



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

011275

10-6-94

OCT 6 1994

#573

MEMORANDUM

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

Subject: Generic Data in Support of Reregistration  
of Methyl isothiocyanate (MITC)

From: Ray Landolt *9/29/94*  
Review Section I  
Toxicology Branch II  
Health Effects Division (7509C)

Barcode D194652  
Submission S447097  
P.C. Code 068103

To: Virginia Dietrich, PM 51  
Reregistration Branch  
Special Review & Reregistration Division (7508C)

Thru: Mike Ioannou, Section Head  
Review Section I  
Toxicology Branch II  
Health Effects Division (7509C)  
and  
Marcia van Gemert, Branch Chief  
Toxicology Branch II  
Health Effects Division (7509C)

*J.M. Ioannou 9/29/94*

*Chab Hunter for 9/30/94*

Registrant: Degussa Corporation

Action Requested: Pazianos Associates, agent for Degussa Corporation, have cited the following toxicology studies sponsored by Nor-Am Chemical Company in support of reregistration of MITC. 81-6 Dermal Sensitization, 82-2 Subchronic (30-day) Dermal, 82-4 Subchronic (90-day) Inhalation, 83-3 Developmental-Rat and Rabbit, 84-2a Gene Mutation, 84-2b Sister chromatid exchange and 84-4 Other Genotoxic Effects.

Recommendations: Data Evaluation Reports for the studies submitted are attached.

81-6 Dermal Sensitization, MRID 412214-04, Acceptable  
MITC is a mild sensitizer when tested by the Guinea Pig Maximization Test.

82-2 Subacute dermal (30-day), MRID 412214-06, Minimum  
The systemic NOEL and LOEL are 1.0 and 10 mg/kg, respectively. At the 10.0 mg/kg level there was decreased serum albumin and increased globulin values and increased relative liver weight. The dermal NOEL and LOEL are 1.0 and 10.0 mg/kg, respectively. Erythema and desquamation were observed at 10.0 mg/kg.



Recycled/Recyclable  
Printed with Soy/Canola Ink on paper that  
contains at least 50% recycled fiber

*D1/24*

8-4 Subchronic inhalation (90-day), MRID 412214-07, Minimum  
The NOEL and LOEL are 2.1 and 20.6 ug/L, respectively.  
Decreased body weight, food efficiency and blood protein  
values accompanied by increased water intake were reported at  
the 20.6 mg/kg level.

83-3a Developmental toxicity-Rat, MRID 00150077, Supplementary  
This study (No.3193-14/10, Acc 257765) was reviewed (005414)  
July 31, 1985 with the request for additional data to upgrade  
the study. Subsequently, these deficiencies were cited in DER  
005415 of August 6, 1986. In response to the FIFRA 88 Phase  
4 Review, the sponsor (Nor-Am Chemical Company) committed to  
provide the additional data requested. The data requested  
to upgrade this study have not been submitted and thus this  
remains a data gap for MITC. Currently, this study was  
evaluated with the conclusion (verbal communication from  
Steve Dapson) that DER 005414 is adequate with concurrence  
with the reviewers request for additional information to  
upgrade the study.

83-3b Developmental toxicity-Rabbit, MRID 00150076, Supplementary  
This study (No. 3687-14/30, Acc 257764) was reviewed  
(DER 005414) July 31, 1985 with the conclusion that the study  
cannot be upgraded. Subsequently, these conclusions were  
reiterated in DER 005415 of August 6, 1986. In response to  
the FIFRA 88 Phase 4 Review, the sponsor (Nor-Am Chemical  
Company) committed to provide the additional data requested.  
Currently, this study was evaluated with the conclusion  
(verbal communication from Steve Dapson) that DER 005414 is  
adequate however, this study may be upgraded if the data  
requested are submitted and found acceptable. The FIFRA 88  
Phase 4 Review determined that for the non-food use of MITC,  
one developmental study (rat) is required.

84-2a Gene mutation, MRID 412214-10, Acceptable  
Negative with Salmonella typhimurium strains TA 1535,  
TA 1537, TA 1538, TA 98 and TA 100 or Escherichia coli WP2  
uvrA with or without mammalian metabolic activation at levels  
up to 1000 ug/disc. Moderate to severe cytotoxicity in all  
strains at  $\geq 50$  ug/disc -S9 and  $\geq 100$  ug/disc +S9 were  
reported.

