(11-23-93)

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DATA EVALUATION REPORT

METHYLISOTHIOCYANATE

Study Type: Mutagenicity: Microbial/Mammalian Microsome Mutagenicity and DNA Damage/Repair (Rec) Assays

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
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Prepared by:

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Methylisothiocyanate GUIDELINE SERIES 84: MUTAGENICITY
SALMONELLA/E. COLI/B SUBTILIS

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Signature: Ray Landolt
Date: 9/22/94

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Date: 9/22/94

DATA EVALUATION REPORT

STUDY TYPE: Microbial/mammalian microsome gene mutation and DNA damage/repair (rec) assays

PC CODE: 068103

MRID NUMBER: 412214-10

TEST MATERIAL: Methylisothiocyanate

SYNONYMS: MITC

STUDY NUMBER: T25

SPONSOR: NOR-AM Chemical Co./Shionogi & Co., Ltd., Osaka, Japan

TESTING FACILITY: Institute of Environmental Toxicology, Tokyo, Japan

TITLE OF REPORT: T25 Methyl Isothiocyanate: Microbial mutagenicity testing on methyl isothiocyanate

AUTHOR: Y. Shirasu

rEPORT ISSUED: Novmber 8, 1978

CONCLUSIONS--EXECUTIVE SUMMARY:

Negative for inducing reverse gene mutatins in spot tests (with precautions taken to prevent compound loss of the volatile test material) with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 or Escherichia coli WP2 uvrA exposed in either the presence or absence of mammalian metabolic activation to test material levels up to 1000 µg/disc. Moderate-to-severe cytotoxicity was demonstrated in all strains at doses ≥ 50 µg/ disc - S9 and ≥ 100 µg/disc +S9.

Reported to be negative for the induction of DNA damage in DNA repair-deficient Bacillus subtilis strain M45 (rec$^-$) up to 2000 µp/disc. However,

cytotoxicity was not achieved, no steps were taken to prevent compound loss, S9 activation was not included, and only single replicates were assayed.

Classification: Acceptable for gene mutations
Unacceptable for primary DNA damage

The gene mutation study satisfies the requirements for FIFRA Test Guideline Series 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data and is acceptable for regulatory purposes.

The bacterial DNA damage/repair study does not satisfy the requirements for FIFRA Test Guideline Series 84-4, and is not acceptable for regulatory purposes.

A. MATERIALS

1. Test Material: Methyl isothiocyanate (MITC)

Description: Not provided
Identification number: None listed
Purity: 94.78%
Receipt date: Not reported
Stability: Not reported
CAS number: Not provided
Solvent used: Dimethylsulfoxide (DMSO)
Other comments: The test material storage conditions were not reported. Dosing solutions were prepared immediately prior to use in sealed tubes. Analytical determinations were not performed.

2. Control Materials:

(a) Solvent/final concentration: DMSO/not specified

(b) Gene mutation

Positive:

Nonactivation:

β-Propiolactone	50	µg/plate	TA1535
9-Aminoacridine	200	µg/plate	TA1537
2-Nitrofluorene	50	µg/plate	TA1538
2-(2-Furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2)	0.25	µg/plate	E. coli WP2 uvrA
	0.10	µg/plate	TA98
	0.05	µg/plate	TA100

Activation:

2-Aminoanthracene (2-AA)	10	µg/plate	all strains

Note: 2-AA was tested with and without S9 activation.

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Methylisothiocyanate SALMONELLA/E. COLI/B. SUBTILIS

(c) DNA Damage/Repair

Negative:

Kanamycin 10 µg/disc B.subtilis H17, M45

Positive:

Mitomycin C 0.1 µg/disc B.subtilis H17, M45

Note: The rec assay was only performed in the absence of S9 activation.

3. Activation: S9 derived from 13-week old male (average weight 427 g) Sprague-Dawley

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ noninduced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___	

The rat liver S9 homogenate was prepared by the performing laboratory. Components of the S9 cofactor mix were as follows:

Component:	Concentration
Sodium phosphate buffer (pH 7.4)	100 mM
KCl	33 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
$MgCl_2$	8 mM
S9 homogenate	30%

4. Test Organism Used: S. typhimurium strains:

___ TA97	x TA98	x TA100	___ TA102	___ TA104
x TA1535	x TA1537	x TA1538		

list any others: E. coli WP2 uvrA
B. subtilis H17 (rec+)
M45 (rec-)

Test organisms were properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Not reported.

5. Test Compound Concentrations Used:

(a) Mutation assay: Eight concentrations (0.5, 1, 5, 10, 50, 100, 500, and 1000 µg/disc) were evaluated with and without S9 activation. Duplicate plates were prepared per strain (S. typhimurium or E. coli), per dose, per condition.

(b) DNA damage/repair assay: Six concentrations (20, 100, 200, 500, 1000, and 2000 µg/disc) were evaluated without S9 activation using single plates per dose.

