

Dose Response Assessment from Effects of Acute Exposure to Methyl Isothiocyanate (MITC)

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ABSTRACT

We determine the short-term health protective concentrations of methyl isothiocyanate (MITC) for airborne exposures to bystanders near treated agricultural fields. This determination is made from an understanding that, at concentrations of environmental relevance, MITC most likely acts via stimulation of the trigeminal nerve, which mediates sensory irritation in the eyes and nose (the critical effect from short-term MITC exposures). The outcome of a clinical study that included sensitive individuals and measured multiple ocular responses to irritation (e.g. perceived irritation, tearing, and blinking of the eyes) is consistent with this proposed mode of action, as are experimental animal data. Databases and studies by the California Department of Pesticide Regulation (CDPR) show that, in accidental exposures, human eye irritation is consistently the most sensitive endpoint at low-modeled acute exposure, and most often the sensitive organ from exposures of unknown, but likely higher, concentrations. In this determination of health protective concentration, sufficient information exists for the overall MITC database and for sensitive individuals, such that an overall uncertainty factor of 1 is appropriate. Based upon benchmark concentration lower limits from the clinical study, health-protective concentrations of MITC are shown to be 0.2 ppm for 4 hours of exposure and 0.8 ppm for 14-minutes of exposure.

Keywords:

Methyl isothiocyanate; MITC; Sensory Irritation; Uncertainty Factor; Benchmark Concentration; lachrymator

INTRODUCTION

Fumigants, such as metam-sodium, metam-potassium, and dazomet, undergo decomposition to the biocide methyl isothiocyanate (MITC) in moist soils. As more fully described by Cain et al. (2009) and the Environmental Protection Agency (EPA, 2008a), at low levels of acute exposure to the vapor, humans may experience irritated or burning eyes, an outcome consistent with the appellation of MITC as a lachrymator. At some low levels, and certainly at higher levels, the spectrum of symptoms may expand to include nasal irritation, throat irritation, rash, headache, nausea, salivation, coughing, and shortness of breath. Not surprisingly, an increase in duration of exposure will exacerbate most symptoms. Their nature implies that they arise directly at the point of contact (EPA, 2008a).

The presence of MITC in certain agricultural areas prompts a need to assess the risk of adverse effects to people following acute exposures. This report uses, as a launching point, an analysis by Cain et al. (2009) of a human study with exposures restricted to the eyes on the grounds that, as a lachrymator, MITC would exert its effects on that organ first and at the lowest concentration (Russell and Rush, 1996). Since the human study looked only at eye irritation, human data from accidental exposures to MITC in California provided a supplement to address the sensitivity of the eye versus other potential target organs, such as the airways. Laboratory data from rodents gave perspective on the vulnerability of the eye vs. the airways at relatively severe levels and durations of exposure.

METHODS

Mode of Action (MOA) and Chemical Specific Adjustment Factors (CSAFs)

We discussed the available information on MITC following the Environmental Protection

Agency (EPA) and International Programme on Chemical Safety (IPCS) mode of action (MOA) frameworks (e.g., Boobis et al., 2008; EPA, 2005) and the related uncertainty factors for non-cancer toxicity, specifically: experimental animal to human extrapolation, use of a LOAEL rather than a NOAEL, use of a study of insufficient exposure durations, database incompleteness, and within-human variability (EPA, 2002). We also followed the IPCS (2005) guidelines on replacement of default toxicokinetics and toxicodynamics uncertainty factors by Chemical Specific Adjustment Factors (CSAFs) for interspecies or intraspecies differences. The approach divides uncertainty into weighted subfactors for toxicodynamic and toxicokinetic information and values them accordingly (see Figure 1). The CSAF can range from either below or above the default subfactor, depending on the toxicodynamic and toxicokinetic properties of the chemical. When the CSAF lies below the default for the critical effect, then IPCS guidelines call for examination of these subfactors for endpoints other than the critical effect.

The IPCS (2005) framework has support in general principles of EPA guidance (EPA, 2002, 2005), and in standard operating procedures for setting Acute Exposure Guideline Levels (AEGs) (National Research Council, 2001) and Reference Concentration (RfC) (EPA, 1994a, 1994b). EPA used the IPCS framework in its consideration of chloropicrin, a chemical which triggers effects similar to those of MITC, and also used aspects of the framework regarding eye irritation as the critical effect for MITC (EPA, 2008a).

In the IPCS (2005) framework, an intraspecies, or interindividual, factor of 1.0 for toxicokinetic variability will often serve for local or contact sensory irritation since absorption, distribution, metabolism and elimination are often of negligible relevance, i.e., variability is not expected in the tissue dose of the active form of the chemical from direct contact. An intraspecies factor of 3.16 ($10^{0.5}$), the default value, may then be used for toxicodynamic

variability, since variation in responses are expected among individuals given the same tissue dose. For MITC as an eye irritant, this framework might support a CSAF below 10, but if so, such consideration would also trigger a review of other toxic endpoints such as irritation-mediated effects on the lungs.

Epidemiology and Experimental Animal Information

We took as a starting point for this dose response assessment the evaluation by Cain et al. (2009) of a clinical study of MITC exposure to the eyes of human volunteers by Russell and Rush (1996). We also sought epidemiology and experimental animal information to address the limited monitoring of effects in this human study. We analyzed several human accidental MITC-specific exposures, including Akanda (2007), O'Malley et al. (2004, 2005) and the California Department of Pesticide Regulation (CDPR, 2001). We also analyzed several experimental animal studies in the EPA database for MITC pesticide submissions, including Jackson et al. (1981), BASF (1987) and Rosskamp et al. (1978).

RESULTS

Human Studies

Cain et al. (2009) found that benchmark dose (BMD) modeling, applied to the number of responders from Russell and Rush (1996) yielded acceptable and nearly identical fits for the logistic and probit models. Modeling results are shown in Table 1, where the ten Berge model shows BMCs based on the integration of all three times points; and the EPA model gives BMCs and BMCLs for individual time points.