84-2b Sister chromatid exchange, MRID 412214-12, Unacceptable  
Negative for the induction of sister chromatid exchange in  
Chinese hamster V79 lung fibroblast cells following 4-hour  
exposure to concentrations of 0.1, 2.0 and 3.5 ug/ml in the  
absence of S9 and 0.1, 2.5 and 5.0 ug/ml in the presence of  
S9 activation. Suppression of cell replication at higher  
concentrations of MITC should have been evaluated. This study  
should be repeated.

84-4 Other gentotoxic effects, MRID 412214-10, Unacceta  
Negative for the induction of DNA damage repair-deficient  
Bacillus subtilis strain M45 (rec<sup>-</sup>) up to 2000 ug/disc.  
Cytotoxicity was not achieved, S9 activation was not  
included and only single replicates were assayed.

### Toxicology Concerns for Methyl Isothiocyanate (MITC)

MITC is highly toxic by the dermal ( $LD_{50}$  145 mg/kg) and inhalation ( $LC_{50} < 0.03$  mg/L) route of exposure. It is also a severe eye and dermal irritant. This chemical is a principal decomposition product and metabolite of Metam-sodium for which neurotoxicity screening studies are recommended. MITC is structurally related to carbon disulfide, a well known and potent neurotoxicant (B. Sette, 7/9/91).

During FIFRA 88 phase four reregistration review of MITC, neurotoxicity studies conducted by the 1982 testing guidelines (81-7 and 82-5) were not requested. However, acute (81-8) and subacute (82-7) neurotoxicity screening battery studies are requested for methyl isothiocyanate to be conducted by the neurotoxicity testing guidelines of March 1991 (EPA 540/09-91-123).

There are no additional Toxicology concerns for MITC at this time.

### Toxicology Data Requirements for Methyl isothiocyanate

Methyl isothiocyanate is a List B chemical for which the data base has been evaluated during FIFRA 88 Phase Four Reregistration process. The following table lists studies requested in support of non-food uses for wood treatment of utility poles.

<u>Guideline Study</u>	<u>MRID/Acc</u>	<u>DER No.</u>	<u>Upgrade- able</u>	<u>Existing Data Gap</u>
81-1 Acute oral	264390	006165	-	No
81-2 Acute dermal	264384	009742	-	No
81-3 Acute inhalation	264386	009742	-	No
81-4 Primary eye irritation	264387	006165	-	No
81-5 Primary dermal irritation	264388	006165	-	No
81-6 Dermal sensitization	41221404	This Review	-	No
81-8 Acute neurotoxicity*	---	---	-	Yes
82-2 21-day dermal	41221406	This Review	-	No
82-4 90-day inhalation	41221407	This Review	-	No
82-7 Subacute neurotoxicity*	---	---	-	Yes
83-3 Teratology-Rat	257765 00150077	005414 005415	Yes	Yes
84-2a Gene mutation	41221410	This Review	-	No
84-2b Chromosomal aberration	41221412	This Review	No	Yes
84-4 Other genotoxic effects	41221410 257758	This Review 005368	No Yes	Yes Yes

---

\* Acute and subacute neurotoxicity studies conducted according to the Neurotoxicity Testing Guideline of March 1991 (EPA 540/09-91-123).

011275

Primary Review by Ray Landolt *9/21/94*  
Review Section I, TB II/HED (7509C)  
Secondary Review by Mike Ioannou *9/26/94*  
Review Section I, TB II/HED (7509C)

DATA EVALUATION RECORD

Study Type: 90-Day Inhalation Study - Rat (82-4)

Test Material: Methyl isothiocyanate

MRID 412214-07

P.C. No. 068103

Synonym: MITC, Methyl mustard oil

Barcode D194652

Submission S447097

Classification: Wood (Timber) Treatment

Study No. 374/77

Date of Study: September 26, 1978

Title of Report: T22 Methyl Isothiocyanate: ZK 3.318  
A 12-13 Week Inhalation Study in the Rat

Authors: G. Rosskamp, G. Schobel, A.S. Bhargava,  
P. Staben and G. Schuppler

Testing Facility: Schering AG, Pharmaceutical Research, Germany

Sponsor: Nor-Am Chemical Company

Registrant: Degussa Corporation

Executive Summary: In a subchronic inhalation study (MRID 412214-07) 3 groups of 10 rats/sex/dose received a nose-only inhalation exposure to MITC at 3.16, 30.67 and 137.13 ug/L 4 hours/day, 5 days/week over a 12 to 13 week period. There were two control groups of 10 rats/sex/dose, one maintained in the laboratory without inhalation exposure and the other in the chamber without exposure to MITC.