B. TEST PERFORMANCE

1. Type of Salmonella Assay:
___ Standard plate test
___ Pre-incubation (_____) minutes
___ "Prival" modification
_x_ Spot test (modified for volatile compounds)
___ Other (describe)

2. Mutation Assay: Frozen cultures of each tester strain were thawed, suspended in phosphate buffer, and used without incubation. The appropriate tester strain in 0.1-mL volumes and 0.5-mL of the S9 cofactor mix, when required, were added to top agar supplemented with 0.5 mM L-histidine and 0.5 mM D-biotin (S. typhimurium strains) or 0.5 mM L-tryptophan (E. coli strain). Mixtures were overlaid on minimal glucose agar plates and allowed to harden. Test material dilutions were prepared in sealed tubes immediately prior to use. Filter discs, impregnated with 0.1 mL of the appropriate test material dose, were placed on the inside of the lid of each petri dish. Plates were individually sealed in vinyl bags and incubated at 37°C for 2 days; revertant colonies were counted. Cultures were reincubated for 1 day and recounted. Solvent and positive controls were incorporated into the agar overlay and these cultures were similarly incubated and counted.

3. DNA Damage/Repair Assay: Frozen cultures of each strain were thawed and streaked onto the surface of B-2 nutrient agar plates. Filter paper discs (10mm) were impregnated with 20 µL of the appropriate test material dose. Solvent, negative, or positive controls were also included in the assay. Plates were incubated overnight at 37°C and the length of the inhibition zones were measured. There was no indication in the report that plates were sealed to prevent compound loss.

4. Evaluation Criteria: No criteria were provided to asses assay validity, a positive response, or the biological significance of the findings.

C. REPORTED RESULTS

1. Mutation Assay: Owing to the volatility of the test material, the standard plate incorporation assay was modified as described. Representative data presented in Table 1 from the 2-day colony counts clearly indicate that the test material was able to interact with the microbial cells. As shown, moderate-to-severe cytotoxicity was seen in all S. typhimurium tester strains and also E. coli WP2 uvrA⁻ at levels ≥50 µg/disc -S9 and ≥100 µg/disc +S9. No increases in reversion to either histidine or tryptophan prototrophy accompanied exposure of the microbial strains to the noncytotoxic doses with or without S9 activation. Colony counts for cytotoxic levels increased, and in a few

Table 1: Representative Results of the Microbial/Mammalian Microsome Mutation Assay (Spot Test) with Methyl Isothiocyanate: 2-Day Incubation

Substance	Dose	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] S. typhimurium TA1535	TA1537	TA1538	TA98	TA100	E. coli WP2 uvrA
Solvent Control								
Dimethylsulfoxide	-	-	17	13	14	21	127	16
	-	+	13	7	15	17	131	10
Positive Controls								
β-Propiolactone	50 µg/plate	-	1576	-	-	-	-	-
9-Aminoacridine	200 µg/plate	-	-	>10,000	-	-	-	-
2-Nitrofluorene	50 µg/plate	-	-	-	>3000	-	-	-
2-(2-Furyl)-3-(5-nitro -2-furyl)-acrylamide	0.25 µg/plate	-	-	-	-	-	-	1202
	0.10 µg/plate	-	-	-	-	263	-	-
	0.05 µg/plate	-	-	-	-	-	1217	-
2-Aminoanthracene	10 µg/plate	+	456	584	>3000	>3000	>3000	105
Test Material								
Methyl isothiocyanate	10 µg/disc[b]	-	8	5	8	22	114	18
	50 µg/disc	-	5	3	5	11	69	12
	100 µg/disc[c]	-	0	3	1	3	54	10
	10 µg/disc[b]	+	12	4	9	22	113	14
	50 µg/disc	+	11	6	5	17	64	11
	100 µg/disc[c]	+	5	1	2	10	52	5

[a] Average values were from duplicate plates; calculated by our reviewers.
[b] Lower levels (0.5, 1, and 5 µg/disc +/-S9) were neither cytotoxic nor mutagenic.
[c] Higher doses (500 and 1000 µg/disc +/-S9) were severely cytotoxic.

Note: No substantive changes in these findings were noted when plates were reincubated an additional ~24 hours and recounted.

Data were extracted from the study report, pp. 11 and 12.

instances approached spontaneous revertant rates when plates were reincubated for an additional ~24 hours. However, this is the expected result for microbial cells exposed to cytotoxic compounds. Furthermore, since methyl isothiocyanate is volatile, it is likely that when the plates were opened for the initial colony counting, the test material evaporated and was not present during the additional incubation time.

As further shown in Table 1, all strains responded to the mutagenic action of the corresponding nonactivated and S9-activated positive controls.

2. DNA Damage/Repair Test: In contrast to the severe cytotoxicity for S. typhimurium and E. coli, the test material had no effect on the survival of DNA repair-competent or DNA repair-deficient strains of B. subtilis at doses up to 2000 μg/disc (Table 2). Because no precautions were taken to prevent compound loss, it is possible that little or no methyl isothiocyanate was available to interact with the bacterial cells.