In an enumeration of adverse effects associated with exposures to agricultural uses of metam-sodium, metam-potassium or dazomet between 1992 to 2003, the California Department

of Pesticide Regulation (CDPR) reported that 657 of 777 cases, or approximately 85%, involved either eye effects alone or eye effects in conjunction with respiratory, skin, or systemic effects (Akanda, 2007). Only 120 cases, or approximately 15%, lacked eye effects, some because exposure involved just the skin. The CDPR information (Akanda, 2007) did not include estimates of levels of exposure.

However, of particular interest within this larger database is an exposure on bystanders following shank-application of metam-sodium in Arvin, CA, reported by O'Malley et al. (2005), where estimates of exposure were given along with symptoms of either burning or tearing eyes alone or in conjunction with sore throat. Residual symptoms included headaches, vomiting, nausea, and asthma or lower respiratory irritation. For this incident, we categorized the responses of people whom had been definitely or probably exposed according to CDPR (personal communications, see Appendix D in *TERA* 2008) by the symptoms of eye irritation alone, eye irritation and any other symptom, and another symptom without eye irritation. We segregated this information by CDPR provided distance codes: Code 1 referred to people who resided on the road bordering the treated field, Code 2 to the people who resided on the second closest road to the treated field, and Code 3 to a packing plant located beyond the second road. These distance Codes were likely to be good indicators of exposures since the incident occurred in the late evening, when more people were likely to be home. As distance from the agricultural field increased (from Code 1 to Code 3), so did the percent of symptoms of just eye irritation. Commensurately, the percent of eye plus other symptoms decreased (Figure 2). Of the 245 responders, only two people reported respiratory symptoms without eye irritation, one was located in Code 1 and the other in Code 2. Of 71 responders working at the 3rd-shift of a carrot packing plant on the outskirts of the residential area (Code 3), 70 reported just eye irritation and

one reported both eye and respiratory symptoms, specifically throat irritation. We computed the average concentrations within the three codes based on information provided by O'Malley et al. (2005); these averages ranged from 0.95 ppm for Code 1 to 0.45 ppm for Code 3. The result suggests that the carrot workers, on average, would have had about half the level of exposure as persons adjacent to the field.

Apart from information on this and other incidents, the CDPR (2008) database included information on asthmatics with respiratory symptoms. *TERA* (2008) compared the notes provided in the CDPR summaries with the full medical reports provided by CDPR and the Pesticide Episode Investigation Supplemental Reports in an effort to piece together the complete story behind each case. Some discrepancies appeared. In some cases, the medical reports or supplemental reports indicated that the person's symptoms were unrelated to the chemical. In other cases, the brief CDPR summaries either omitted information relevant for risk assessment or the reports contained no additional information. Of the 13 asthmatics identified in the complete medical records, only four had symptoms unrelated to eye irritation that were clearly connected to MITC exposure. Two of the four also had severe health problems unassociated with asthma that may have lead to hypersensitivity. All four cases also show effects at non-portal of entry tissues, suggesting that they may have been individually exposed to concentrations greater than the estimated minimal levels (*TERA*, 2008).

Experimental Animal Studies

A review of experimental animal data for eye or lung effects of MITC identified one well-designed study of acute toxicity (Jackson et al., 1981) and two of short-term to subchronic

exposure (BASF, 1987; Rosskamp et al., 1978). The acute study of Jackson et al. (1981)¹ is relevant for acute exposure assessment because the authors reported the duration to onset of clinical signs, including eye irritation, in addition to mortality. These authors conducted a four-hour inhalation study with MITC on seven groups of 10 (five per sex) Sprague-Dawley rats. Rats were exposed to 0, 94, 166, 190, 210, 263 or 548 ppm (corresponding to 0, 282, 496, 570, 628, 786, or 1640 mg/m³, respectively).

Figures 3a and 3b show our modeled pattern for eyes and lungs effects from Jackson et al. (1981). At the lowest concentration (93 ppm) and shortest time (15 minutes), 100% of the rats showed eye effects. This percentage decreased as concentration and time increased, most likely due to response fatigue. The number of rats that showed lung effects in Figure 3b increased from zero at the lowest concentration and shortest time and then increased as both a function of time and concentration. For the shortest duration examined, 15-minutes, LOAELs for eye irritation and lung irritation were approximately six-fold different.

Figure 3c combines both Figures 3a and 3b. Only at combinations of exceedingly high concentrations and long durations does the potency of MITC to irritate or otherwise impact the lung catch up with its ability to irritate or impact the eye. The pattern of convergence of potency in the direction of high concentrations and long durations should of course reflect itself in the opposite direction of divergence. At the much lower levels of stimulation pertinent to the human study, the pattern of divergence would likely show itself even more extremely, namely an even larger ratio of the eye vs. lung effect levels. This study clearly illustrates that eye symptoms are the first to develop in time; signs of lung irritation develop later.

¹ See *TERA* (2008) for a more in-depth analysis of the Jackson et al. 1981 study.

Mode of Action

A first step in determining a safe concentration for MITC is to establish the mode of action (MOA) for its critical effect of eye irritation. This MOA appears to be stimulation of the trigeminal nerve as discussed above. We analyze several criteria related to this MOA below.

Key events: The human study (Russell and Rush, 1996), the experimental animal data, and the available human incidents all support sensory ocular irritation as the choice for MITC's critical effect. Moreover, all of the quantitative data show that eye irritation occurs first as MITC concentration increases (e.g., Figures 2 and 3c), with the exception of one person in the carrot factory who showed both eye irritation and sore throat. Figure 4 outlines the key events for MITC's eye irritation.

Concentration-Response and Temporal Association: Figures 2 and 3 of Cain et al. (2009) and Figures 3a,b,c of this paper show a clear onset of effects as a function of both MITC concentration and exposure time, including those of the key events highlighted in Figure 4. As concentration and time increase, the severity of effects also increases. Sensation in the eyes evolves into eye irritation and then respiratory irritation. Acutely, eye effects are more sensitive than the expected or observed lung effects by between 2 and 6-fold.

Strength, consistency, and specificity of association of key events: Observations in humans support the key events outlined in Figure 4. A comparison of Figures 2 and 3 of Cain et al. (2009) shows that MITC evokes the sensation of ocular discomfort without producing clinical signs of eye irritation. Four or eight-hour exposures to 0.22 ppm failed to produce even sensations of eye irritation. Similar progressions, but among effects of higher severity, are found with modeled concentrations for either humans (Figure 2) or rats (Figure 3c).