By extrapolation from a four to six hour exposure (82-4 Guideline Data Recommendation for 6 hour exposure) the NOEL and LOEL levels are 2.1 (low dose) and 20.6 ug/L (mid dose), respectively. 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 include decreased total protein with increased alkaline phosphatase and alanine aminotransferase values.

This study is classified Core Minimum and satisfies the 82-4 Guideline Data Requirement 82-4 for a 90-day inhalation study.

A. Materials:

1. Test Compound: Methyl isothiocyanate (ZK 3.318) with a purity of 95.69% was used in this study. Contaminants are [REDACTED]
2. Test Animals: Male and female Wistar rats weighing between 142 and 239 g were used in this study.

B. Study Design - Allocation of animals

1. Ten rats/sex were maintained in the laboratory as untreated controls.

<u>Test Group</u>	<u>Analytical Concentration</u>		<u>Male</u>	<u>Female</u>
	<u>ppm</u>	<u>ug/L</u>		
Chamber Control	-	-	10	10
Low Dose	1.04	3.16	10	10
Mid Dose	10.09	30.69	10	10
High Dose	45.11	137.13	10	10

The doses selected for this study were based on a range finding study in which two groups of 5 rats/sex/group were exposed to 100 ppm MITC. One group that received a 1-hour daily exposure for 4-days exhibited "moist noses and mouths" with an apathetic appearance. The other group received a 4-hour daily exposure for 4-days exhibited "bloody noses and mouths" with death of 2/10 animals within 4-days.

When not exposed to the test material all animals were housed individually in air conditioned rooms (20-23°C and relative humidity of 50-56%) with a 12 hour light/dark cycle provided for a uniform environment. Food and water were available ad libitum.

2. Exposure: The animals received a nose-only exposure to a gas-air mixture generated in a dynamic air flow chamber for 4-hours/day, 5-days/week over a 12 to 13 week period. The 82-4 Guideline Data Recommendation for a 90-day inhalation study is 6-hour exposure. To correct for the four exposure in this study one may use Haber's rule which states that the animal response is constant when the product of the concentration (C) of a substance, multiplied by time (t), during which the exposure experienced is a constant ( $C \times t = K$ ). By extrapolation, the inhalation exposure of the low, mid and high dose in this study would be 2.1, 20.6 and 91.9 ug/L, respectively for a 6-hour exposure.

Relative humidity and temperature within the chamber ranged between 46-53% and 21-22°C, respectively. Chamber concentrations of the air-gas mixture of MITC were monitored from samples drawn for 10 minutes during the first and third hour exposure for infra-red analysis. Chamber design and generation of the air-gas mixture of MITC on pages 7 and 8 of this report are attached.

### C. Methods and Results:

#### 1. Observations:

- a. Gross signs of toxicity were generally limited to the high dose with all animals apathetic in appearance during days 27 to 85 accompanied by salivation and nasal discharge during days 1 to 85 of the study. Stimulated vocalization was reported for 6/20 animals during days 58 to 78. Additional signs observed at the high dose include dyspnea, stiff-legged gait and enlarged abdomen.
- b. One animal died during week 4 at the high dose due to experimental error (blood sampling).
- c. Body weight was recorded weekly. A significant ( $p < 0.01$ ) decrease in body weight gain was reported for males (63%) and females (47%) at the high dose as compared to the chamber control group. At the mid dose body weight gain was decreased (not significant) for males (11%) and females (15%) as compared to the chamber controls.
- d. Food consumption was recorded weekly. A significant ( $p < 0.05$ ) decrease in food consumption was reported for males (6%) and females (8%) at the high dose as compared to the chamber controls. Food intake for the low and mid dose animals were comparable to the chamber control group.
- e. Food efficiency calculated from the mean values reported in the table on the following page show that food efficiency was affected to a great extent at the high dose males and females and to a lesser degree in the mid dose males and females with a decrease of 15 and 12%, respectively.

#### Food Efficiency (%)

	Chamber Control	Exposure (ug/L)		
		3.16	30.69	137.1
Males	7.5	7.6	6.4	2.9
Females	5.7	5.5	5.0	3.0



The following table from the text of this study summarizes the tables, in German, submitted of the mean values and standard deviations for changes in body weight gain, food consumption and water intake.

