. A total of seven California application site studies were considered for this report. These included 4 soil injection studies and 3 fixed-set sprinkler studies done under cool and warm air / soil conditions (one study, #B9 [Site F], conducted in Kern County in June 1999, monitored both application methods and so is considered to contain two separate studies; for details on the monitoring conditions, see Parts A and B of this report [DPR, 2002a and 2002b] and the footnote to Table 11). Monitoring distances for the soil injection studies were from 12 yards to 970 meters (different units of distance were used in different studies) from the perimeter of the field. Monitoring distances for the fixed-sprinkler studies were as indicated in the tables. One-hour, 8-hour and 24-hour air concentrations based on application site measurements appear in Table 11 These values, which ranged between 41 and 2853 ppb for 1-hour, between 32 and 2348 ppb for 8-hours and between 12.7 and 1102 ppb for 24 hours, were markedly higher than the short term ambient values shown in Table 9 (as would be expected). Similarly, application site measurements of seasonal MITC concentrations, with mean values ranging between 11 and 419 ppb (note that in two cases at Site F mean values were not calculated), were, in general, much higher than the seasonal ambient site exposures shown in Table 10.

Not all of the applications were performed under conditions which would be considered compliant with more recent Technical Information Bulletins (TIBs) and product labels for metam sodium. These require the soil to be "sealed" immediately following application to minimize off-site movement of odors and the imposition of buffer zones. In addition, applications during inversion conditions is prohibited. For four of the six studies (sites A, B, C, and E, which are equivalent to study designations B1, B3, B6, and B5 used in the Exposure Assessment) the soil was not "sealed" following application, as is currently required. Therefore, the air concentrations measured during those applications may not be representative of current practices. They were included in the present document to provide historical perspective. Interestingly, the air concentration *ranges* did not change when the four studies were excluded from the analysis because the remaining two studies included the high and low MITC air levels for both acute and seasonal estimates. However, with respect to one of the two remaining studies (site D [Exposure Assessment designation B4]), information provided during the preparation of this report indicated a potential for an inversion during the application period, though whether or not an actual inversion existed at that time could not be confirmed. The presence of an inversion would, as mentioned, be inconsistent with current requirements. In view of this uncertainty, caution should be exercised with respect to the air concentrations and MOE values calculated from this study. Finally, it should be noted that buffer zones of up to 0.5 miles are currently required for sprinkler applications of metam sodium

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(buffer zones are not currently required for injection applications). Many of the sprinkler application measurements were made within these currently recommended buffer zones.

<u>Table 11. Application site 1-hour, 8-hour and 24-hour air concentrations of MITC</u>
<u>for the general public from 3 soil injection and 2 sprinkler applications</u>

	MITC, ppb		
	1-hour	**8-hour**	**24-hour**
Site A[a]	646	646	618
Site B[b]	827	827	472
Site C[c]	236	236	236
Site D[d]			
5 meters	2853	2321	1102
75 meters	2813	2348	878
150 meters	1760	1534	468
Site E[e]			
5 meters	1255	811	186
25 meters	1043	701	171
125 meters	762	513	118
500 meters	163	106	22.8
Site F[f] - sprinkler irrigation			
150 meters	281	195	101 (30-101)[g]
300 meters	200	133	52 (8-52)[g]
700 meters	99	90	31 (3.6-31)[g]
970 meters	41	32	12.7 (0.08-12.7)[g]
Site F[f] - shank injection			
150 meters	281	244	175 (5.3-175)[g]
300 meters	216	151	106 (5.4-106)[g]
486 meters	199	123	84 (6-84)[g]
837 meters	242	149	106 (4-106)[g]

Explanatory notes: (1) In cases where the 8-hour or 24-hour concentrations are lower than the 1- hour point, the former values are time-weighted averages of two or more consecutive measurements, whereas the latter value is always the highest single value measured. Where the 8-hour and 1-hour concentrations are equal (Sites A-C), there were no sampling times less than 8 hours. Consequently, both concentrations were derived from the same sampling time. Sampling times are provided in the following footnotes. (2) Where appropriate, MITC air concentrations were corrected for the maximum application rate of metam sodium (see DPR, 2002b). (3) Current metam sodium Technical Information Bulletins, which are part of the label when metam sodium is used in California, specifically require the soil to be "sealed" immediately following application to minimize off-site movement of odors. During the studies referred to at Sites A, B, C, and E, the soil was not sealed following application, as is currently required. Therefore, the air concentrations measured during these applications may not be representative of current practices. These studies were included to provide historical perspective. With respect to one of the two remaining studies (site D), information provided during the preparation of this report indicated a potential for an inversion during the application period, though whether or not an actual inversion existed at that time could not be confirmed. The presence of an inversion would be inconsistent with current requirements. In view of this uncertainty, caution should be exercised with respect to the air concentrations and MOE values calculated from this study.

[a]Contra Costa Co., March 1993 (#B.1); soil injection; cool air / soil (53-55EF); sampling was done 15 yards from the

field perimeter. The 1- and 8-hour points were derived from a single 625-minute sampling time. The 24-hour point is a time-weighted average of two consecutive sampling time points, 625 and 815 minutes.

[b]Kern Co., Summer 1993 (#B.3); soil injection; warm air (61-92EF) / warm soil (79-88EF); sampling was done 20 and 40 yards from the field perimeter. The 1- and 8-hour points were derived from a single 785-minute sampling time. The 24-hour point is a time-weighted average of three consecutive sampling time points, 345, 370 and 370 minutes.

[c]Bakersfield, August 1995 (#B.6); soil injection; warm air (59.7-98.8EF) / warm soil (78-86EF); sampling was done 12, 13 and 20 yards from the field perimeter. The 1-, 8- and 24-hour points were derived from a single 760-minute sampling time.

[d]Kern Co., August 1993 (#B.4); fixed-set sprinklers; warm air / soil (80-86EF); sampling distances from the field perimeter were as indicated. The 1-hour point for each distance was derived from 6-hour sampling times. The 8-hour point for each distance was the time-weighted average of consecutive 1.5- and 6-hour sampling times. The 24-hour points was time-weighted average of four consecutive 6-hour and one 1.5-hour sampling times.

[e]Madera Co., May 1992 (#B.5); fixed-set sprinklers; warm air (53-94EF) / warm soil (58-88EF); sampling distances from the field perimeter were as indicated. The 1-hour point for each distance was derived from 4-hour sampling times. The 8-hour point for each distance was the time-weighted average of two consecutive 4-hour sampling times. The 24-hour point was the time-weighted average of four consecutive 6-hour sampling times.