Biological plausibility and coherence: The postulated MOA is biologically plausible as evidenced by both the temporal concordance and concentration dependence of eye irritation, and from considerations of dosimetry from similar, soluble, irritating, inhaled chemicals, such as chloropicrin (EPA, 2008c; *TERA*, 2005) and carbon dioxide (CO₂) (Stevens and Cain, 1986; Kjaergaard et al., 1992; Shusterman et al., 2001; Frasnelli and Hummel, 2003; Shusterman et al., 2003). Coherence is addressed by consistent portal-of-entry effects in both the rodent and human studies; although recognition of eye sensations is not possible in experimental animals.

Uncertainties, inconsistencies, and data gaps: While gaps in the database exist regarding the concentration and onset of nasal and lung irritation in humans, and regarding eye irritation beyond eight-hours exposure, human incident reports are somewhat helpful. These reports show a progression of eye, nasal and lung irritation that is roughly similar to what is observed in experimental animals. With the exception of the Arvin incident as shown in Figure 2, however, these incident reports cannot be used to make definitive statements, since exposures are generally not known or modeled.

Controlled exposures to humans for concentrations much above 3.3 ppm are not available, since such concentrations might be expected to produce severe irritation. However, the current temporal concordance data as shown in Figures 2 and 3 of Cain et al. (2009) suggest that as duration of exposure continues, the sensation of eye feeling would not progress unless the concentration also increased. This suggestion is supported by roughly similar NOAELs and LOAELs for nasal and lung irritation after exposure to rats for 20 and 90 days, respectively (BASF, 1987; Rosskamp et al., 1978---data not shown but available in *TERA*, 2008).

Uncertainty factors

A second step in determining a safe concentration for MITC is to judge the appropriate uncertainty factors based on the available data, following EPA (2002) and IPCS (2005) guidelines. Since we judge, as do others (e.g., EPA 2008a, 2008b) that the human study of Russell and Rush (1996) is the best basis for the development of short-term safe concentrations, and it contains a NOAEL, uncertainty factors for extrapolation from experimental animals to humans, LOAEL to NOAEL and duration of exposure, are not needed.

An uncertainty factor for sufficiency of database might be needed, if some question existed as to whether symptoms in the eye are not the critical effect. However, review of the available literature, summarized above and in previous reports (e.g., EPA, 2007a, 2007b; *TERA*, 2007) indicates that eye symptoms occur first, or at least concurrently with other symptoms, in the field exposures where exposures are quantified (see Figure 2). Eye effects also occur first in controlled exposures to experimental animals in both time and concentration (see Figure 3c). No study has indicated any adverse effect below the threshold for eye irritation in short-term exposures. Accordingly, the use of any uncertainty factor for deficiency of the database seems unwarranted.

In contrast, an uncertainty factor for within human variability, which protects sensitive populations, needs consideration. Toxicokinetic and toxicodynamic variations among individuals are involved when choosing this factor to protect sensitive populations. Both EPA (2002) and IPCS (2005) guidelines indicate that the usual factor of 10-fold for within-human variability can be considered as two equal parts, one for toxicokinetic variability and one for toxicodynamic variability, and that each subfactor is “assigned” a value of $\frac{1}{2}$ log base 10, or an approximate value of 3. Separate evaluations of specific data on toxicokinetic and toxicodynamic components are conducted.

In general, a reduced factor for the toxicokinetics component is often used for irritants, because the irritation is due to direct contact and the expected variability in toxicokinetics is nil to minimal. In the case of MITC, direct contact with the eye stimulates the trigeminal nerves as discussed in Cain et al. (2009). Figure 2 shows that eye effects are the first response in modeled human exposures; human incident reports from unquantified exposures show that eyes are likely the first response, or perhaps the first concurrent response. That eye effects are the first response is unequivocally demonstrated in acute experimental animal inhalation exposures. Thus, based on general principles and specific data, nil to minimal toxicokinetic variation among humans is expected and a CSAF of 1-fold, rather than the default value of 3-fold, is reasonable for the lack of toxicokinetic variability among humans to this critical effect of eye irritation.

Toxicodynamics are also important in determining and quantifying the sequence of events at the cellular and molecular levels that lead to a toxic response. This is because variations in receptor binding, number of receptors, and other conditions that might affect neurological response are known to exist among humans. Three main areas of investigation are outlined in the IPCS (2005) guidance document for the determination of the adequacy of the human and experimental animal data for refinement of the toxicodynamic component: relevance of population, adequacy of concentration-response data, and adequacy of number of subjects/samples. We address these questions for the critical effect, eye irritation. If the resulting CSAF for this endpoint is less than the default, we then consider other potential critical effects and what the appropriate uncertainty factor(s) would be, in order to determine the final CSAF.

Relevance of population: Ocular irritation shows only minor variation in sensitivity among subjects aged 18 to 35 years and screened for ocular health (e.g., Cain et al., 2005, 2007). The

chemesthetic studies also show quite steep stimulus-response (psychometric) functions at threshold. Furthermore, chemesthetic sensitivity diminishes little, if at all, from early to late adulthood, with some acceleration of loss in the seventh decade (Wysocki et al., 2003). Persons less than 18 years appear to have no greater sensitivity to irritating stimuli than do young adults. Children between five and 14 years evinced essentially the same sensitivity as subjects aged 15 to 20, and 21 to 54 (Hummel et al., 2007). Further support for a smaller factor than the default value of 3, for toxicodynamics of irritation endpoints, is provided by Kjaergaard et al. (1992), who demonstrated that the threshold for sensitive individuals lies within a factor of 2 of the (average) response of young adults. Note that the Kjaergaard ratio is between the mean response of the young adults and a highly sensitive group. Thus, based on the literature, toxicodynamic variability for irritation endpoints is likely to be smaller than the default toxicodynamic value of 3-fold would indicate.

