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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

April 13, 2006

MEMORANDUM

SUBJECT: **Human Studies Review Board:** Weight of Evidence Discussion for Methyl isothiocyanate (MITC).

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This document describes the scientific support for deriving a point of departure for methyl isothiocyanate (MITC) using a human odor threshold and eye irritation study (MRID no. 44400401). This point of departure is applicable for use in acute (1-day) risk assessments to by-standers and occupational workers exposed to MITC in air.

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I. Background and Introduction:

There are several pathways of exposure for methyl isothiocyanate (MITC, see figure 1 for chemical structure). MITC can be used as a pesticide directly to treat wood poles. MITC is also a key degradate of several fumigant pesticides (i.e., metam sodium, metam potassium and dazomet). Following application, metam sodium, metam potassium and dazomet break down quickly to a variety of degradates, including the major degradate, MITC. It is believed that MITC that provides the fumigating properties of the parent active ingredients. Metam sodium and metam potassium are used to treat sewage systems. Metam sodium, metam potassium, and dazomet are used as a soil fumigants. Soil fumigants are pesticides which, when injected or incorporated into soil, form a gas which permeates the soil and kills soil-borne pests, such as insects, microorganisms, weeds, and nematodes. After the fumigant dissipates from the soil in a few days to a couple of weeks, planting can take place. Soil fumigants have the potential to move off site following field applications, resulting in exposure to bystanders near treated areas and to people far away from treated areas through ambient air. Use of the soil fumigants also results in exposure to those handling the pesticides or working in treated fields. Acute inhalation exposures to bystanders and workers appear to present the greatest risk concern. Based on pounds of active ingredient applied, metam sodium is the most widely used soil fumigant in the U.S and in 2001 was the 3rd most commonly used pesticide in the country. As such, due to the extensive use of metam sodium as a soil fumigant, the major pathway of exposure to MITC is acute inhalation exposure from the off-gassing following metam sodium application.

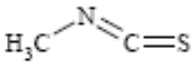

Figure 1. Chemical structure of MITC.

The Agency is currently undertaking a systematic effort to evaluate the human health risks from exposure to soil fumigants. The public comment period of the Agency's preliminary risk assessments for metam sodium and MITC ended in October, 2005. The Agency's risk assessments for metam sodium, metam potassium, dazomet, and MITC have relied on the MITC eye irritation study as the basis for the point of departure (PoD) for acute inhalation exposures to by-standers and occupational workers from off-gassing of MITC. The California Department of Pesticide Regulation (CDPR) has also used this study in the CDPR human health risk assessment for exposure to MITC from metam sodium applications. In accordance with the human studies rule, the Agency is asking the Human Studies Review Board to review the scientific conduct and design of the eye irritation study and its potential utility in assessing human health risk.

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II. Hazard Characterization and Database Summary

The mode of toxic action for MITC is not known at this time. However, reactivity with biological nucleophiles such as sulfhydryl groups of glutathione or proteins has been proposed as the potential mode of action (Valentine, et al., 1995). Consistent with this proposed mode of action, MITC is primarily an irritating compound that produces non-specific systemic effects in oral toxicity studies such as changes in body weight, food consumption, and hematological parameters. Following air exposures to MITC, consistent effects are observed in rats and humans. For example, clinical signs and pathological changes of the respiratory tract consistent with an irritant have been observed in laboratory studies in rat. Humans exposed to MITC complain of symptoms such as itchy and burning eyes, rash and burning skin, nausea, scratchy throat, salivation, coughing, and shortness of breath. In acute toxicity testing with animals, MITC is considered Acute Toxicity Category I (corrosive) for skin and eye irritation. MITC is also a skin sensitizer in guinea pigs.

A. Animal database

The majority of animal studies available for metam sodium/potassium, dazomet, and MITC are for oral exposure and are not considered relevant for assessing acute inhalation exposure for MITC. At the present time, the data base of acceptable animal inhalation toxicology studies for MITC is very limited. A 28-day inhalation study with MITC in rats (MRID no. 45314802) is available. There are several acute lethality studies available with MITC. However, one acute inhalation lethality study (MRID no 45919410) is considered the most reliable. There is also a 90-day inhalation study with MITC which is considered unacceptable due a variety of deficiencies including lack of nasal pathology and poor analytical data (MRID no. 41221407). Executive summaries of the two subchronic studies are provided in Appendix 1.