[f]Kern Co., June 1999 (#B9); sprinkler irrigation or shank injection as indicated; for sprinkler irrigation, air temperature was <90EF; for shank injection, soil temperature was <90EF; sampling distances from the field were as indicated. For sprinkler irrigation, 150 meters, the 1-hour point came from a 244-minute sampling time, the 8-hour point a time-weighted average of consecutive 244- and 246-minute sampling times, and the 24-hour point a time-weighted average of five consecutive sampling times of 209-290 minutes. For sprinkler irrigation, 300 meters, the 1-hour point came from a 239-minute sampling time, the 8-hour point a time-weighted average of consecutive 239- and 251-minute sampling times, and the 24-hour point a time-weighted average of six consecutive sampling times of 225-258 minutes. For sprinkler irrigation, 700 meters, the 1-hour point came from a 236-minute sampling time, the 8-hour point a time-weighted average of consecutive 236- and 249-minute sampling times, and the 24-hour point a time-weighted average of five consecutive sampling times of 232-261 minutes. For sprinkler irrigation, 970 meters, the 1-hour point came from a 252-minute sampling time, the 8-hour point a time-weighted average of consecutive 252- and 248-minute sampling times, and the 24-hour point a time-weighted average of six consecutive sampling times of 224-252 minutes. For shank injection, 150 meters, the 1-hour point came from a 252-minute sampling time, the 8-hour point a time-weighted average of consecutive 252- and 228-minute sampling times, and the 24-hour point a time-weighted average of six consecutive sampling times of 226-258 minutes. For shank injection, 300 meters, the 1-hour point came from a 252-minute sampling time, the 8-hour point a time-weighted average of consecutive 252- and 228-minute sampling times, and the 24-hour point a time-weighted average of six consecutive sampling times of 228-252 minutes. For shank injection, 486 meters, the 1-hour point came from a 259-minute sampling time, the 8-hour point a time-weighted average of consecutive 259- and 223-minute sampling times, and the 24-hour point a time-weighted average of six consecutive sampling times of 223-259 minutes. For shank injection, 837-meters, the 1-hour point came from a 274-minute sampling time, the 8-hour point a time-weighted average of consecutive 274- and 243-minute sampling times, and the 24-hour point a time-weighted average of six consecutive sampling times of 192-287 minutes.

[g]The ranges provided for the 24-hour data, Site F, were established from twelve (150, 300 and 700 meters for sprinkler irrigation; 150, 300 and 486 meters for shank injection) separate or eight (970 meters for sprinkler irrigation; 837 meters for shank injections) separate time-weighted average determinations using two or three sampling stations over four days. MOE values (see Table 14) were calculated using the highest time-weighted average value.

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<u>Table 12. Seasonal application site MITC air concentrations
from 4 soil injection and 3 fixed-sprinkler sites</u>

	MITC, ppb	**n**
Site A[a]	47 (.03-124)	7
Site B[b]	44 (.23-170)	8
Site C[c]	16 (.11-45)	6
Site D[d]		
5 meters	80 (.58-547)	9
75 meters	65 (.25-539)	9
150 meters	62 (.51-337)	9
Site E[e]		
5 meters	19 (1.69-80)	13
25 meters	18 (1.97-67)	13
125 meters	12 (.67-52)	13
500 meters	3 (.25-10)	13
Site F[f] - sprinkler irrigation		
150 meters	11 (10-12)	3[h]
300 meters	6 (5-7)	3[h]
700 meters	2 (1.59-3)	3[h]
970 meters	(0.38-0.78)[g]	2[h]
Site F[f] - shank injection		
150 meters	13 (10-18)	3[h]
300 meters	7 (6-11)	3[h]
486 meters	6 (4-7)	3[h]
837 meters	(6-10)[g]	2[h]

Explanatory note: Moderate term values were the time weighted averages (TWAs) of several MITC air concentration determinations established over several days of monitoring. For example, at site A (study #B1-Contra Costa Co.), 7 concentration determinations averaging ~593 minutes each were carried out over a 187-hour period. The TWAs were then multiplied by the ratio of 23 days / 120 days (to account for the number of exposure days per application site exposure period) to generate the mean moderate term air concentration. Where appropriate, MITC air concentrations were corrected for the maximum application rate of metam sodium (see DPR, 2002b). Current metam sodium Technical Information Bulletins, which are part of the label when metam sodium is used in California, specifically require the soil to be "sealed" immediately following application to minimize off-site movement of odors. During the studies referred to at Sites A, B, C, and E, the soil was not sealed following application, as is currently required. Therefore, the air concentrations measured during these applications may not be representative of current practices. These studies were included to provide historical perspective. With respect to one of the two remaining studies (site D), information provided during the preparation of this report indicated a potential for an inversion during the application period, though whether or not an actual inversion existed at that time could not be confirmed. The presence of an inversion would be inconsistent with current requirements. In view of this uncertainty, caution should be exercised with respect to the air concentrations and MOE values calculated from this study.
Footnotes a-f are as appear in Table 11.
[g]Mean MITC values were not calculated from this distance.
[h]The numbers of replicates at site F are the number of monitors from which measurements were taken. Four measurements were taken from each monitor, from which time weighted averages were calculated. Consequently, the mean MITC air concentration that is expressed at distances up to 700 meters is the grand mean of 3 sets of 4 measurements. The absence of a grand mean value at 970 and 837 meters reflects the fact that only 2 sets of measurements were done.

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    C.   Risk Characterization
        1.   Acute (short term) toxicity

The risk for non-oncogenic human health effects is expressed as a margin of exposure (MOE). The MOE is the ratio of the NOEL from experimental human or animal studies to the human exposure dosage or, for local effects (as is the case in the current assessment), air concentration.

$$\text{Margin of Exposure} \quad = \quad \frac{\text{NOEL (local)}}{\text{Air concentration}}$$

Ambient air exposures. MOEs for acute (1-, 8-, and 24-hour) exposure to MITC in ambient air were calculated using the short-term ambient exposure values shown in Table 9 and the acute NOEL for eye irritation of 220 ppb. These values appear in Table 13. The lowest acute ambient MOE value was 15, obtained from the 1-hour and 8-hour determinations at the Shafter outdoor site in the summer of 1997.

Table 13. Acute margins of exposure for the general public from ambient air concentrations of MITC at 1, 8, and 24 hours

| | Margin of Exposure | | |
	1-hour	8-hour	24-hour
Kern County, summer 1993 (#B.2)			
Shafter	200	200	200
Bakersfield	76	76	76
Lamont	27	27	27
Weedpatch	25	25	25
Kern County, summer 1997 (#B.7)			
Lamont			
houses	23	23	37
environment	44	44	88
Weedpatch			
environment	23	23	47
Shafter			
houses	17	17	33
environment	15	15	29
Kern County, winter 1998 (#B.7)			
Lamont			
houses	116	116	183
Weedpatch			
environment	129	129	138
Arvin			
houses	157	157	314
environment	733	733	2200
Lompoc, summer 1999 (#B.8)			
environment	733	733	2200

[a]MOE (Margin of Exposure) = NOEL ÷ ambient air concentration (from Table 9). Acute NOEL = 220 ppb (eye irritation in humans).

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Application site air exposures. One-hour, 8-hour, and 24-hour acute margins of exposure in application site air are presented in Table 14. With one exception (Site F, sprinkler irrigation, 970 meters, MOE = 17), all of the mean MOEs were below 10, and many were less than 1.

Exclusion of MOEs calculated from sites A, B, C, and E due to non-compliance with the updated TIB and product label application recommendations did not result in any change in MOE *ranges* for application site exposures to MITC. However, with respect to one of the two remaining studies (site D), information provided during the preparation of this report indicated a potential for an inversion during the application period, though whether or not an actual inversion existed at that time could not be confirmed. The presence of an inversion would, as mentioned above, be inconsistent with current requirements. In view of this uncertainty, caution should be exercised with respect to the air concentrations and MOE values calculated from this study.