Group	Food consumption (kg)		Water consumption (kg)		Bodyweight gain (g)	
	M	F	M	F	M	F
I (untreated control)	1.44/0.08	1.12/0.06	2.58/0.26	2.34/0.20	158/18	77/17
II (sham dose)	<u>1.24/0.08</u>	1.07/0.05	2.45/0.16	2.31/0.24	<u>93/16</u>	<u>62/7</u>
III ZK 3.318 (~ 1 ppm)	<u>1.22/0.09</u>	<u>1.03/0.05</u>	2.37/0.31	2.35/0.26	<u>93/21</u>	<u>57/13</u>
IV ZK 3.318 (~ 10 ppm)	<u>1.29/0.05</u>	<u>1.05/0.06</u>	2.79/0.23*	<u>2.8 /0.5*</u>	<u>83/19</u>	<u>53/11</u>
V ZK 3.318 (~ 45 ppm)	<u>1.17/0.05*</u>	<u>0.98/0.05**</u>	2.71/0.28	2.69/0.27*	<u>14/26**</u>	<u>33/13**</u>

--- p < 0.05 ;      — p < 0.01      (comparisons with group I)  
 • p < 0.05 ;      \*\* p < 0.01      (comparisons with group II)

f. Water intake (recorded twice weekly) increased at the mid dose significantly ( $p < 0.05$ ) for males (14%) and females (21%) and at the high dose for males (16%) as compared to the chamber controls. An increase (not significant) in water intake was reported for the high dose females (11%).

g. Ophthalmoscopic examination of all animals during weeks -2, -1 and 12 of the study were negative.

## 2. Clinical Findings:

a. Hematology - Blood was withdrawn during weeks -2, 4 and 12 from 5 animals/sex/group for determination of the following parameters (the checked (\*) parameters are recommended by Subdivision F Testing Guidelines of November 1989).

* Erythrocyte count	* Hemoglobin concentration
* Leukocyte count	* Hematocrit
Reticulocyte count	Mean corpuscular hemoglobin
* Differential count	Mean cell volume
* Thrombocyte count	

A significant ( $p < 0.05$ ) increase in leucocyte and lymphocyte count during week 4 accompanied by a significant ( $p < 0.01$ ) increase in neutrophil count during weeks 4 and 12 were reported for the high dose females. Hematocrit and erythrocyte values were significantly ( $p < 0.05$ ) increased for the high dose males by week 4. Bone marrow analysis revealed a significant ( $p < 0.05$ ) reduction of eosinophilic granulopoiesis in the high dose females by week 12. These changes are considered spurious events and not dose related.

Thromboplastin time, partial thromoplastin time, thrombin time and fibrinogen determined during weeks -1, 4 and 12 from 5 animals/sex/dose were without an effect on the parameters determined.

- b. Blood Chemistry - Blood was withdrawn during weeks -1, 4 and 12 from 5 animals/sex/dose for determination of the following parameters (the checked (\*) parameters are recommended by Subdivision F Testing Guidelines of November 1989). The check (\*\*) parameters were omitted.

* Alkaline phosphatase	* Total Protein
* Aspartate aminotransferase	* Albumin
* Cholesterol	* Urea
* Lactic acid dehydrogenase **	* Phosphate **
* Glucose (fasting)	* Calcium **
Alanine aminotransferase	* Sodium **
Protein electrophoresis	* Bilirubin **

A significant ( $p < 0.05$ ) decrease in total protein was reported for the mid and high dose males and for the high dose females by week 12 of the study. Fasting glucose values were significantly ( $p < 0.05$ ) reduced by week 12 for the high dose females. At the high dose a significant ( $p < 0.05$ ) increase in alkaline phosphatase values were reported for males and females accompanied by significantly ( $p < 0.01$ ) increased alanine aminotransferase values in males by week 12.

- c. Urinalysis parameters were examined during weeks 4 and 12 from 5 animals/sex/dose for examination of the following parameters (checked (\*) parameters are recommended by Subdivision F Testing Guidelines of November 1989).

* Blood	* Appearance	* Sediment
* Protein	* Glucose	* Specific Gravity
* Ketone bodies	* Urobilinogen	pH
* Total bilirubin (omitted)		

No changes were recorded related to the test material.