There are no studies with laboratory animals available which specifically evaluate the dose-response relationship and the continuum of potential *acute, single-day* respiratory effects (i.e., progression to more serious clinical outcomes) from exposure to MITC. Beginning on day 3 of exposure, observations of clinical signs of eyelid closure, somnolence, and ruffled fur were noted in the 28-day inhalation study in rat. Acute lethality studies are not designed to evaluate sub-lethal dose response effects. At the lowest exposure concentration of 282 mg/m^3 (approximately 80 ppm) in the acute lethality study, no mortality or gross pathological changes were observed but lung weights were increased and rales were noted on day one and reoccurred on day six post-dosing.

B. Human information

Epidemiological and incidence reporting data indicate that eye and respiratory irritation are common complaints among individuals exposed to MITC. Humans exposed to MITC complain of symptoms such as itchy and burning eyes, rash and burning skin, nausea, scratchy throat, salivation, and coughing. According to California EPA's Risk Characterization Document for MITC (2003), following a spill of metam sodium in the Sacramento River in 1991, 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. Measurements of MITC air levels in the area were not available for the first three days after the spill; modeling estimates of MITC air concentrations by CDPR and the Metam Sodium Task Force range significantly (3 ppm up to 4000 ppm) depending on the assumptions used in different modeling simulations. It is, therefore, not possible to correlate reported signs and symptoms with exposed air concentrations based on these data. Cone *et al.* (1994) performed a follow-up study of 197 adults referred to health practitioners for evaluation of potentially spill-related health problems. Thirty of these were considered positive for persistent effects including 10 who reported persistent exacerbation of asthma and met the criteria for reactive airways dysfunction syndrome (RADS). An additional MITC drift incident occurred in the town of Earlimart, California in 1999; reported symptoms by individuals exposed were consistent with those in the Sacramento spill. Again, no air concentration measurements are available; modeling simulations with EPA's ISC model estimate that air concentrations of MITC ranged from 0.5 to 1 ppm (O'Malley et al, 2004).

In order to evaluate human odor threshold and eye irritation produced by MITC vapors, human volunteers were exposed to air concentrations of MITC in a laboratory setting. A brief summary of this study is provided below.

Summary of the MITC eye irritation and odor threshold study :

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 eye irritation study, 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. Some individuals were tested for more than one exposure duration. The subject identification numbers were changed with each session and thus, the reader can not determine which subjects were included repeatedly. Sessions consisting of three exposure durations (14 minutes, 4 hours and 8 hours) were included. A range of MITC concentrations were used (see Table 1).

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An olfactometer which permitted the operator to dispense the test material through a manifold system was used. The test material could thus be diluted over a 100-fold concentration range. 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). Subjects marked the level of perceived irritation from 'no irritation' to 'so much that you feel you should end the exposure' with mid-level similar to that for cutting up an onion;
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 ("zero-time controls") and upon exposure to the negative control ("air-only controls") 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 pre-exposure and greater than could 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 and greater than could be expected from individual differences observed within the group.

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 performed in a double-blind manner. Table 1 below provides the design for the study.

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Table 1: Study design for the eye irritation human study with MITC.

Duration	Dates	Concentration(s) Tested	Number of subjects
8 hour trial	12/8/94 – 1/9/95	Air only control	12
		Acetic acid control	7
		0.22 ppm	16
4 hour trial	3/13/95 – 3/31/95	Air only control	12
		Acetic acid control	14
		0.23 ppm	12
		0.8 ppm	9
14 minute	4/18/95 – 4/26/95	Air only control	10
		Acetic acid control	0
		0.6 ppm	9
		1.9 ppm	9
		3.3 ppm	9

Subjective (Likert scale) responses. 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 (Table 2). In that test, as many as 8 out of 9 subjects 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 at 0.8 ppm 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.