Table 14. Acute margins of exposure for the general public from application site air concentrations of MITC at 1, 8, and 24 hours

	Margin of Exposure		
	1-hour	**8-hour**	**24-hour**
Site A[a]	<1 (0.3)[h]	<1 (0.3)	<1 (0.4)
Site B[b]	<1 (0.3)	<1 (0.3)	<1 (0.5)
Site C[c]	<1 (0.9)	<1 (0.9)	<1 (0.9)
Site D[d]			
5 meters	<1 (0.1)	<1 (0.1)	<1 (0.2)
75 meters	<1 (0.1)	<1 (0.1)	<1 (0.3)
150 meters	<1 (0.1)	<1 (0.1)	<1 (0.5)
Site E[e]			
5 meters	<1 (0.2)	<1 (0.3)	1
25 meters	<1 (0.2)	<1 (0.3)	1
125 meters	<1 (0.3)	<1 (0.4)	2
500 meters	1	2	10
Site F[f] - sprinkler irrigation			
150 meters[i]	<1 (0.8)	1	2
300 meters[i]	1	2	4
700 meters[i]	2	2	7
970 meters[i]	5	7	17
Site F[f] - shank injection			
150 meters	<1 (0.8)	<1 (0.9)	1[i]
300 meters	1	2	2[i]
486 meters	1	2	3[i]
837 meters	<1 (0.9)	2	2[i]

Footnotes a-f are as appear in Table 11.
[g]MOE (Margin of Exposure) = NOEL ÷ application site air concentration (from Table 11). Acute NOEL = 220 ppb (eye irritation in humans).
[h]In cases where the MOE is less than one, the actual calculated value is shown in parentheses.
[i]MOEs calculated for 24-hour exposure times for Site F used the highest exposure value from the ranges provided in Table 11. For more details, see footnote *g* in that table.

2.   Subchronic toxicity

Ambient air exposures. Margins of exposure for seasonal ambient air scenarios appear in Table 15. These MOEs were calculated using the critical estimated NOEL of 100 ppb established in Wistar rats by Klimisch *et al.* (1987). It was based on the irritation-related increased incidence of nasal epithelial atrophy in both sexes after subchronic inhalation exposure. Because of the higher exposures measured at Weedpatch in the Kern County 1993 study (#B.2), the mean MOE of 28 at that site was lower than the corresponding MOEs from the other sites. Interestingly, the Weedpatch site also showed the lowest MOE (MOE=83) in the Kern County, summer 1997 study. One other site, Lamont (Kern County, 1993; #B.2) also dipped below 100 (MOE=40).

Table 15. Seasonal margins of exposure for the general public
from ambient air exposures to MITC

	MOE[a]
Kern County, summer 1993 (#B.2)	
Shafter	667 (106-33,333)
Bakersfield	114 (395-769)
Lamont	40 (14-588)
Weedpatch	28 (13-123)
Kern County, summer 1997 (#B.7)	
Lamont	
houses	108 (19-2500)
environment	123 (46-1111)
Weedpatch	
environment	83 (24-1111)
Shafter	
houses	250 (18-3333)
environment	196 (15-5000)
Kern County, winter 1998 (#B.7)	
Lamont	
houses	313 (95-2500)
Weedpatch	
environment	233 (71-3333)
Arvin	
houses	625 (156-3333)
environment	909 (435-3333)
Lompoc, summer 1999 (#B.8)	
environment	166,667

[a]MOE = ENOEL from the Wistar rat subchronic inhalation study of Klimisch *et al*., 1987 ÷ Ambient concentration. Subchronic ENOEL = 100 ppb (based on nasal epithelial atrophy in both sexes at the low dose). Exposure data are from Table 10.

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Application site air exposures. MOEs for seasonal exposure to MITC in application site air are presented in Table 16. Mean MOEs at all sites were below the benchmark of 100. As mentioned above, the applications at sites A, B, C, and E were not considered to be in conformance with current Technical Information Bulletins and product labels and thus are presented only to provide historical perspective. In addition, with respect to one of the two remaining studies (site D), information provided during the preparation of this report indicated a potential for an inversion during the application period, though whether or not an actual inversion existed at that time could not be confirmed. The presence of an inversion would, as mentioned above, be inconsistent with current requirements. In view of this uncertainty, caution should be exercised with respect to the air concentrations and MOE values calculated from this study. The measurements at site F were in conformance with current TIBs and product labels.

Table 16. Seasonal margins of exposure for the general public from application site air exposures to MITC

	MOE[g]
Site A[a]	2 (1-3333)
Site B[b]	2 (1-435)
Site C[c]	6 (2-909)
Site D[d]	
5 meters	1 (.18-172)
75 meters	2 (.19-400)
150 meters	2 (.30-196)
Site E[e]	
5 meters	5 (1-59)
25 meters	6 (1-51)
125 meters	8 (2-149)
500 meters	33 (10-400)
Site F[f] - sprinkler irrigation	
150 meters	9 (8-10)
300 meters	17 (14-20)
700 meters	50 (33-63)
970 meters	(128-263)[h]
Site F[f] - shank injection	
150 meters	8 (6-10)
300 meters	14 (9-17)
486 meters	17 (14-25)
837 meters	(10-17)[h]

Footnotes a-f are as appear in Table 11. Exposure data is taken from Table 12.
[g]MOE = ENOEL from the Wistar rat subchronic inhalation study of Klimisch *et al.*, 1987 ÷ Ambient concentration. Subchronic ENOEL = 100 ppb (based on nasal epithelial atrophy in both sexes at the low dose).
[h]Mean values for the measurements for Site F, 970 and 837 meters, were not provided. For explanation, see footnote h, Table 12.

3. Chronic toxicity

MOEs based on chronic toxicity studies were not developed in this document because chronic exposure numbers were not provided in the Exposure Assessment (DPR, 2002b). These values will be available in the SB 950 MITC risk assessment currently under preparation (DPR, 2002d).

D. Risk Appraisal

Risk assessment is the process used to evaluate the potential for human exposure and the likelihood that the adverse effects of a substance will occur in humans under the specific exposure conditions. Every risk assessment has inherent limitations on the application of existing data to estimate the potential risk to human health. Therefore, certain assumptions and extrapolations are incorporated into the hazard identification, dose-response assessment, and exposure-assessment processes. These, in turn, result in uncertainty in the risk characterization, which integrates all the information from the previous three processes. Qualitatively, risk assessments for all chemicals have similar uncertainties. However, the degree or magnitude of the uncertainty can vary depending on the availability and quality of the data, and the types of exposure scenarios being assessed.

In the following section, the specific areas of uncertainty associated with the characterization of health risks from exposure of the general public to airborne MITC under ambient and application site scenarios are delineated.

1. Toxicology / hazard identification

Use of the human eye irritation study to establish the acute regulatory endpoint. In the DPR Interim Risk Assessment Document for MITC (DPR, 1994), the NOEL selected for evaluation of potential acute human exposure to airborne MITC was obtained from a published account of the toxicity of MITC in the Russian literature (Nesterova, 1969). It was selected as the best available source of information at that time. However, for regulatory purposes, the Russell and Rush (1996) study is preferable to the Nesterova study. Besides being obviously more relevant to humans, Russell and Rush utilized modern carbon tube sampling and gas chromatographic analysis to verify MITC levels. Nesterova, on the other hand, employed an ill-defined colorimetric methodology. Russell and Rush also provided extensive documentation of their methods for determining eye irritation, which included 5 different irritation parameters, as well as appropriate documentation of their results. Nesterova provided neither. Dosing was clearly demarcated in Russell and Rush; such was not the case in Nesterova. Finally, the test article in the human study was pure MITC, which was directed to the eye masks by a series of tubes, valves and gauges under controlled pressure. The test article in the Nesterova study was not MITC, but metam, which was added to soil and subsequently heated to generate MITC. It is virtually certain (but again not documented by the investigator) that the test animals in the Nesterova study were exposed not only to MITC, but to other degradation products of metam. Indeed, it seems possible that the

degradation products in the Nesterova study might cross-react in the colorimetric determination of MITC (or, at least, this possibility was not excluded), which would invalidate the method. In conclusion, the recent availability of the experimental study of MITC-induced eye irritation in human volunteers provided a more contemporary evaluation of the hazard of potential exposures of workers, and the public, to MITC vapors following agricultural use of metam sodium.