### 3. Terminal Findings:

Histopathological examination of the following tissues are recommended by Subdivision F Guidelines of November 1989. The checked (\*) tissues were examined from all animals of the control and high dose. In addition lungs, liver and kidneys of all low and mid dose animals are to be examined. The checked (X) organs were weighed from 10 rats/sex/dose.

* aorta	* X pituitary	* mammary gland
eyes	* ileum	* X spleen
caecum	* trachea	* muscle
* colon	jejunum	* X adrenals
* duodenum	* bone marrow	* uterus
* X brain	* X liver	* ovaries
* skin	* lung	oviduct
* X heart	* X lymph node	* pancreas
* testes	* stomach	* spinal cord
epididymis	* X kidneys	* peripheral nerve
esophagus	rectum	* thyroid/parathyroid
* thymus	salivary glands	* urinary bladder

Bronchi and prostate were examined in addition to the recommended tissues.

- Macroscopic findings - No gross pathological findings were reported relative to the test material.
- Organ Weights - Absolute and relative organ weight changes correlate with the decrease in body weight for the high dose males and females.
- Histopathological examination of the untreated controls and the high dose animals were without effect on the tissues examined. Tissues of the low and mid animals were not examined.

D. Discussion: This study was conducted with 10 rats/sex/dose of 3.16, 30.67 and 137.13 ug/L. The animals received a nose-only inhalation exposure of MITC 4-hours/day, 5-days/week over a 12 to 13 week period. To correct for the four hour exposure the doses for a six hour exposure for the low, mid and high dose would be 2.1, 20.6 and 91.9 ug/L, respectively

Clinical determinations of hematology, chemistry and urinalysis parameters were conducted on 5 rather than the 10 animals/sex/dose. No definitive data were provided from the terminal observations to support the clinical findings reported. Adequate information was provided from the hematology, urinalysis and blood chemistry values to determine the NOEL and LOEL for this study.

The low and mid dose animals were not subjected to histopathologic evaluation of lungs, liver and kidenys. However, the high dose animals were without an effect on histopathological examination and preclude the need to examine the low and mid dose tissues.

E. Conclusions: NOEL = 2.1 ug/L

LOEL = 20.6 ug/L with decreased body weight, food efficiency and blood protein values accompanied by increased water intake.

Classification of Data - Minimum

This study was conducted prior to the November 1989 Guidelines for testing and was not classified as a Guideline study because of the deficiencies cited in the discussion. This study does contain useful information and is acceptable for a 90-day inhalation study.

RIN 3426-95

MITC REVIEW

Page \_\_\_\_\_ is not included in this copy.

Pages 13 through 14 are not included.

The material not included contains the following type of information:

- \_\_\_\_ Identity of product inert ingredients.
- \_\_\_\_ Identity of product impurities.
- \_\_\_\_ Description of the product manufacturing process.
- \_\_\_\_ Description of quality control procedures.
- \_\_\_\_ Identity of the source of product ingredients.
- \_\_\_\_ Sales or other commercial/financial information.
- \_\_\_\_ A draft product label.
- \_\_\_\_ The product confidential statement of formula.
- \_\_\_\_ Information about a pending registration action.
- ☒ FIFRA registration data.
- \_\_\_\_ The document is a duplicate of page(s) \_\_\_\_\_.
- \_\_\_\_ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Primary Reviewer: Ray Landolt *9/28/94*  
Review Section I, TB II/HED (7509C)  
Secondary Reviewer: Mike Ioannou  
Review Section I, TB II/HED (7509C) *J.M.L. 9/28/94*

## DATA EVALUATION RECORD

Study Type: 30-Day Dermal Toxicity Study - Rat (82-2)

Test Material: Methyl isothiocyanate

MRID 412214-06  
P.C. Code 068103  
Barcode D194652  
Submission S447097

Synonym: MITC, Methyl mustard oil

Classification: Wood (Timber) Treatment

Study No. 4411, Report No. PF 15/86

Date of Study: November 26, 1975, Revised: July 16, 1986

Title of Report: T20/2-METHYL ISOTHIOCYANATE-Subacute  
(28-30 Day) Dermal Toxicity Study in the Rat

Authors: Chr. Schobel and H. Schweinfurth

Testing Facility: Schering AG, Germany

Sponsor Nor-Am Chemical Company

Registrant: Degussa Corporation

Executive Summary: In a 30 day dermal toxicity study (MRID 412214-06) MITC (95.5%) was applied dermally for a 5-hour daily exposure to 3 groups of 10 rats/sex/dose 5-days/week at 1.0, 10.0 and 100 mg/kg.