Exposure to 0.22 ppm (220 ppb) did not result in a statistically significant mean Likert scale response when compared against air-only controls. Despite the fact that statistical significance *was* achieved at 1 hour when compared against zero-time controls (13%±15% *vs.* 4%±8% among zero-time controls), the lack of statistical significance when compared against air-only controls (which registered 6%±9%) resulted in a judgement of no response.

Shorter exposures to 0.6 ppm (600 ppb) did not result in statistically significant Likert scale changes, though 1 of 9 individuals appeared to respond at 4 and 14 minutes. Exposure to 1.9 ppm (1900 ppb) or 3.3 ppm (3300 ppb) MITC for 4 or 14 minutes 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.

Table 2. Mean perception-of-eye-irritation (Likert scale) data, human subjects

Units: % of total line distance (standard deviation)

4-hr trial

	Time points, hours 0	1	2	3	4	# subjects
Air-only control	1% (2%)	6% (9%)	5% (8%)	5% (6%)	4% (6%)	
p-value #1[a]	n/a	n/a	n/a	n/a	n/a	
p-value #2[b]	n/a	0.08	0.07	0.02	0.07	12
0.22 ppm	4% (8%)	13% (15%)	8% (10%)	6% (8%)	6% (7%)	
p-value #1[a]	0.21	0.16	0.43	0.55	0.49	
p-value #2[b]	n/a	0.02	0.05	0.16	0.42	12
0.8 ppm	2% (2%)	25% (14%)*	26% (14%)*	39% (19%)*	39% (26%)*	
p-value #1[a]	0.57	0.00	0.00	0.00	0.00	
p-value #2[b]	n/a	0.00	0.00	0.00	0.00	9

[a]p-value #1, t-test against air-only control subjects
[b]p-value #2, t-test against zero-time values
*Judged a positive irritation response. An irritation effect is considered to have been detected only if both statistical tests indicate significant differences and if mean is higher than the zero-time mean.

8-hr trial

	Time points (hours) 0	1.5	3	3.5	6	8	subjects
Air-only control	1% (1%)	9% (10%)	12%(15%)	6% (10%)	15%(19%)	8% (13%)	
p-value #1[a]	n/a	n/a	n/a	n/a	n/a	n/a	
p-value #2[b]	n/a	0.04	0.03	0.16	0.03	0.09	12
0.22 ppm	2% (2%)	5% (4%)	5% (4%)	4% (4%)	8% (8%)	6% (5%)	
p-value #1[a]	0.23	0.18	0.10	0.44	0.18	0.46	
p-value #2[b]	n/a	0.01	0.00	0.24	0.01	0.00	16
0.8 ppm							
p-value #1[a]							
p-value #2[b]	Not done						n/a

[a]p-value #1, t-test against air-only control subjects
[b]p-value #2, t-test against zero-time values

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Eyeblink responses. Mean blink rate determinations at 0.8 ppm were statistically significantly increased at the 2- and 3-hour time points compared both to air-only and zero-time controls (Table 3), 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 indicated 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.

Table 3. Mean eyeblink data, human subjects

Units: Blinks per minute minus zero-time rate (standard deviation)

4-hr trial

	Time points, hours 0	1	2	3	4	# subjects
Air-only control		3 (6)	3 (9)	3 (8)	3 (8)	
p-value #1[a]		n/a	n/a	n/a	n/a	
p-value #2[b]	n/a	0.13	0.24	0.23	0.18	12
0.22 ppm		-5 (6)	-2 (6)	-5 (5)	-3 (4)	
p-value #1[a]		0.00	0.13	0.01	0.03	
p-value #2[b]	n/a	0.02	0.35	0.01	0.04	12
0.8 ppm		7 (7)	16 (11)*	14 (13)*	12 (11)	
p-value #1[a]		0.15	0.01	0.03	0.052	
p-value #2[b]	n/a	0.00	0.00	0.01	0.01	9

[a]p-value #1, t-test against air-only control subjects
[b]p-value #2, t-test against zero-time values
*Judged a positive irritation response. An irritation effect is considered to have been detected only if both statistical tests indicate significant differences and if mean is higher than the zero-time mean.