Specific data for MITC also argue for a reduced toxicodynamic factor. For example, Russell and Rush (1996) tested human volunteers of both sexes of various ages and health conditions. The severity of eye irritation was quantified by both subjective and objective criteria. The subjective scales were clearly more sensitive than objective clinical signs. This fact is consistent with the MOA of MITC being stimulation of the trigeminal nerve. Furthermore, Russell and Rush (1996) included both males and females, younger and older subjects, smokers and nonsmokers, allergic and non-allergic subjects, and those exposed and not exposed to chemicals in the workplace. This inclusiveness suggests enough diversity in the Russell and Rush (1996) study to represent a range in the population. In particular, the age of the majority (58%) of subjects in Russell and Rush (1996) lay below 35 years, which is the sensitive age range for this kind of testing (Cain, unpublished observations). An initial analysis of these data

showed that the younger members of the Russell and Rush (1996) study showed an increased sensitivity (TERA, 2007). It follows that the resulting NOAELs or BMC_{10s} are more representative of those from sensitive subpopulations.

MITC incident reports support a consistent finding of eye irritation across all age groups, and also support the findings of Russell and Rush (1996). Irritation to the eyes is the apparent first effect in many children and adults at similar exposures. Thus, both data from literature reviews on irritation endpoints and specific data for MITC indicate that little toxicodynamic variability is expected and that a reduced factor is appropriate.

Adequacy of concentration-response data: All three exposure durations of the Russell and Rush (1996) human study provide timing of the critical effect in the relevant range of environmental exposure. An integration of three of these eye effects allowed adequate, duration-specific, quantitative information in order to develop BMCL_{10s} (see Table 1). We use these BMCL_{10s} of approximately 0.2 or 0.8 ppm as the points of departure to establish safe short-term inhalation levels. The results of the human study are consistent with the available literature regarding higher concentrations of MITC. This literature, both for humans and rats, suggests that both concentration and duration of MITC exposure are key components in the development of irritation, the onset of irritation, and the severity of the irritation.

In addition, the chosen BMCL_{10s} are from the individual time trials found in Table 1, and are expected to be lower than BMCL_{10s} that might be projected from the BMC_{10s} calculated using the preferred approach. This added conservatism in choice of BMCL₁₀, although modest, further supports the conclusion that a reduced factor for toxicodynamics will adequately protect the sensitive end of the general population.

Adequacy of number of subjects/samples: The human study of Russell and Rush (1996) evaluated a total of 70 subjects of both sexes and various ages and health backgrounds. Some subjects participated in more than one phase of the study. This study provided sufficient information for a sensitive subpopulation for sensory irritants, because the design of the study monitored effects based on a known sensitive subgroup, young adults. As expected, variability existed in the ability of these subjects to detect MITC, with individuals less sensitive to the feeling of MITC being unable to detect it at the lower concentrations. At the lowest concentrations at each time point, no convincing irritation was produced, most likely due to the fact that concentrations were not high enough to achieve surface reactions in the eye or that other defensive mechanisms of the eye were not overwhelmed.

Incident data provide support for the use of the findings of Russell and Rush (1996). As previously discussed, a majority of over 700 people in these incidents reported experiencing eye irritation (Akanda, 2007; O'Malley et al., 2004, 2005); several asthmatics also report eye irritation and other symptoms. The Arvin incident also shows increasing severity of effects with relative increase in concentration of MITC (Figure 2). Additionally, the acute animal study by Jackson et al. (1981) shows that eye effects occur first, but that with increased concentrations and time respiratory effects will develop (Figure 3c). These effects are parallel to those seen in the human incident reports.

Summary of the intra-species factor for eye irritation: Based on the known MOA for MITC, the consistency and specificity of eye irritation in both young and older individuals from a relevant population, the adequacy of concentration response information, an adequate number of individuals, along with matching eye and irritation effects from experimental animals, and the choice of conservative BMCL_{10S}, it is reasonable to reduce the toxicodynamic component of the

intraspecies factor to 1.

Therefore, the overall intraspecies factor for MITC, based on both toxicokinetics and toxicodynamics, is reduced to 1-fold.

Uncertainty factor for lung irritation: The IPCS (2005) guidelines recommend additional evaluations for effects closely related to the critical effect, in this case eye irritation, when the CSAF for the critical effect is less than the overall default value of 10-fold. For MITC, lung irritation is a closely related effect. Thus, it is appropriate to consider whether an uncertainty factor for this endpoint would suggest a safe concentration after short-term exposure that is lower than that based on eye irritation.

As shown in figure 3c, a clear 6-fold or greater separation exists in rats among LOAELs for eye and lung irritation after 15 minutes from Jackson et al. (1981); although this difference wanes at longer exposures, it is likely due to fatigue of the eye's protective responses (e.g. blinking, tearing, and eye closure). This is confirmed by benchmark concentrations calculated in TERA's response to an EPA memorandum (TERA, 2009). Using the data from Jackson et al. (1981), a benchmark concentration (BMC) of 48 ppm was calculated for eye effects and a BMC of 650 ppm was calculated for lung effects following 1.5 hours of exposure; this is a 14-fold difference in effect levels. BMCs calculated from the Jackson et al. (1981) data for 2.5 hours of exposure are 48 ppm and 140 ppm for eyes and lungs, respectively; though there is only a 3-fold difference between these effects at this time-point, it is still clear that eyes react prior to the onset of lung irritation and the effects of MITC are dependent on both time and concentration of exposure. See TERA (2009) for more information on the methods used in developing these BMCs. Information from the Arvin incident also show a separation between eye effects and eye

and other effects, but the difference between the average concentrations of distance codes 1 and 3 are only about 2-fold.

A potential concern is for effects on asthmatics, since Russell and Rush (1996) excluded persons with symptoms of cold or allergy during the exposures, or who had recent asthma attacks, and the MITC exposures were only to the eyes. Data of case reports from CDPR show the majority of asthmatics exhibited eye effects only, but some of them noted effects, in the absence of reported eye effects. Unfortunately, details in these cases are scant and actual or estimated exposure concentrations are not available. Thus, it is possible that these asthmatics were exposed to a concentration above that needed to evoke eye irritation, and that the expected eye irritation was not recorded, or its lack resulted from fatigue, as is apparently the case in the acute experimental animal study. Relatively few and inconsistent data are available in the literature on this issue and the relative sensitivity of asthmatics versus healthy individuals to the respiratory irritant effects of sensory irritants is not generally known. An analysis on chloropicrin, a related irritating fumigant, indicated that respiratory effects only occurred at concentrations above the $BMCL_{10}$ for ocular irritation *TERA* (2005). Similarly, EPA (2008c) evaluated the incident reports for chloropicrin and determined that the data do not suggest that individuals with asthma are more sensitive to chloropicrin.