The systemic NOEL and LOEL are 1.0 and 10.0 mg/kg, respectively. At the 10.0 mg/kg level there was decreased serum albumin and increased globulin values and increased relative liver weights.

The dermal NOEL and LOEL are 1.0 and 10.0 mg/kg, respectively. Erythema and desquamation were observed at the 10.0 mg/kg level.

The affect of dermal exposure to the 100 mg/kg dose of MITC is severe erythema and necroses accompanied by signs of severe pain, decreased body weight and food intake, increased water intake, increased blood glucose, decreased serum albumin, increased globulin values, increased blood and urinary lactate dehydrogenase values, increased absolute and relative liver and adrenal weights.

This study is classified as Core-Minimum and satisfies the 82-2 data requirement for a 21-day dermal toxicity study.

#### A. Materials:

1. Test Compound: Methyl isothiocyanate (ZK 3.318) of batch No. 24962 and a purity of 95.5% was used in this study. MITC was dissolved in sesame oil for dermal application at 0.2, 2.0 and 20 mg/ml for days 1 and 2 then changed to 0.5, 5.0 and 50 mg/ml from day 3 through the termination of the study.
2. Test Animals: Male and female Wistar rats weighing between 150-243g for males and 140-175g for females were used in this study.

#### B. Study Design - Allocation of animals

<u>Test Group</u>	<u>Test Material</u>	<u>Dose mg/kg</u>	<u>Males</u>	<u>Females</u>
1. Control	Sesame oil	-	10	10
2. Low dose	MITC	1.0	10	10
3. Mid dose	MITC	10.0	10	10
4. High dose	MITC	100.0	10	10

The test material was applied to the clipped intact dorsal skin (20-30 cm<sup>2</sup>) of 10 rats/sex/dose level at 1.0, 10 or 100 mg/kg for a 5 hour exposure 5 days/week for 30 days. Dose levels were adjusted weekly based upon individual body weight. The test site was covered with a mull bandage. After the 5-hour exposure the bandage was removed and the test site was washed with luke warm water and dried with soft tissue. Due to the volatility of MITC, gas masks were worn by laboratory personnel during all procedures in the animal room. All animals were housed individually with food and water available ad libitum. Temperature (21-25°C), humidity (40-65%) and 12 hour light/dark cycle were provided for a uniform environment.

Statistical analysis: The Duuett-test was used to assess the significance of differences between group mean values.

## C. Methods and Results

### 1. Observations:

- a. All animals were observed daily for mortality, signs of toxicity and dermal irritation. No clinical signs of toxicity were reported for the low and mid dose animals. The 100 mg/kg animals exhibited signs of severe pain during the 5-hour exposure period with vocalization, jumping, reaching for test site and "a supine position under the water bottle". One male at the high dose appeared apathetic, emaciated and cold to touch prior to death on day 26.

Dermal response of the test site to the low dose was yellow desquamation. Light-colored desquamation and erythema were reported for the mid dose during days 5 through 17. At the 100 mg/kg dose by day 5 the test sites were red and coriaceous with desquamation, fissures, eschar and necrosis through day 15 followed by sloughing of the necrotic tissue through day 26. Necrosis developed on the regenerated treated skin during days 26 to 29. Severity of the dermal response was greater in males than females.

- b. Body weight was recorded weekly. A significant ( $p < 0.01$ ) decrease in mean body weight gain was reported for males (-4 vs 60 g for controls) and females (23 vs 45 g for controls) at the high dose.
- c. Food consumption was recorded every 2 to 3 days. A significant ( $p < 0.01$ ) decrease in food consumption was reported for the high dose males.
- d. Water intake was recorded every 3 to 4 days. A significant ( $p < 0.01$ ) increase in water intake was reported for females at the high dose.

### 2. Clinical Findings

Blood was withdrawn from the jugular vein during weeks -2, 2 and 4 from 5 animals/sex/dose for determination of hematology and clinical chemistry parameters.

- a. Hematology - The checked (\*) parameters are recommended by Subdivision F Guidelines of November 1989.
 