8-hr trial

	Time points (hours) 0	1.5	3	3.5	6	8	# subjects
Air-only control		-2 (7)	-3 (7)	-1 (5)	-1 (7)	0 (7)	
p-value #1[a]		n/a	n/a	n/a	n/a	n/a	
p-value #2[b]	n/a	0.42	0.15	0.48	0.54	0.97	12
0.22 ppm		-3 (6)	-2 (5)	-2 (5)	-2 (4)	-2 (5)	
p-value #1[a]		0.62	0.67	0.55	0.81	0.48	
p-value #2[b]	n/a	0.07	0.15	0.10	0.12	0.19	16
0.8 ppm							
p-value #1[a]							
p-value #2[b]	Not done						n/a

[a]p-value #1, t-test against air-only control subjects
[b]p-value #2, t-test against zero-time values

Tearing, ocular morphology, and visual acuity. 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."

Recovery. 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.

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Table 4. Summary of MITC eye irritation effects from human subjects

Exposure time	NOAEL (ppm)	LOAEL (ppm)	Source of observed Effect
1 minute	3.3	-	-
4 minutes	0.6	1.9	Subjective eye irritation
14 minutes	0.6	1.9	Subjective eye irritation
1 hour	0.23[a]	0.8	Subjective eye irritation
1.5 hours	0.22[a]	-	-
2 hours	0.23[a]	0.8	Subjective eye irritation and blink rate
3 hours	0.23[a]	0.8	Subjective eye irritation and blink rate
3.5 hours	0.22[a]	-	-
4 hours	0.23[a]	0.8	Subjective eye irritation
6 hours	0.22[a]	-	-
8 hours	0.22[a]	-	-

[a]The slightly different values obtained at the low dose NOAEL level (0.22 and 0.23 ppm) reflected the fact that they were derived from tests performed on different days.

Conclusions from the eye irritation portion of the study include:

- For a one-minute exposure, the NOAEL for eye irritation is 3.3 ppm due to a lack of response in any parameter tested.
- For exposures 4-14 minutes, the NOAEL for eye irritation is 0.6 ppm based on responses on the Likert subjective scale at 1.9 ppm.
- For exposures of 1-8 hours, based on the statistically significant subjective (Likert scale) responses at 0.8 ppm MITC at 1-4 hours and the statistically significant eyeblink responses at 2 and 3 hours, 0.22 ppm was designated as the NOAEL for this study.

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C. Point of Departure and Uncertainty Factor(s)

As stated above, there are no studies with laboratory animals available which specifically evaluate the dose-response relationship and the continuum of potential *acute, single-day* respiratory effects (i.e., progression to more serious clinical outcomes) from exposure to MITC. In most cases, the Agency does not consider an acute lethality study as appropriate for deriving a PoD for assessing human health risk assessment. For purposes of characterization the Agency has evaluated the potential that the acute inhalation lethality study in rat could provide a PoD for acute risk assessment of MITC. If the lowest dose in the acute lethality study of 282 mg/m^3 is treated as an acute low-observed-adverse-effect-level (LOAEL) and a typical LOAEL to NOAEL 10X factor is applied along with 100X for inter- and intra-species variation, then the final value would be 0.24 mg/m^3. The 1-8 hour NOAEL from the eye irritation study is 0.66 mg/m^3 (0.22 ppm). Using an intra-species factor of 10X, the final regulatory value from the eye irritation study is 0.066 mg/m^3. Thus, in the case of MITC, use of the human eye irritation study provides a more sensitive endpoint for assessing acute human health risk to MITC.

Eye irritation is not an effect from inhalation exposure *per se*. However, eye irritation can result from exposures in the air. With respect to respiratory impairment, arguably, eye irritation is less severe compared to other possible effects associated with inhalation exposure to MITC, particularly given the expected reversible nature of the eye irritation effects at lower concentrations. Nonetheless, eye (as well as nose and throat) irritation is uncomfortable and could potentially interfere with everyday tasks or activities. OPP notes that the Agency's Reference Concentration (RfC) methodology (1994) includes eye, nasal, and throat irritation in the list of adverse effects-- albeit at the lower end of the hierarchal list which ranks effects from most to less severe. Due to the limitations in the existing *inhalation* toxicology database for MITC, the degree to which eye irritation predicts more serious outcomes is unclear. However, given that humans exposed to MITC complain of symptoms such as itchy and burning eyes in addition to rash and burning skin, nausea, scratchy throat, salivation, coughing, and shortness of breath, in the absence of more robust dose-response data from acute exposures, eye irritation can be considered as a *biomarker* and *surrogate for potential respiratory effects*.