Perhaps the most reasonable explanation of the observed difference in effect levels between the eye and lungs, however, comes from dosimetry understanding. MITC is highly water-soluble and at low ambient concentrations, the upper airways will strongly attenuate the dose to the lung. Thus, a NOAEL or BMDL for eye effects in sensitive humans, will very likely be health protective for lung effects in asthmatics.

Health Protective Concentrations

The best estimate of a health protective concentration for a four-hour exposure is 0.2 ppm. This is determined by dividing the average of the BMCL₁₀s of either 0.20 or 0.22 ppm for the four-hour trial found in Table 1 by an uncertainty factor of 1 for eye irritation and rounding to one digit. The best estimate of a health protective concentration for a 14-minute exposure is 0.8 ppm. This value is determined by dividing the average of the BMCL₁₀s of either 0.83 or 0.78 ppm for the 14-minute trial found in Table 1 by an uncertainty factor of 1 and rounding to one digit.

DISCUSSION

The currently available database suggests that sensory irritation in the eye is the immediate precursor to an adverse eye effect and thus a critical effect consistent with the EPA (2009) definition. Sensory eye irritation might also be seen as a biomarker or surrogate for respiratory effects, and, thus, a valid endpoint for protection against these more serious effects. Categorizing such irritation as only a biomarker, however, may unnecessarily limit analysis of MITC and its plausible MOA. In essence, the consideration of sensory irritation as only a biomarker, rather than the critical effect, might serve to raise the safe concentration. Admittedly, such movement upwards might be scientifically appropriate.

In addition, the most likely MOA for response to MITC, based on both human and animal data, is stimulation of the trigeminal nerve. A long-latency of response to MITC found in Russell and Rush (1996) may lead one to question this MOA. However, ratings of perceived magnitude of irritations from Russell and Rush (1996) indicated that time-dependence occurs for concentrations as low as 0.8 ppm. The function for that concentration suggests that irritation

may emerge in the range of tens of minutes and perhaps as long as an hour. The increase continued until about two hours when the response began to wane. A trade-off between latency and rate of increase of response characterizes TRP ion channels as well; the lower the concentration, the longer the latency. For example, in what Brône et al. (2008) presented as a representative case, a 32-fold increment in concentration of the agent CS reduced latency by perhaps 100-fold. All agents studied by Brône et al. (2008) showed similar time- and concentration-dependence responses. In addition, Cain (unpublished observations) showed a 20-minute latency for irritant response in humans to a 50 ppb exposure of chloropicrin. The latency decreased to 3 minutes for the level 150 ppb. Only at levels into the hundreds of ppb did latency drop below about 30 seconds. Hence, this concern that a long-latency of response might question the trigeminal nerve basis for our proposed MOA, seems misplaced.²

Another issue for consideration is the usefulness of the human incident reports. For example, several of the MITC incident reports are from skin exposures, and in some cases from skin exposures involving people wearing goggles. Such exposures do not help in determining a safe air concentration, but instead may confound the determination of the critical effect from inhalation exposures. In contrast, other MITC incident reports allow some quantification and,

² A more important question arising from this second issue, however, concerns whether long latency affords further protection to the exposed person over the case where the agent has its maximum effect immediately, i.e., with no appreciable latency. To answer this question, one needs to consider scenarios of exposure. Presumably, many exposures last for minutes or tens of minutes. In such cases, the person may never experience the effects of exposure. This will depend upon concentration. As the data of Russell and Rush (1996) show, higher concentrations will cause responses at shorter latencies and the responses will increase with time. Even at the higher concentrations in the study, MITC would warn with low-level sensations before these become high level. For the sensations to become intense the person needs the passage of time in contact with the agent. Again, this is consistent with studies that found TRPA1 as a receptor for plant-derived noxious deterrents, such as iso-thiocyanates, the pungent ingredients in mustard, wasabi, and horseradish, and thiosulfinates in garlic and onions (Escalera et al., 2008). We make the point that MITC occurs naturally in capers, a pungent edible material.

more importantly, a higher degree of confidence in the estimated relative exposures. For example, the Arvin incident included medical reports and surveys of the residents with the residents' addresses. Since this exposure occurred in the late evening, when residents were likely to be at home, the medical reports can be categorized by resident-based distance from the epicenter of contamination, providing a confident measure of relative exposure.³

It is also important to consider whether the use of the experimental animal studies as supporting information for the development of a safe concentration based on the clinical human study is appropriate. We acknowledge that the Jackson et al. (1981) study in rats has limitations for supporting the development of safe air concentrations from the clinical studies, as it was designed to study acute lethality. More sensitive measures of eye and lung parameters in the Jackson et al. (1981) would have suggested more appropriate ratios than what we calculate with the existing data. However, more sensitive and appropriate measures of lung parameters, when compared with more sensitive and appropriate measures of eye parameters, may yield the same ratios as we calculate. In either case, a consideration of all data shows it unlikely that sensitive lung effects would precede sensitive eye effects as concentrations increase.

In addition, alternative MOAs, and principally one regarding the co-occurrence of eye effects, respiratory effects, and skin effects at similar concentrations, should be considered, and some support for this MOA is provided by the incidence data from Akanda et al. (2007) that show eye and other effects at apparently similar concentrations. In contrast to this apparent

³ Use of distance from the site of fumigation as a surrogate for concentration is a well-accepted epidemiological technique. For example, ongoing research on the effects of radiation on Hiroshima and Nagasaki survivors bases its dosimetry estimates on distance from the center of the explosion (see Radiation Effects Research Foundation at http://www.rerf.or.jp/general/research_e/raditiondose.html).

finding, however, is that MITC vapor is highly water-soluble. Water-soluble vapors and gases deposit themselves largely in the mucus-lined upper airways. As Medinsky and Bond (2001) illustrated, “[e]xperimental studies and mathematical models have shown that highly water-soluble vapors such as ethanol and methanol are almost entirely scrubbed by the nose on inspiration.” Furthermore, MITC is reactive, a property that favors even further its capture by the upper airways. As Medinsky and Bond also illustrate: “Once formaldehyde contacts the mucosal surface, it dissolves because it is highly water-soluble. Because it is a reactive gas, formaldehyde and its reactive products stay in that region of deposition.” These examples pertain to principles of inhalation toxicology and one needs only to know the properties for the materials of interest. Thus, the upper airways will afford protection of the lower airways from incident airborne MITC, particularly at low concentrations, and this alternative, co-exposure MOA is not favored.