Using eye irritation as a regulatory endpoint may be viewed as being unnecessarily health protective, since it may represent a local reversible nuisance response rather than a serious systemic adverse effect. However, it must be recognized that the human subjects in the critical study of Russell and Rush were exposed *only* through the eyes. Nasal, esophageal, tracheal and bronchial irritation were not evaluated because exposure of those tissues did not occur. As mentioned elsewhere in this report, respiratory irritation was a primary acute response to MITC at the Sacramento River spill and in incident reports of exposed agricultural workers. Time weighted average high estimates of MITC levels during the first three days following the spill ranged between 1300 and 4500 ppb at 100 meters and between 340 and 1240 ppb at 500 meters from the river, based on Gaussian plume modeling. However, substantial uncertainty accompanied these estimates. Whether or not the symptoms reported after the spill would be relevant at the LOEL level of 800 ppb is unknown. However, the presence of potentially serious persistent effects after the spill, including reactive airways dysfunction syndrome (RADS) and perhaps other debilitating conditions, suggests that protection against a less serious condition like eye irritation is advisable, as it may reasonably be expected to protect against the more serious effects that could appear at the same or higher concentrations.

The human eye irritation study identified a NOEL of 220 ppb after MITC exposure times of 1 to 8 hours. DPR used this value for evaluation of potential acute toxic effects. In risk assessment, 24-hour exposures are often used for estimation of "acute" effects, and experimental NOELs from shorter exposure times are normalized to an estimated 24-hour value using some form of Haber's Law (in simplest form, 8-hour NOEL x 8/24). Such a normalization was not done in this risk assessment; the estimated 24-hour NOEL was considered equivalent to the 8-hour value. This was done because the NOEL for human eye irritation remained constant at 220 ppb between 1 and 8 hours of exposure. It was considered unlikely that the NOEL would decline to one-third of that level if exposure was lengthened to 24 hours. However, since 24-hour exposure data were not obtained in the critical acute study, the 24-hour MOEs calculated using 8-hour toxicity data may underestimate the risk (*i.e.*, generate an inappropriately high MOE).

Two additional points bear mentioning. First, the reversibility of the irritation response that was evident upon removal of the MITC stimulus in the human study is interpreted as an indicator of the transient nature of that response. However, this is not an adequate reason to discount the adverseness of the response. Second, the level of confidence that is invested in the eye irritation LOEL/NOEL determination is impacted by the low number of human subjects (9-16) at each dose level and exposure

period. It is thus possible that sensitive individuals in the general population are not accounted for in the LOEL/NOEL designations. Furthermore, the great variability in eye blink rates and subjective perception of irritation in any single control subject makes it difficult to ascertain at low irritation levels whether or not a dosed subject has responded positively. The possibility that there are individuals in the general population that might show a positive irritation response at the NOEL dose cannot be excluded. Conversely, and for the same reason, the high incidence of positive respondents in the Likert scale and eye blink determinations at 0.8 ppm MITC, as reported above in section IV.A.2., may conceivably overestimate the actual incidence.

Finally, it is worth noting that the mean olfactory threshold, 1.7 ppm (range, 0.2 - 8 ppm) (Russell and Rush, 1996), would not provide adequate warning to many people of an imminent ocular or respiratory irritation hazard.

Use of the rat subchronic inhalation study to establish the critical subchronic and chronic endpoints. The critical estimated subchronic NOEL of 100 ppb, derived from the rat 4-week inhalation toxicity study (Klimisch *et al.*, 1987), was calculated from the study LOEL of 1.7 ppm by applying Haber's Law and dividing the result by an uncertainty factor of 3. The decision to use an apparent increase in incidence of nasal epithelial atrophy at the low dose as the basis for the LOEL determination had prominent weaknesses: 1) No increase in incidence rate was observed between the low dose and the mid dose when the data were examined both on a section plane basis (Table 5) and on a number-of-rats-per-dose basis (Table 6) . This lack of dose response called into question the reality of the alleged low dose response, particularly in view of the unaccounted for finite level of atrophy among control animals, especially males. Without historical control data, it was difficult to know if the control incidence rates were anomalous. (It should nonetheless be noted that one of the control males appeared to have a respiratory ailment, expressed as widespread rhinitis and epithelial atrophy. If so, this would have contributed to the high control values.)   2) No other histopathological irritation responses were evident until the high dose (34 ppm), where nasal epithelial atrophy, bronchopneumonia, emphysema, bronchial and tracheal epithelial proliferation, rhinitis, and focal metaplasia were observed, in addition to increases in lung weight and decreases in body weight. The only other indication of lung pathologic changes at the mid dose (6.8 ppm) was the statistically increased polymorphonuclear granulocyte count in males (females showed a statistical increase at the high dose only). This sign was interpreted to reflect sub-histopathologic inflammation in the lung, though even this explanation was inferential. Thus not only were there serious questions about the increase in nasal epithelial atrophy at the low dose, but even the response at the mid dose was not altogether certain.   3) Pairwise comparison using Fischer exact tests did not reveal statistically significant atrophy until the high dose. This was the case in both the section plane (Table 5) and rats-per-dose analyses (Table 6).

Even in light of these weaknesses, it was not possible to rule out an MITC-driven increase in

nasal epithelial atrophy at the low dose. Such an increase was particularly apparent when the development of focal and non-focal lesions were considered as part of the same process, *i.e.*, one in which the less severe focal lesions gave way to the more severe non-focal lesions at higher doses[1]. Thus the incidence of focal atrophy declined to zero at the high dose in section planes 2 and 3, while non-focal atrophy increased to 100% at that dose, implying that atrophy had become so extensive that individual foci were no longer distinguishable. The data from section plane 2 indicate that an increase in focal atrophy occurred in both sexes at the low dose. There was a decline in this character at the mid dose, which may reflect the increased severity (*i.e.*, the focal-to-non-focal progression) at higher doses. An increase in non-focal atrophy among females at the low dose in section plane 3 was also evident.

It should be noted that the various section planes were probably not equivalent with respect to focal atrophy, showing what may have amounted to a systematic decrease in responsiveness between section planes 2 and 4. The increase in focal atrophy at the low dose in section plane 2, particularly in females, was not evident either in section planes 3 or 4, possibly because those areas of the nasal passages were less sensitive or were exposed to lower effective MITC concentrations. The latter possibility would have been the case if, as it is assumed here, section plane 2 was closer to the outer, MITC-containing, air (*i.e.*, if the section plane numbers indicated an outer-to-inner histological sectioning sequence). Unfortunately, since the report provided no information concerning the anatomical disposition of these planes, this remained supposition[2].

Interpretation of focal and non-focal atrophy as part of the same process allows one to combine incidence data for these two lesions (Table 6). When incidence of atrophy was expressed on a number-of-rats-per-dose basis, where double-counting of animals with lesions in more than one section plane was strictly avoided, increases at the low dose for all lesion categories were evident when the sexes were considered together. When focal and non-focal data were combined (row 3, "total"), a doubling in incidence occurred (though, as mentioned, a further increase at the mid dose was not observed).