From the overall findings, the study author concluded that methyl isothiocyanate was negative in the rec-assay and also in the reverse-mutation tests.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that methyl isothiocyanate was adequately tested in the mutation assay, and caused moderate-to-severe cytotoxicity at levels ≥50 μg/disc -S9 or ≥100μg/disc +S9, but did not induce a mutagenic effect. A negative in the spot test is generally not considered to be a sufficient indication of nonmutagenicity in S. typhimurium or E. coli because the procedure is only qualitative and limits the number of microorganisms that can be exposed to the test material. However, the method employed by the study director (i.e., test material dilutions prepared in sealed tubes and individually-sealed dishes) assured that all organisms on a given dish were exposed to methyl isothiocyanate. Similarly, the induced cytotoxic response supports the assessment that conditions were adequate for test material-cell interaction. It is, therefore, doubtful that performance of the more elaborate desiccator assay for gases and/or vapors would substantively alter the outcome of the methyl isothiocyanate gene mutation spot test.

The DNA damage/repair assay, however, is not acceptable for the following reasons:

1. No precautions were taken to prevent compound loss.
2. There was no indication of cytotoxicity at any level.
3. Only a nonactivated test was performed.
4. Only single replicates were used.

Based on these considerations, we conclude that the gene mutation assay is acceptable and that the microbial DNA damage/repair assay is unacceptable.

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Table 2: Representative Results of the Bacillus subtilis DNA Damage/Repair (REC) Assay with Methyl Isothiocyanate

Substance	Dose (μg/disc)	Inhibition Zone (mm)[a] M45 (rec⁻)	H17 (rec⁺)	Difference (mm)
Solvent Control				
Dimethylsulfoxide	-	0	0	-
Negative Control				
Kanamycin	10	6	4	2
Positive Controls				
Mitomycin C	0.1	10	0	10[b]
Test Material				
Methyl isothiocyanate	2000[c]	0	0	-

[a] Results were from single plates containing both H17 and M45.
[b] Positive results were assumed by our reviewers to be a minimum of 5 mm difference in the zone of inhibition between the rec⁻ and the rec⁺ strains (see Nishioka, H. 1975. Mutagenic activities of metal compounds in bacteria. Mutat. Res. 31:185-189).
[c] Findings for lower doses (20, 100, 200, 500, and 1000 μg/disc) were negative.

Data were extracted from the study report, p. 10.

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E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? No (A statement from the sponsor signed and dated August 24, 1989 indicated that the study was conducted prior to the US EPA requirements of GLPs).

F. APPENDIX: None attached

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CASWELL FILE

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

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Methylisothiocyanate (MITC)
Phase IV Data Response

Submission No. S421556
Chem No. 068103
Tox Chem No. 573

FROM: Ray Landolt 9/3/92
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)

TO: Barbara Briscoe PM 51
Accelerated Reregistration Branch
Special Review and Reregistration Division (H7508W)

THRU: Mike Ioannou, Section Head
Review Section I
Toxicology Branch II
Health Effects Division (H7509C) 9/3/92
and
Marcia van Gemert, Branch Chief 9/8/92
Toxicology Branch II
Health Effects Division (H7509C)

Registrant: Degussa Corporation

Action Requested: 1. Data waiver requests for two generation reproduction (83-4) and general metabolism (85-1) studies.

2. Provide comments on protocol for Estimation of Inhalation (231) and Dermal (232) Exposures for MITC at Outdoor Sites.

Conclusion: 1. Data waiver requests for two generation reproduction (83-4) and general metabolism (85-1) studies are not applicable to the Phase IV Toxicology Data Review which concluded that these two studies are not required for the terrestrial nonfood use registered by Degussa Corporation.

2. The protocol for estimation of dermal and inhalation exposure assessment should provide for continuous monitoring during the treatment of each timber.

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Consideration Given this Request

1. The Accelerated Reregistration Branch (SRRD) requests review and comment on data waivers for a two generation reproduction (83-4) and general metabolism (85-1) studies.

   Methylisothiocyanate is registered as a "Fungicide for Wood". The directions for use recommend treating structural timbers such as utility poles, bridge timbers and laminated wood products (MITC-FUME label).

   This use is considered a terrestrial nonfood use. These two studies (83-4 and 85-1) were not data requirements with Phase IV Toxicology data request.

   The registrant's correspondence reply to the requirements status for a two generation reproduction and general metabolism study has cited Reregistration Phase III comments that these studies are "not required for the registrant's use pattern". This is correct.

2. Protocol for Estimation of Inhalation (231) and Dermal (232) Exposure for MITC at Outdoor Sites.

   To be effective in the control of decay in wooden timbers, MITC would be expected to migrate through the untreated wood. Exposure to the applicator may occur during the application of each vial (5 to 10 seconds) as well as following the release from each broken vial through fissures in the timber. Monitoring for MITC should be continuous for each timber treated.