The last and final issue relates to the choice of uncertainty factors for the development of the safe concentrations. A toxicokinetic factor of 1-fold is supported in the case of MITC, since our proposed MOA is receptor mediated at the portal of entry. Quite simply, since the response does not depend on kinetics to move the active chemical to the target tissue, variability in toxicokinetics among individuals is not likely to be relevant. Likewise, the most probative study for the determination of these safe concentrations, that of Russell and Rush (1996), supports a dynamic uncertainty factor of 1-fold because the critical effect is eye irritation, sensitive individuals were tested, and irritation is not expected to be greater in children (Hummel et al., 2007). MITC is a known lachrymator (which is why the Russell and Rush study used only eye exposures), and highly water-soluble. At low ambient concentrations, the upper airways should strongly attenuate the dose to the lung and may even prevent exposure to the lung.

The effect of MITC on sensitive subpopulations, such as asthmatics, is also an important consideration in this assessment. Although asthmatics might react more strongly than non-asthmatics to MITC at higher concentrations, the available data do not indicate their airways are more sensitive than their eyes. For example, out of the approximately 800 incident reports on MITC we previously summarized, 13 were known to be asthmatics (*TERA*, 2008). This response of ~2% (13/~800) is much less than the identified percentage of about 8% for asthmatics in the background population of Kern County (EPA, 2007a, 2007b), and Tulare County (American Lung Association, ALA, 2007), the sites of many incidents. Furthermore, as described in *TERA* (2008), 4 asthmatics had symptoms other than eye effects. These symptoms are likely to have been due to MITC exposures above the level needed to cause eye irritation, since all 4 described symptoms at other organs in addition to the portal of entry.

The current assessment has considered the human data in sufficient depth that an uncertainty factor can be derived based on the entirety of the data, and additional conservatism is unwarranted. Our judgment of a four-hour health protective value of 0.2 ppm is four-fold lower than that determined by the National Advisory Committee (EPA, 2008b) for its Acute Exposure Guideline Levels (AEGL) of 0.8 ppm. However, our judgment of a 14-minute health protective value of 0.8 ppm is identical to this committee's 10-minute value (EPA, 2008b). In both cases, the Russell and Rush (1996) study formed the basis of the evaluation and an uncertainty factor of 1 was the collective best judgment.

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REFERENCES

- Akanda, R. (2007) An evaluation of illnesses associated with exposure to agricultural uses of metam-sodium, metam-potassium, and dazomet. California EPA, DPR. HS-1862.
- American Lung Association (ALA). (2007). Estimated prevalence and incidence of lung disease by lung association territory. Epidemiology and Statistics Unit, Research and Program Services. April, 2007. Available at: <http://www.lungusa.org/atf/cf/%7B7A8D42C2-FCCA-4604-8ADE-7F5D5E762256%7D/ESTPREVLUNGDISSEASE0407.PDF>
- BASF. (1987) Study on the subchronic inhalation toxicity of methyl isothiocyanate in Wistar rats. Abteilung Toxikologie, Department of Toxicology, West Germany. Report no. 40I0231/ 8539.
- Boobis, A.R., Doe, J.E., Heinrich-Hirsch, B., Meek, M.E., Munn, S., Ruchirawat, M., Schlatter, J., Seed, J., and Vickers, C. (2008). IPCS Framework for Analyzing the Relevance of a Noncancer Mode of Action for Humans. *Crit Rev Tox* 38, 87–96.
- Brône, B., Peeters, P.J., Marannes, R., et al. (2008). Tear gasses CN, CR, and CS are potent activators of the human TRPA1 receptor. *Toxicol Appl Pharmacol* 231, 150-156.
- Cain, W.S., de Wijk, R.A., Jalowayski, A.A., Pilla Caminha, G., Schmidt, R. (2005) Odor and chemesthesis from brief exposure to TXIB. *Indoor Air* 15(6), 445-457.
- Cain, W.S., Schmidt, R., Jalowayski, A.A. (2007) Odor and chemesthesis from exposures to glutaraldehyde vapor. *Int Arch Occup Environ Health* 80(8), 721-731.

Cain, W.S., et al. (2009) Stimulation of the trigeminal nerve as a likely mode of action for acute sensory effects following exposure to MITC. Manuscript in Preparation.

CDPR (California Department of Pesticide Regulation). (2001) Illnesses related to exposure to metam-sodium by-products in Earlimart, California in November, 1999. CalEPA. HS-1808.

EPA (U.S. Environmental Protection Agency). (1994a). Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. Office of Health and Environmental Assessment. Washington, DC. EPA/600/8-90-066F. October.

EPA (U.S. Environmental Protection Agency). (1994b) Memorandum: Generic data in support of reregistration of methyl isothiocyanate (MITC). 011275.

EPA (U.S. Environmental Protection Agency). (2002). A review of the Reference Dose (RfD) and Reference Concentration (RfC) processes. Risk Assessment Forum. EPA/630/P-02/002F, December.

EPA (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment. Washington D.C. EPA/630/P-03/001B.

EPA (U.S. Environmental Protection Agency). (2007a). Metam-Sodium: Phase 5 Revised Chapter of the Reregistration Eligibility Decision Document (RED); DP Barcode: D337533, Metam-Sodium PC Code: 039003, Metam-Potassium PC Code: 039002, MITC PC Code: 068103. Memorandum from Charles Smith to Veronique LaCapra. Office of Pesticide Programs. April 12.

EPA (U.S. Environmental Protection Agency). (2007b). Human health risk assessment: Chloropicrin. Office of Pesticide Programs, Health Effects Division. Docket No. EPA-HQ-OPP-2007-0350-0005

EPA (U.S. Environmental Protection Agency). (2008a). Reregistration eligibility decision (RED) for the methyldithiocarbamate salts (metam-sodium, metam-potassium) and methyl isothiocyanate (MITC). Office of Pesticide Programs. Washington, DC. EPA 738-R-08-006.