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[1]That focal atrophy induced at low doses gave way to non-focal atrophy at higher doses was never explicitly stated in the pathology report and should be viewed as inferential. Indeed, the pathology report never even used the term "non-focal", choosing instead to define atrophy either as "focal atrophy" or "atrophy". It was, therefore, a decision of the Medical Toxicology Branch of DPR to apply the description "non-focal" to those nasal passages that were listed as merely atrophic. However, as stated above (sections V.A. and XIII.A.2), the fall-off of focal atrophy and its apparent replacement by the non-focal variety provided extremely strong support for this interpretation, particularly in view of the decline of focal atrophy to near-zero at the high dose while non-focal incidence rose to near-100%.

[2]It is not known why nasal atrophy was not reported at any dose for section plane 1. Since the incidence of nasal atrophy was 100% at the high dose in the other three section planes, it follows that the tissues represented in section plane 1 were simply not capable, for whatever reason, of demonstrating this characteristic. Interestingly, nasal cavity metaplasia was reported exclusively in section plane 1 in males, and only in section planes 1 and 2 in females, supporting the contention that the outer nasal planes were more apt to show pathologic lesions.

Interestingly, combination of the gender and lesion data in section plane 2 (Table 5) generated the same incidence curve as the combined gender and lesion data in Table 6 (3/10, 6/10, 6/10, 10/10 at increasing MITC air concentrations). This implied that sensitivity at the low dose resided in section plane 2. The combined gender and focal + non-focal atrophy data from section plane 3 also showed an increase at the low dose (2/10, 4/10, 4/10, 10/10). In this case, the increase at the low dose was driven largely by the increase in non-focal atrophy in females noted above. Finally, the total number of section planes exhibiting atrophy also rose by almost 2-fold between the controls and the low dose (Table 6, row 4, "total").

The most basic form of Haber's Law, $C^n \times t = K$, with the exponential modifier set at 1, was used to estimate the subchronic LOEL in the rat study. The 6 hours per day, 5 days per week exposure regimen was converted to a 24 hours per day, 7 days per week regimen using this relation, effectively lowering the LOEL from 1700 ppb to 300 ppb. This might be viewed as a health conservative measure, since it explicitly accepts the Haberian assumption that the longer the exposure time, the less MITC is required to elicit a similar response. However, the universality of Haber's Law, *i.e.*, whether it is applicable to all chemicals under all exposure regimens, is susceptible to criticism (see, for example, Miller *et al.* [2000]). Nonetheless, an inverse concentration *vs.* time relation was operative for short exposure times in the human eye irritation experiment. Exposure to 1.9 ppm or 3.3 ppm MITC resulted in positive subjective (Likert scale) responses at 4 and 14 minutes. One hour was required for a positive subjective response at 800 ppb. Also, a statistically significant blink rate response that occurred in 4 and 14 minutes at 3.3 ppm required 2 hours at 800 ppb. Exposures longer than 1 hour did not, however, result in further changes in the LOEL. Under the considerably longer exposure scenario of the rat subchronic inhalation study there was no reason either to confirm or refute the applicability of Haber's Law, to say nothing of the appropriateness of designating an exponential modifier with the concentration term. It was decided, therefore, to invoke Haber's Law as a default, with the understanding that it could be abandoned or modified with further experimental data.

The use of an irritation-related endpoint did not preclude the possibility that MITC exposure by the inhalation route had systemic effects. Indeed, there was some indication to this effect in both the Klimisch *et al.* (1987) and Rosskamp (1978) subchronic inhalation toxicity studies. In Klimisch *et al.* (1987), a non-statistically significant 14% rise in prothrombin time observed in males at the mid dose (6.8 ppm) became a statistically significant 16% rise at the high dose (34 ppm) (no effect was discerned in females). Total bilirubin also rose while urea and glucose concentrations fell in non-statistically significant fashion at the mid dose, also becoming statistically significant at the high dose. Furthermore, liver and kidney weights were statistically increased in males at the high dose. Finally, high dose male mean body weight declined by 6% compared to controls during the first week. The males continued to lose weight during the second and third weeks (body weights were 7% and 9% less than controls at those times), recovering slightly by the fourth week (when body weights were 5% less than controls). In Rosskamp *et*

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al. (1978), body weight effects were discerned at the mid and high doses (10 and 45 ppm). It was not known if the body weight effects resulted from systemic or irritative toxicity, or whether or not they were a function of lower food consumption (decreases in food consumption were noted at the high dose in the Rosskamp study; food consumption was not measured in the Klimisch study). However, in the critical Klimisch *et al.* (1987) study, the putative systemic effect at the mid dose (*i.e.*, the LOEL dose) was, at best, slight. As the high dose observations will attest, the irritative effect of MITC was clear and prominent. For these reasons, it was concluded that the most defensible approach to the calculation of subchronic and chronic MOEs should rely upon the irritative effects and thus utilize the MITC air concentration data rather than absorbed doses.

       <u>Toxicologic risk from metam sodium and breakdown products</u>. This risk assessment document focuses on the toxic effects of MITC vapors, and does not address potential toxicity resulting from simultaneous exposure to MITC and the parent compound metam sodium. Metam sodium is a non-volatile salt, making inhalation exposure very unlikely, except perhaps in association with aerosolized water droplets. However, the most likely scenario for simultaneous exposure would occur under occupational conditions where dermal exposure to metam is likely. While the toxicologic implications of simultaneous exposure are unknown, it is extremely unlikely that it would occur under the non-occupational scenarios that are the subject of the current assessment. It bears mentioning, however, that metam sodium has toxicologic properties that are different from those of MITC. Metam sodium by the oral route causes angiosarcomas in mice and hemagiosarcomas in rats, and is clastogenic, hepatotoxic and embryotoxic (see the DPR Risk Characterization Document for metam sodium (DPR, 2002c)).

       Both MITC and the photolytic breakdown product methyl isocyanate (MIC) have similar toxic effects in humans and experimental animals. For risk assessment purposes it is reasonable to believe that the toxic effects might be additive (Geddes *et al.*, 1995). Numerical summing of the effects was not, however, attempted in this document. This was due to uncertainty regarding the actual magnitude of MIC air levels that occur following agricultural use of metam sodium. Preliminary application site measurements in one Kern County study revealed MIC levels between 0.09 and 2.5 ppb (ARB, 1995; DPR, 2002a). The potential highest level of MIC to be found under the conditions of this study, estimated by correcting for application rate and recovery similarly to MITC (DPR, 2002b), was 5 ppb. This exceeds by about 5-fold the 1-hour acute REL of 0.98 ppb calculated above. However, it should be recognized that the particular application examined in the ARB study would not comply with current Technical Information Bulletin and label requirements. Clearly, more extensive monitoring under various exposure scenarios will be necessary before a reliable estimation of human risk from MIC alone or from combined exposures to MIC and other metam sodium breakdown products can be made.

       Toxicologic effects of simultaneous exposure to hydrogen sulfide (H_2S) and MITC are also not considered for this document, nor is there any indication whether such exposure might compound the

effects of either chemical. Nonetheless, it appears that H$_2$S levels occurring in association with metam applications may exceed the minimum risk levels established by the ATSDR (see discussion of H$_2$S toxicity above, section XII.C.)