The MITC odor threshold and eye irritation study (MRID 44400401) evaluated the dose-response relationship for eye irritation at exposure durations ranging from 4 minutes to 8 hours. The results of the odor threshold study indicate that the eyes are likely to become irritated prior to detecting the odor; thus the odor threshold study has not be used for PoD derviation. The eye irritation study with MITC appears to have been scientifically well conducted with sensitive methods similar to those used by other laboratories (Doty et al, 2004). The study provides a dose and time-related response to MITC for the subjective scale and the blinking rate. Thus, the Agency has selected the human eye irritation study as the basis for the PoD in acute risk assessment to MITC in air.

The Agency has established the following PoDs for varying durations of exposure:

- For a one-minute exposure, the NOAEL (no-observed-adverse-effect-level) for eye irritation is 3.3 ppm due to a lack of response in any parameter tested.

- For exposures 4-14 minutes, the NOAEL for eye irritation is 0.6 ppm based on responses on the Likert subjective scale at 1.9 ppm.

- For exposures of 1-8 hours, based on the statistically significant subjective (Likert scale) responses at 0.8 ppm MITC at 1-4 hours and the statistically significant eyeblink responses at 2 and 3 hours, 0.22 ppm was designated as the NOAEL.

For acute inhalation exposures to MITC, because a study using human subjects is being used, an interspecies factor is not necessary. A standard 10X was assigned for intraspecies variability.

III. Conclusions

The general public may be exposed to fumigants, like MITC, in air following application because of their volatility since these chemicals can off-gas into ambient air and can be transported off-site by wind to non-agricultural areas. The inhalation database for MITC for acute exposures is limited. The Agency has selected the human eye irritation study as the appropriate study for deriving a PoD for assessing acute risk to MITC.

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References:

California Environmental Protection Agency, (2003) Risk Characterization Document for MITC. Department of Pesticide Regulation, California Environmental Protection Agency July 25, 2003.

Cone, J.E., L. Wugofski, J.R. Balmes, R. Das, R. Bowler, G. Alexeeff and D. Shusterman. 1994. Persistent respiratory health effects after a metam sodium pesticide spill. *Chest* 106:500-508

Doty RL, Cometto-Muniz JE, Jalowayski AA, Dalton P, Kendal-Reed M, Hodgson M. (2004) Assessment of upper respiratory tract and ocular irritative effects of volatile chemicals in humans. Crit Rev Toxicol. 2004 Mar-Apr;34(2):85-142.

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. American Journal of Industrial Medicine. 46: 1-15.

41221407 Rosskamp, G.; Schobel, G.; Bhargava, A.; et. al. (1978) T22 Methyl Isothiocyanate: ZK 3.318: A 12_13 Week Inhalation Study in the Rat: Project ID 374/77. Unpublished study prepared by Schering AG. 202 p.

44400401 Russell, M.; Rush, T. (1996) Methyl Isothiocyanate: Determination of Human Olfactory Detection Threshold and Human No Observable Effect Level for Eye Irritation: Lab Project Number: MITC_UCD_1A_1993: MITC_UCD_1B_1994: RR 96_049B. Unpublished study prepared by University of California and Western Research Center (Zeneca). 136 p.

45314802 Klimisch, H. (1987) Study of the Subchronic Inhalation Toxicity of Methyl Isothiocyanate in Wistar Rats (4_Week Study): Lab Project Number: 87/0244: 40I0231/8539. Unpublished study prepared by BASF Aktiengesellschaft. 272 p.