EPA (U.S. Environmental Protection Agency). (2008b). Acute exposure guideline levels (AEGLs) for methyl isothiocyanate. National Advisory Committee. Proposed April 2008.

EPA (U.S. Environmental Protection Agency). (2008c). Reregistration eligibility decision (RED) for Chloropicrin. Office of Pesticide Programs. Washington, DC. EPA 738-R-08-009.

EPA (U.S. Environmental Protection Agency). (2009). Integrated Risk Information System (IRIS). Available at www.epa.gov/iris.

Escalera, J., von Hehn, C.A., Bessac, B.F., et al. (2008). TRPA1 mediates the noxious effects of natural sesquiterpene deterrents. *J Bio Chem* 283, 24136-24144.

Frasnelli, J., Hummel, T. (2003) Age-related decline of intranasal trigeminal sensitivity: is it a peripheral event? *Brain Res.* 987(2), 201-206.

Hummel, T., Roudnitzky, N., Kempter, W., Laing, DG. (2007) Intranasal trigeminal function in children. *Developmental Medicine & Child Neurology* 49, 849-853.

- IPCS (International Programme on Chemical Safety). (2005) Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration-response assessment. World Health Organization. Geneva.
- Jackson, G.C., Clark, G.C., Prentice, D.E., Read, R.M., Gopinath, C., Cherry, C.P. (1981) Methyl isothiocyanate acute inhalation toxicity in rats: 4 hour exposure. Huntingdon Research Centre. RZ- No: 81/082.
- Kjaergaard, S.K., Pedersen, O.S., Molhave, L. (1992) Sensitivity of the eyes to airborne irritant stimuli: the influence of individual characteristics. Arch. Environ. Health. 47, 45-50.
- Medinsky, M.A., Bond, J.A. (2001). Sites and mechanism for uptake of gases and vapors in the respiratory tract. Toxicology 160, 165-172.
- NRC (National Research Council). (2001) Standard operating procedures for developing acute exposure guideline levels for hazardous chemicals. Washington, DC, National Academy Press.
- O'Malley, M., Barry, T., Verder-Carlos, M., Rubin, A. (2004) Modeling of methyl isothiocyanate air concentrations associated with community illnesses following a metam-sodium sprinkler application. Am J Ind Med 46, 1-15.
- O'Malley, M., Barry, T., Ibarra, M., Verder-Carlos, M., Mehler, L. (2005) Illnesses related to shank application of metam-sodium, Arvin, California, July, 2002. Journal of Agromedicine, 10(4), 27-42.

Radiation Effects Research Foundation (RERF). (2007). Estimate of radiation exposure dose.

Available at: http://www.rerf.or.jp/general/research_e/raditiondose.html. Accessed April 3, 2009.

Roskamp, G., Schobel, G., Bhargava, A., et al. (1978) Methyl Isothiocyanate: ZK 3.318: A 12-28 13 Week Inhalation Study in the Rat, Project ID 374/77. Schering AG. (Summarized in 29 U.S. EPA 2006b).

Russell, M., Rush, T. (1996) Methyl Isothiocyanate: Determination of human olfactory detection threshold and human no observable effect level for eye irritation. University of California and Western Research Center, Zeneca. Lab Project Number: MITC_UCD_1A_1993: MITC_UCD_1B-1994: RR 96_049B, unpublished.

Shusterman, D. (2001) Odor associated health complaints: competing explanatory models. *Chem. Senses* 26(3), 339-343.

Shusterman, D., Murphy, M.A., Balmes, J. (2003) Differences in nasal irritant sensitivity by age, gender, and allergic rhinitis status. *Int. Arch. Occup. Environ. Health* 76(8), 577-583.

Stevens, J.C., Cain, W.S. (1986) Aging and the perception of nasal irritation. *Physiology and Behavior* 37(2), 323-328.

ten Berge, W. (2007). <http://home.planet.nl/~wtberge/doseresp.html>

TERA (Toxicology Excellence for Risk Assessment). (2005) Use of benchmark concentration modeling and categorical regression to evaluate the effects of acute exposure to chloropicrin vapor Part I. Technical report. August 2005. Available at <http://www.tera.org/Publications/Publications.html>.

TERA (Toxicology Excellence for Risk Assessment). (2007) Benchmark concentration modeling on the effects of acute exposure to Methyl Isothiocyanate (MITC). November 2007. Available at <http://www.tera.org/Publications/Publications.html>.

TERA (Toxicology Excellence for Risk Assessment). (2008) Benchmark concentration modeling on the effects of acute exposure to Methyl Isothiocyanate (MITC). Supplemental. September 2008. Available at <http://www.tera.org/Publications/Publications.html>.

TERA (Toxicology Excellence for Risk Assessment). (2009) Memo regarding the US EPA memorandum: Response to post-RED MITC toxicology related comments *March 2009*. Available at <http://www.tera.org/Publications/Publications.html>.

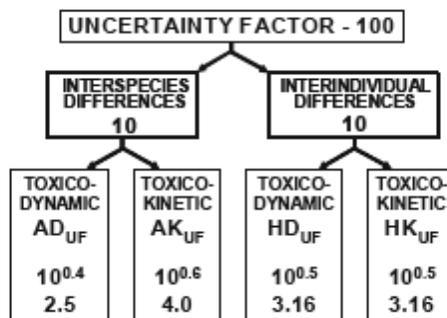
Wysocki, C.J., Cowart, B.J., Radil, T. (2003). Nasal trigeminal chemosensitivity across the adult lifespan. *Perception & Psychophysics*. 65(1), 115-122.

Table 1

BMC estimates (ppm) at the three exposure durations. BMCLs are in parentheses, when appropriate.