Toxicologic risk from co-exposure to metam sodium and its breakdown products. Co-exposure to any combination of metam sodium, MITC, MIC, and H$_2$S (or other metam sodium breakdown products) could elicit additive or synergistic effects. These might particularly be expected in respiratory and ocular tissues, which are known to be sensitive to the irritative effects of these compounds in isolation. Unfortunately, no clear experimental or epidemiologic data are available to suggest the presence of, or potential for, additive / synergistic interactions. At this point it can only be said that such effects are plausible. Furthermore, meaningful estimation of the potential for toxicologic effects arising from simultaneous exposure, even in a single tissue, would be contingent on careful measurements of the air levels of each chemical under both application site and ambient scenarios. Only preliminary measurements are available to this date.

Possible oncogenicity of MITC (weight of evidence considerations). An apparent elevation in female rats bearing multiple fibroadenomas and carcinomas was noted in the 2-year rat drinking water study (Brown, 1981). Fibroadenomas are considered to be benign tumors in both rats and humans. They contain both connective tissue and epithelial elements, and can, infrequently, evolve into sarcomas or carcinomas. The neoplastic nature of rat fibroadenomas is indicated by their transplantability (Foulds, 1975). As noted in the discussion of the rat drinking water study (section VI.B.), the question of a possible role for MITC in the induction of these tumors is unresolved. Arguing in favor of a role is the observation that the treatment groups tended to show higher incidence rates than controls, both for multiple fibroadenomas in terminal survivors and for carcinomas in survivors and decedents. Marginal statistical significance (p=0.054) was attained at the high dose for the former condition, but not for the latter. When all of the dose groups were combined, such that the incidence of animals with multiple fibroadenomas in controls was 6/25 vs. 37/83 in dosed animals, a Fisher exact test yielded a p-value of 0.052. When incidence rates for multiple and single fibroadenomas in survivors and decedents were combined, the top two doses were higher than controls, but statistical significance was achieved only at the mid dose. With total carcinoma incidence (survivors + decedents), statistically significant increases were seen at the low and mid doses. If all doses were combined, such that the control incidence was 0/60 vs. 12/180 in dosed animals, the resultant Fisher p-value was 0.029.

Arguing against a role for MITC as an inducer of mammary tumors were the following considerations: 1) In no case was dose responsiveness evident. Cochran-Armitage trend tests were always negative, 2) Approximately 50% of the animals in each dose group died before termination of the study at 104 weeks. As only 60 animals per group initiated the study, the small number still present at

the end decreased the interpretive and statistical power of the study, 3) Fibroadenoma incidence rates were extremely high, even among controls. In fact, their occasional appearance outside of the visible subcutaneous masses in randomly chosen tissues used for histopathology suggests that the reported incidence rates may actually have been underestimated. If actual incidence approached 100% in all groups, it would be difficult to claim a treatment effect on animals with fibroadenomas. The most that could be said in that case would be that MITC increased the incidence of fibroadenomas that were big enough to be visible as subcutaneous masses, 4. Carcinoma incidence rates fell close to the mean and within the historical control range established at a separate laboratory, 5. Mammary histopathology, especially the incidence of hyperplasia and ectasia, was not influenced by MITC exposure, and 6. Except for a single weakly positive *in vitro* chromosome aberration test, MITC was not shown to be mutagenic or clastogenic, nor did it induce sister chromatid exchange or unscheduled DNA synthesis. Genotoxicity was thus discounted as a driving force in any putative oncogenicity.

Cutaneous fibrosarcomas were noted at the high dose of 200 ppm in the mouse drinking water study. While statistical significance by Fisher exact test was not achieved in either sex when they were considered separately, combining the sexes resulted in significance at a level of $p < 0.05$. Because of the appearance of these tumors in both sexes only at the high dose (incidence was naught at all other doses), and the statistical significance of the combined data when compared to controls, MITC exposure is considered a likely cause of the fibrosarcomas. Interestingly, incidence of fibromas appeared to rise at the high dose in the rat drinking water study, lending weight to the possibility that the fibrosarcoma rise in mice was due to MITC exposure.

2. Exposure assessment

Some of the uncertainties associated with the exposure assessment have already been discussed in Part B of this document (DPR, 2002b). The following uncertainties are considered noteworthy:

1. The 1-hour exposure level may be underestimated because the sampling times were much greater than 1 hour. Thus the peak MITC level was not known. For example, in the ambient monitoring the sampling times were not less than 11 hours (though at Lompoc the sampling times were not known), while in the application site monitoring the sampling times never dipped below 239 minutes.

2. Sample collection in some of the application site studies was done at distances that would currently be considered inappropriately close to the field for sprinkler applications (*i.e.*, within current buffer zones) and/or without use of recommended water seals (sites A, B, C, and E). In one case, an inversion layer may have been present during the application, though this was not confirmed (site D). In those cases, estimates of MITC air concentrations may have been inappropriately high. On the other hand, siting of sampling apparatuses in the remaining studies (sprinkler and injection

applications at site F) were considered inappropriate because they were not downwind of the application. In that case, estimates of MITC air concentrations may have been inappropriately low.

3.   The value used in this document for number of exposure days per season for application site measurements (23 days per 120-day season), may be an underestimate if, under some meteorologic conditions, MITC is more stable than under others.

4.   MITC air concentrations in one Kern County air monitoring study were collected at roof-top level. This could produce an under- or over-estimate of potential toxicity, if MITC levels at roof-top levels vary significantly from those at ground level. In all likelihood, however, mixing in the atmospheric boundary layer (the lowest 10-100 meters of the atmosphere) would assure uniformity of concentration at these distances from the application sites.

3.   Risk characterization

The margins of exposure presented in this document are listed as both the average expected value and, when available, as the range of potential values based on the range of measured values in the exposure studies. This was done to demonstrate the uncertainty that exists for estimation of potential risk due to the wide range of measured values for airborne MITC.

Generally, a margin of exposure of 10 is considered sufficiently protective of human health when data are derived from human studies. It allows for a 10-fold spread in sensitivity between the most and the least sensitive individuals. Acute MOE values for 1- and 8-hour exposures under currently permissible application site scenarios (however, see the caveat for site D described in section XIII.B.2. and in the notes to Table 11), with their range of <1-2 (with the only exception deriving from exposure estimates done 970 meters from a field undergoing a sprinkler application, with corresponding MOEs of 5 and 7 for 1- and 8-hour estimates), are clearly below that standard, indicating a potential health concern. Acute application site 24-hour MOEs, which ranged between <1 and 10 (again with the exception deriving from the 970 meter measurement in Site F for sprinkler application, with its MOE of 17) also indicated a health concern. Acute ambient MOEs, which ranged from 15 to 2200, did not indicate immediate cause for health concern.

A margin of exposure of at least 100 is considered sufficiently protective of human health when data are derived from animal studies. The MOE of 100 assumes that humans are 10 times more sensitive than laboratory animals and that the range of least to most sensitive individual within the human population is 10. This standard was not achieved for any of the application site seasonal exposure scenarios, at any measurement distance out through 700 meters when using the animal-based estimated critical NOEL of 100 ppb. Ambient exposure scenarios indicated less reason for concern, though measurements at Weedpatch in 1993 and 1997 and at Lamont in 1993 showed MOEs below the benchmark of 100.