45919410 Jackson, G.; Clark, G.; Prentice, D.; et al. (1981) Methyl Isothiocyanate: Acute Inhalation Toxicity in Rats__4 Hour Exposure: Lab Project Number: 81/082: 378/801109. Unpublished study prepared by Huntingdon Research Center. 115 p.

US Environmental Protection Agency. 1994. Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Office of Research and Development. EPA/600/8-90/066F, October 1994.

Valentine, W. M., Amarnath, V., Amarnath, K., and Graham, D. G. 1995. Characterization of protein adducts produced by N-methyldithiocarbamate and N-methyldithicarbamate esters. Chem. Res. Toxicol. 8: 254-261.

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Appendix 1: Executive summaries of sub-chronic inhalation studies with MITC.

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CITATION: Klimisch, H.J. (1987). Study of the Subchronic Inhalation Toxicity of Methyl Isothiocyanate in Wistar Rats (4 weeks study). Department of Toxicology. BASF Aktiengesellschaft, D-W6700 Ludwigshafen, Federal Republic of Germany. Project No 4010231/8539, BASF Reg. Document Number 87/0244, January 29, 1987. MRID 45314802. Unpublished.

EXECUTIVE SUMMARY: In a 28 day inhalation toxicity study (MRID 45314802), Methyl Isothiocyanate [96.9 % a.i.] was administered to 5/sex/dose of SPF Wistar/Chubb:THOM rats by whole body exposure at analytical concentrations of 0, 5.0, 20, or 100 mg/m^3 equivalent to 0, 5.0, 20, or 100 ug/L (measured concentrations 0, 5.1, 19.9 or 100 ug/L) and (equivalent to concentrations of 0, 1.7, 6.8, and 34 ppm) for 6 hours per day, 5 days/week for a total of 28 days.

All animals survived to study termination. Mid and high dose rats demonstrated clinical signs during exposure from the third exposure period day. No clinical signs were observed in the low dose animals. According to the study report,

> "During exposure, the animals of test group 2 showed eyelid closure, somnolence, and ruffled fur from the third day of exposure onwards. On the next morning before exposure nothing abnormal was found in the animals.....At 20 mg/m^3 the animals showed first indications of an irritating effect of the test substance and a slightly deteriorated general state of health."

Additional clinical signs observed at the high exposure concentration included reddish nasal discharge, salivation, eye discharge, and difficulty in breathing or whooping respiration, and stretched posture. In the high dose rats, although signs recovered between exposures at the beginning of the study, towards the end of the study ruffled fur and respiratory sounds were no longer reversible.

Body weight and body weight gain were significantly decreased ($p<0.05$) at the high dose. Food consumption and feed efficiency were not measured. There were decreases in plasma urea, glucose, triglyceride, and albumin the high dose males. In high dose females, urea and glucose were also decreased. In the males of mid exposure group, there was a decrease in urea concentration in the plasma.

At the mid and high exposure concentrations, increase in neutrophilic polymorphonuclear granulocytes in the peripheral blood was observed in males; this was also observed in the high exposure concentration for females.

There was increased lung weight at the high exposure concentration. Histopathology revealed an increase in incidence and severity of rhinitis in the nasal cavity at the high exposure concentration 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 exposure concentration included: atrophy of the olfactory epithelium, metaplasia of the nasal respiratory 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 exposure concentration),bronchopneumonia and bronchial and bronchiolar epithelial proliferation (5 males, 2 females), and emphysema (3 males, 2 females).

The systemic LOAEL is 19.9 mg/m^3, (6.8 ppm), based on clinical signs consistent with irritation in both sexes and increased neutrophilic polymorphonuclear granulocytes in the blood of males. The systemic NOAEL is 5 mg/m^3 (1.7 ppm).

The LOAEL for effects in the extrathoracic (ET) region is 100 mg/m^3, (34ppm), based on observation of pathological changes of the nasal cavity (metaplasia of respiratory epithelium and atrophy of the olfactory epithelium). The ET NOAEL is 19.9 mg/m^3 (6.8 ppm).

The LOAEL for effects in the tracheabronchial (TB) region is 100 mg/m^3 (34ppm), based on observation of pathological changes (tracheal epithelial proliferation and single cell necrosis, bronchopneumonia and bronchial and bronchiolar epithelial proliferation). The TB NOAEL is 19.9 mg/m^3(6.8 ppm).