	Model	14 minutes	4 hours	8 hours
ten Berg	Logistic	1.5	0.53	0.36
	Probit	1.4	0.53	0.38
EPA BMDS 1.4.1	Logistic	1.4 (0.83)	0.37 (0.22)	NA
	Probit	1.4 (0.78)	0.33 (0.20)	NA

NA = not applicable



AD_{UF} - Uncertainty factor for animal to human differences in toxicodynamics
 AK_{UF} - Uncertainty factor for animal to human differences in toxicokinetics
 HD_{UF} - Uncertainty factor for human variability in toxicodynamics
 HK_{UF} - Uncertainty factor for human variability in toxicokinetics

Figure S-1. Subdivision of the usual uncertainty factor of 100 used in setting guidance values for the exposure of the general population, such as ADIs, TDIs or RfDs. Different numerical values could be derived if the usual total default uncertainty factor were not 100 — for example, in the risk assessment of occupational exposures (based on IPCS, 1994).

Figure 1. The IPCS (2005) framework for Chemical Specific Adjustment Factors (CSAFs).



Figure 2. Percent of responders in the Arvin incident who experienced eye irritation only, eye irritation in conjunction with other symptoms, or other symptoms without eye irritation, in relation to their distance from the treated field. Average MITC concentrations for distance codes 1, 2, and 3 are approximately 0.95, 0.60, and 0.45 ppm, respectively.

Eye Effects Observed in Rats

Eye Effects: Lacrimation and Eye Closing

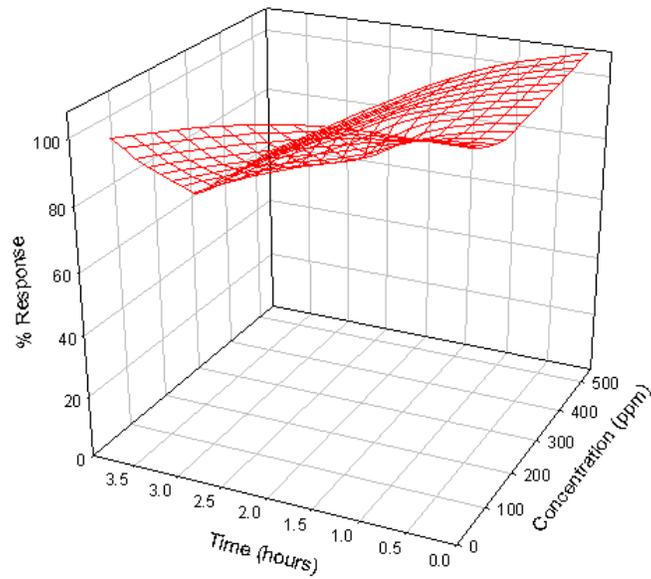


Figure 3a. Eye responses, manifested as lacrimation or lid closing, vs. concentration and duration of exposure. The combined percentage of responses of the two measures assumed independence of each and was corrected for joint-responses. Based upon data from Jackson et al. (1981).

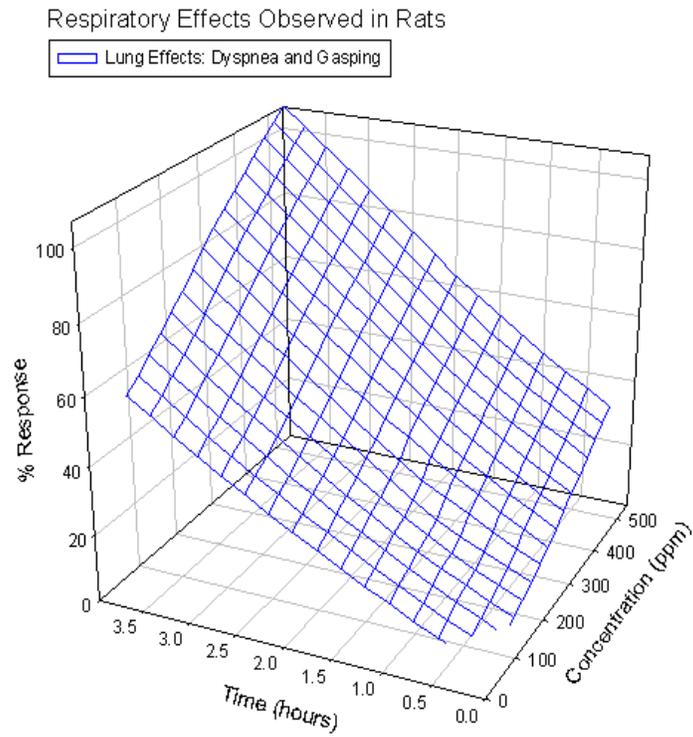


Figure 3b. Lung responses, manifested as dyspnea or gasping, vs. concentration and duration of exposure. The combined percentage of responses of the two measures assumed independence of each and was corrected for joint-responses. Based upon data from Jackson et al. (1981).

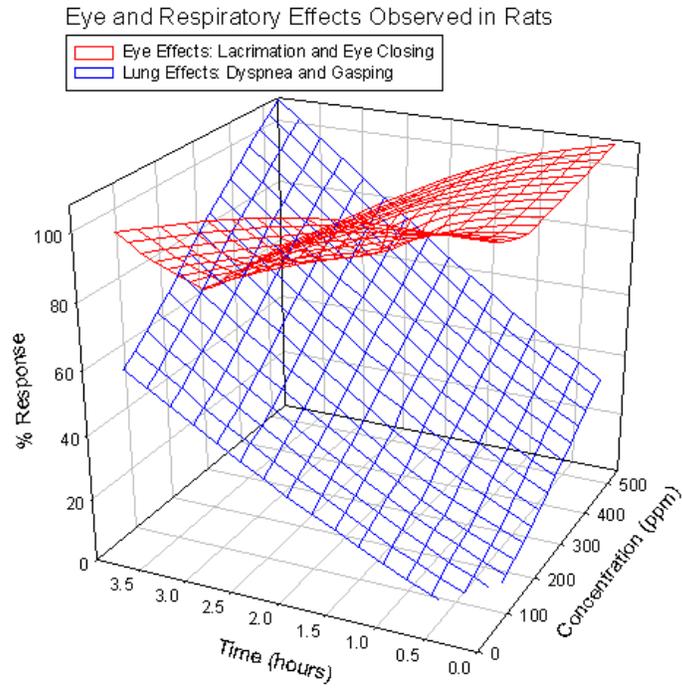


Figure 3c. Response surfaces from Figures 3a and 3b plotted together.

Acute Air MITC Exposure



Trigeminal Nerve Stimulation



Eye Detection (Feeling)



Eye Irritation

Figure 4. Key events in the MOA of MITC