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E.   Reference Exposure Levels

The reference exposure level (REL) is defined by the Office of Environmental Health Hazard Assessment as "the concentration level at or below which no adverse health effects are anticipated for a specified exposure duration" (OEHHA, 1999). RELs are based on the best available medical and toxicological studies and "are designed to protect the most sensitive individuals in the population by the inclusion of margins of safety." Air concentrations of MITC below the REL are considered sufficiently low to protect human health. The REL for acute toxicity was calculated by dividing the definitive acute NOEL (220 ppb, based on the occurrence of eye irritation in humans at 800 ppb) by an uncertainty factor of 10 to account for intraspecies variation in sensitivity. The REL_{acute} was 22 ppb. Because eye irritation is a local effect, it was not necessary to adjust the calculation to take into account different breathing rates in different segments of the population. The NOEL did not change at 1, 4 and 8 hours. Consequently, the REL of 22 ppb was relevant for potential exposure times of up to 8 hours. Ambient exposure estimates for 1 and 8 hours did not exceed 14.6 ppb. A human health concern was therefore not indicated under those conditions. However, the estimated application site exposure ranges for 1 and 8 hours, 41-2853 ppb and 32-2348 ppb, respectively, clearly exceeded the acute REL. A human health concern under application site scenarios was thus indicated[3].

The 24-hour REL for subchronic toxicity was calculated by dividing the 24-hour critical subchronic NOEL of 100 ppb by a combined uncertainty factor of 100 (10-fold to account for interhuman variability and 10-fold for the possibility that humans are more sensitive than animals) to yield a value of 1 ppb. Seasonal time-weighted average (TWA) MITC concentrations ranged between 0.11 and 3.54 ppb for ambient determinations in Kern County (0.0006 ppb in Lompoc), indicating the potential for human health concerns. Similar measurements done under application site scenarios resulted in a range of mean TWA air concentrations between 2 and 80 ppb, clearly above the subchronic REL in most cases[4].

Because chronic exposure estimates were not available (DPR, 2002b), chronic MOEs were not calculated for this assessment. Nonetheless, chronic REL values, which do not rely upon exposure estimates, were estimated in the eventuality that use patterns or air monitoring at some future time would indicate a potential for chronic exposure. In the absence of a chronic inhalation toxicity study, the 24-hour chronic REL was estimated by dividing the subchronic REL by a default uncertainty factor of 10, analogous to the suggestion of Dourson and Stara, 1983, to invoke an uncertainty factor of 10 when calculating a chronic Acceptable Daily Intake using less-than-chronic toxicity data. Thus the 24-hour $REL_{chronic}$ is 1 ppb ÷ 10 = 0.1 ppb.

The REL values for MITC, calculated above, appear below in Table 17.

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[3]However, see the caveats in section XIII.B.2. regarding the compliance or non-compliance of the application site studies with respect to the requirements in current Technical Information Bulletins.

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Table 17. Summary of definitive studies, regulatory endpoints, LOEL and NOEL concentrations, and reference exposure levels (RELs) for airborne MITC

| Exposure time/species | Endpoints | LOEL | NOEL | REL | | | | Reference |
				1-hour	4-hour	8-hour	24-hour	
Acute, human 1-8 hr study	Eye irritation	800 ppb	220 ppb	22 ppb[a]	22 ppb[a]	22 ppb[a]	----	Russell & Rush, 1996
Subchronic. 4-wk rat subchronic study	Nasal epithelial atrophy	300 ppb	100 ppb	----	----	----	1 ppb[b]	Klimisch, 1987
Chronic, 4-wk rat subchronic study	Nasal epithelial atrophy	300 ppb	100 ppb	----	----	----	0.1 ppb[c]	Klimisch, 1987

[a]The 1-hour acute REL was calculated by dividing the definitive acute NOEL (220 ppb) by an uncertainty factor of 10, which accounts for variations in sensitivity within the human population. Because the level of eye irritation was unchanged at 1, 4 and 8 hours, those REL values are the same as the 1-hour value.
[b]The 24-hour subchronic REL was calculated by estimating the 24-hour subchronic NOEL from the LOEL and dividing the result by a combined uncertainty factor of 100 (see text). One hour and 4-hour subchronic NOELs were not calculated.
[c]The 24-hour chronic REL was calculated by dividing the subchronic REL by an uncertainty factor of 10.

XIV. OTHER MITC RISK ASSESSMENTS

A. US EPA MITC Risk Assessment.

US EPA has issued two documents relating to human risk from MITC exposure. The second of these documents (US EPA, 1995), provided MOE values for acute and subchronic exposures. It is a revision of the first (US EPA, 1994c), which provided MOEs only for acute exposures. Because the toxicity studies used to gauge acute risk were different in the two EPA documents, it is of interest to examine both and compare them to the values generated in the current document by DPR.

In 1994, the US EPA issued an assessment of risk to workers and residential / bystanders from metam sodium following soil applications (US EPA, 1994c). Included in that document was a risk assessment for short-term (acute) exposure to MITC. EPA based its risk calculations on a critical short-term NOEL of 3 mg/kg/day derived from a rabbit developmental toxicity study. EPA did not have the benefit of the human eye irritation study, issued in 1996, upon which DPR has based its current acute risk assessment. Exposure values developed by EPA were based on one of the application site monitoring studies also used by DPR. Short-term MOEs calculated by EPA for residents and bystanders ranged from 16 to 135. EPA revised its risk estimates after it reviewed the Rosskamp (1978) 12-13 week rat subchronic toxicity study (US EPA, 1995), basing the revised MOE calculations on that study. MOEs were calculated using the same exposure numbers as before. Unlike the current DPR document, acute and subchronic risk ("short and intermediate term toxicity" in their terminology) were not distinguished by EPA, *i.e.*, the same NOEL and MOEs were considered appropriate to both exposure scenarios. EPA derived the critical NOEL of 1 ppm from the Rosskamp study, considering the observed toxicity to be systemic in nature. In converting the air concentration to an estimate of internal dose, EPA assumed that the length of exposure was not 4 hours (which was the exposure time used by Rosskamp [1978]), but 24 hours. This resulted in an internal dose estimation of 2.4 mg/kg/day. The final MOE values calculated by EPA ranged between 13 and 108. In comparison, using the NOEL provided by the human eye irritation study and several additional exposure scenarios provided in the DPR Exposure Assessment, DPR calculated acute ambient MOEs between 15 and 2200 and acute application site MOEs between <1 and 17 (current document). DPR's ambient seasonal MOEs ranged between 28 and 166,667, while the application site seasonal MOEs ranged between 1 and 50.

the discomfort category, endpoints were derived for upper respiratory tract irritation, lacrimation and eye irritation. Within the disability category the endpoints were growth retardation and upper respiratory tract irritation. Within the lethality category the endpoints were LC_{50} and LC_{80}. In contrast to this approach, DPR, as presented in the current document, established regulatory endpoints based on the length of exposure, separating these into effects resulting from acute, subchronic or chronic exposures. The actual endpoints chosen represent the lowest measured concentration in each category, theoretically protecting the population from any effects that may occur at higher concentrations.

Direct comparisons are possible where regulatory endpoints were based on the same studies or where the aim was to protect the public from similar types of damage. OEHHA generated an REL for ocular irritation of 0.35 ppb, with a corresponding 1-hour REL of 0.5 ppb. These figures were based on an observation of eye irritation in cats at 67 ppb, with a corresponding NOEL of 35 ppb (Nesterova, 1969). The REL was calculated by dividing the NOEL by 100 to account for intra- and interspecies variations in sensitivity. Presumably, OEHHA used the Nesterova study because no others were available at the time to provide a threshold for eye irritation. DPR's acute REL value of 22 ppb (applicable as a 1, 8 or 24-hour REL) was based on direct experimental observations of eye irritation in humans after controlled exposures which established a NOEL of 220 ppb and LOEL of 800 ppb (Russell and Rush, 1996). For a discussion of the comparative aspects of the Nesterova *vs.* the Russell and Rush studies, see section XVIII.D.1. above.