This subchronic toxicity study is **Acceptable but does not satisfy** the guideline requirement for a subchronic inhalation study (82-4) in the rat. The study duration was too short and the number of animals used were inadequate to satisfy the Guideline requirement. Detailed tables of the clinical signs were not provided in the study report.

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CITATION:

Rosskamp, G.; Schobel, G.; Bhargava, A.; et. al. (1978) T22 Methyl Isothiocyanate: ZK 3.318: A 12-13 Week Inhalation Study in the Rat: Project ID 374/77. Prepared by Schering AG. November 26, 1975 MRID no. 41221407. Unpublished

Davis, C. (1990) Nor-Am Chemical Company Phase 3 Summary of MRID 41221407. Methyl Isothiocyanate: A 12-13 Week Inhalation Study in the Rat (T22); Lab ID. No. 374/77. Prepared by Schering AG, Berlin, Federal Republic. September 26, 1978 MRID no. 92114013. Unpublished

EXECUTIVE SUMMARY:

In a subchronic inhalation study (MRID no. 41221407) 4 groups of 10 Wistar rats/sex/dose received a nose-only inhalation exposure to MITC (95.69% a.i., Batch no. 26,300) at 0, 3.16, 30.67, and 137.13 ug/L for 4 hours/day, 5 days/week over a 12 to 13 week period (By extrapolation from four to six hours of exposure, the dose levels are 0, 2.1, 20.6, and 91.9 ug/L). There were two control groups of 10 rats/sex/dose, one maintained in the laboratory without inhalation exposure and the other in chamber without MITC.

No effects on urinalysis parameters, gross or microscopic pathology or organ weights were noted at any dose level. Although no clinical signs were noted in the low or middle dose groups, clinical signs of apathetic appearance accompanied with salivation and nasal discharge was observed throughout the study in high dose rats. Six of 20 rats in the high dose exhibited stimulated vocalization during the last 30 days of exposure.

A treatment related decrease in body weight gain was noted in male and females of the middle (11% M and 15% F, not statistically significant) and the high (63% M and 47% F, $p < 0.01$) dose groups. A treatment related increase in water consumption was noted in males and females of the mid dose group (14% and 21%, $p <0.05$) and males of the high dose group (16%, $p<0.05$). Food consumption, however, was decreased ($p < 0.05$) in only the high dose group.

Hematological parameters of leucocyte and neutrophil counts and hematocrit and RBC values were significantly increased in the high dose group only. A significant decrease in total blood protein was noted in males and females of the mid dose group ($p<0.05$) and females of the high dose group. Changes in fasting glucose, alkaline phosphatase, and alanine aminotransferase were also in the high dose females.

Effects reported at the mid dose were decreased body weight, food efficiency and blood protein values accompanied by increased water intake. At the high dose (91.9 ug/L) the animals exhibited apathy, salivation, nasal discharge, and stimulated vocalization. These animals exhibited a decrease in body weight, food intake, and food efficiency accompanied by an increase in water intake. Alterations in clinical chemistry values at this dose included decreased total protein with increased alkaline phosphatase and alanine aminotransferase values.

Overall, the results of this study are questionable for a variety reasons detailed by California Department of Pesticide Regulation. The following text was extracted from California Environmental Protection Agency, (2003) RISK CHARACTERIZATION DOCUMENT Methyl Isothiocyanate (MITC) Following the Agricultural Use of Metam Sodium, Sacramento, California July, 2003.

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.

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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.

The LOAEL is 30.67 ug/L/day (extrapolated to 20.6 ug/L/day for 6 hour exposure), based on decreased body weight, food efficiency and blood protein values accompanied by increased water intake. The NOAEL is 3.16 ug/L/day (extrapolated to 2.1 ug/L/day for 6 hour exposure).

This subchronic inhalation toxicity study in the rat is **acceptable-guideline** and satisfies the guideline requirement for a subchronic inhalation study OPPTS 870.3465; OECD 413 in the rat.

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