As stated above, OEHHA did not provide a subchronic NOEL *per se* for MITC. The only studies of regulatory significance cited by OEHHA in which the animals received more than acute exposures were developmental toxicity studies. Nine-day exposures in pregnant rats and rabbits leading to maternal and fetal growth retardation (Irvine, 1983 and 1984) generated NOELs of 5 and 3 mg/kg/day, respectively, with corresponding RELs of 50 and 30 μg/kg/day (Alexeeff, 1994). However, DPR does not generally view such short-term exposures as amounting to a subchronic exposure scenario. In addition, oral studies such as these are not clearly applicable in cases where the primary exposure is by inhalation. Only in the absence of a subchronic inhalation toxicity study might endpoints be derived from studies using an alternate exposure route. Interestingly, OEHHA, in its full risk assessment document (OEHHA, 1992), did discuss Rosskamp (1978), one of the two subchronic inhalation toxicity studies that were reviewed by DPR in the current document. OEHHA suggested that the 1 ppm exposure level be considered a LOEL due to evidence of "changes in organ weights" at that dose (OEHHA, 1992; page E-16). A closer reading of the OEHHA document suggests, however, that OEHHA only had access to a summary of the Rosskamp study; consequently they were not aware that the only statistically significant absolute organ weight changes occurred in male pituitaries. Statistically significant relative pituitary weight changes were not observed at any dose, nor were any statistically significant organ weight changes at all observed at the next higher dose of 10 ppm. It is, consequently, very unlikely that a LOEL of 1 ppm was appropriate in this case. DPR designated 1 ppm as the NOEL,

not LOEL, dose in the current document. In any case, Rosskamp (1978) was not used by either agency to characterize risk associated with MITC exposure.

XV.   CONCLUSIONS

Acute exposure. The acute NOEL selected for evaluating potential adverse health effects in humans exposed for short periods to vapors of MITC following the agricultural use of metam sodium was 220 ppb. This was based on the finding of eye irritation at a LOEL dose of 800 ppb in a human experimental toxicity study. Results of short-term ambient air monitoring for MITC from 3 studies (2 from Kern County utilizing several receptor sites, one from Lompoc) were used in the estimation of 1-hour, 8-hour and 24-hour ambient residential / bystander exposures. These concentrations ranged from 0.1 to 14.6 ppb, yielding MOEs between 15 and 2200. Because the MOEs did not go below the benchmark of 10, which is recommended for health protection when the toxicity evaluation derives from human data, human health concerns were not indicated under ambient scenarios. Furthermore, exposure levels did not exceed the acute REL value of 22 ppb, which was considered to be relevant for exposure times between 1 and 8 hours. Results of application site air monitoring from 4 soil injection studies and 3 fixed-set sprinkler studies were used in the estimation of 1-hour, 8-hour and 24-hour application site residential / bystander exposures. Air concentrations for these studies ranged between 41 and 2853 ppb for 1-hour exposures, between 32 and 2348 ppb for 8-hour exposures, and between 12.7 and 1102 ppb for 24-hour exposures. MOE ranges for 1-, 8-, and 24-hour application site exposures were <1 to 5, <1 to 7, and <1 to 17, respectively. These low MOEs indicate a potential for human health impacts under application site scenarios. The application site exposure levels were, moreover, above the acute REL of 22 ppb under many conditions. (Not all of the applications were performed under conditions which would be considered compliant with more recent Technical Information Bulletins (TIBs) and product labels for metam sodium. Several applications would be currently illegal because they did not use a post-application water seal; they were included in this document for comparative purposes. An additional study was conducted under conditions that were possibly consistent with the presence of an inversion layer. The results of this study should be interpreted with caution. Finally, it should be noted that buffer zones of up to 0.5 miles are currently required for sprinkler applications [buffer zones are not currently required for injection applications]. Many of the sprinkler application measurements were made within these currently recommended buffer zones.)

Exposure estimates within the first three days of the Cantara Loop spill were as follows: the Office of Environmental Health Hazard Assessment estimated the peak 1-hour time weighted average concentration to be 1300 ppb for a distance of up to 100 meters from the river, dipping to 340 ppb at 500 meters; DPR's maximal 1-hour time weighted average estimates were 4500 ppb at 100 meters and 1240 ppb at 500 meters downwind from the river; the Metam Sodium Task Force estimated maximum 1-hour time weighted average air concentrations of MITC at the river's edge to be between 3 ppm and 650 ppm. Although there was considerable uncertainty associated with these values, derived as they were from plume modeling estimations, they may account for the plethora of short-term and possible persistent irritative symptoms that were reported. In addition, air concentration modeling after the Earlimart

incident suggested that the symptoms, which were largely irritative in nature, were induced after exposure to MITC concentrations in the 0.5-1 ppm range (lower bound estimates). It is plausible that, under the current use and exposure conditions, irritative endpoints, very likely not restricted to ocular irritation, will be realized in the field.

Seasonal exposure. The estimated critical subchronic NOEL of 100 ppb, derived from the rat 4-week inhalation toxicity study (Klimisch *et al.*, 1987), was calculated from the study LOEL of 1.7 ppm by applying Haber's Law and dividing the result by a default uncertainty factor of 3. The LOEL was based on a finding of nasal epithelial atrophy subchronic inhalation exposure of rats at that dose. Results of ambient air monitoring from the same 3 studies as were used in the acute risk analysis study generated moderate term MITC concentration estimates of 0.0006-3.54 ppb. Corresponding ambient MOE ranges were 28-166,667. Because an MOE of 100 is generally considered to be protective of human health for adverse effects observed in animal studies, the former range indicated a potential for ambient health effects (three MOE determinations, at Weedpatch in 1993 and 1997 and at Lamont in 1993 dipped below 100). Application site monitoring showed mean seasonal air levels to range between 2 and 80 ppb depending on site and distance from the application. These values resulted in MOEs between 1 and 50, indicating a potential impact on human health. This was further emphasized by the subchronic REL determination of 1 ppb, clearly below levels measured at many application sites. (However, the caveats mentioned above under Acute Exposure also apply for subchronic exposure.)

Chronic exposure. MOEs based on chronic toxicity studies were not developed in this draft because chronic exposure estimates were not presented in the Exposure Assessment. However, a conditional chronic REL, which did not rely upon exposure estimates, was estimated in the eventuality that air monitoring at some future time would indicate a potential for chronic exposure. This was done by using a default procedure of dividing the subchronic REL by an uncertainty factor of 10, generating the chronic REL value of 0.1 ppb. Whether or not a chronic human health concern is indicated will be evaluated when chronic exposure data become available.

The 2-year rat drinking water study provided weak evidence that MITC may have induced mammary fibroadenomas and carcinomas in females. A small increase in subcutaneous fibromas was also noted at the high dose, though it was unclear if MITC was responsible for the rise. The 2-year mouse drinking water study provided evidence that MITC may have induced cutaneous fibrosarcomas in both sexes. However, the data from neither long-term drinking water study were sufficient to trigger a quantitative oncogenic risk evaluation. In mice, various serum chemical, hematologic and histologic alterations were also noted at the top 2 doses, with amyloid degeneration in the kidney possibly increased at all doses in males and ovarian cysts in high dose females.

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