* Erythrocyte count	* Hemoglobin concentration
* Leukocyte count	* Hematocrit
* Differential count	Mean corpuscular hemoglobin
* Thrombocyte count	Mean corpuscular volume
Mean corpuscular hemoglobin concentration	

No significant dose related changes were reported.



At necropsy femoral bone marrow was evaluated from all animals of the low, mid and high dose groups and from 10 male and 6 female control rats. Female bone marrow of the test levels was not subject to statistical evaluation due to the low (6) number of samples obtained. A significant ( $p < 0.01$ ) increase in the erythropoiesis index was reported for the 100 mg/kg males accompanied by a significant ( $p < 0.01$ ) increase in the neutrophil granulocytopenia index was reported for the 10 and 100 mg/kg males. These changes were not considered related to the test material due to the low indices reported in the control animals.

- b. Clinical chemistry - The checked (\*) parameters are recommended by the Subdivision F Testing Guidelines of November 1989.

* Alkaline phosphatase	* Total protein
* Lactic dehydrogenase	* Albumin
* Cholesterol	* Urea
* Glucose	* Phosphate (omitted)
* Alanine aminotransferase	* Calcium (omitted)
* Bilirubin (omitted)	* Sodium (omitted)
Protein electrophoresis	
* Aspartate aminotransferase (omitted)	

A significant ( $p < 0.01$ ) increase in blood glucose values were reported for the high dose males and females. Albumin values decreased significantly ( $p < 0.05$ ) for the mid and high dose males and females during week 4 and for the high dose females during week 2. These decreases in albumin values were accompanied by significant ( $p < 0.05$ ) increases in gamma globulin values for the mid dose males and females and high dose females. Beta globulin values were increased significantly ( $p < 0.05$ ) for the mid dose males and high dose males and females during week 4. A non-significant increase in lactate dehydrogenase was reported for high dose males during week 4. The statistically significant and percent changes as compared to the control group in blood glucose, albumin and globulin values are summarized in the following table.

Dose (mg/kg) Sex Week	10				100			
	male		female		male		female	
	2	4	2	4	2	4	2	4
<u>Glucose</u>						44↑		28↑
<u>Albumin</u>		16↓		19↓		17↓	15↓	14↓
<u>Globulin-beta</u>		77↑				74↑		40↑
gamma		33↑		65↑				60↑

In addition, plasma and erythrocyte cholinesterase (ChE) values were determined during week -1, 2 and 4. Brain cholinesterase was determined at the termination of the study. A significant ( $p < 0.01$ ) decrease in plasma cholinesterase activity was reported for the high dose females during weeks 2 and 4. These changes in plasma cholinesterase activity were without corresponding changes in erythrocyte and brain ChE activities.

Statistically significant changes in blood enzyme activity, cholesterol and total protein values were not considered clinically significant or dose related.

- c. Urinalysis parameters were examined during weeks -2, 2 and 4 from 5 animals/sex/dose. The checked (\*) parameters are recommended by Subdivision F Testing Guidelines of November 1989.

* Blood	* Appearance	* Sediment
* Protein	* Glucose	* Specific gravity
* Ketone bodies	* Urobilirubin	pH
* Total bilirubin (omitted)		

No changes in the above parameters were reported related to the application of the test material. Quantitative measurement of lactate dehydrogenase excretion was determined on days -1, 2 and 4 from 5 animals/sex/dose. A significant ( $p < 0.01$ ) increase in lactate dehydrogenase excretion was reported for the high dose males during week 4. A non-significant increase in lactate dehydrogenase excretion was reported for the mid (1/5) and high dose females (1/5) during week 4.

3. Terminal Findings: The following checked (\*) tissues are recommended for histopathologic examination of the control and high dose animals by Subdivision F Testing Guidelines of November 1989. The following tissues were subjected to histopathological examination on all animals. The checked (x) organs were weighed on all animals.

* x liver	* x kidneys	* lung
x adrenals	x heart	pancreas
stomach	duodenum	jejunum
ileum	caecum	colon
x thyroid	thymus	spleen
lymph node	x brain	spinal cord
peripheral nerve	skin	x ovaries
x testes		

- a. Macroscopic observations were limited to the severe dermal necroses of all the high dose animals.

- b. Organ Weights - A significant ( $p < 0.01$ ) increase in relative liver weights of the mid dose females and the absolute and relative liver weights of the high dose females was reported.

Male relative liver weights were increased significantly ( $p < 0.01$ ) at the high dose.

Absolute and relative adrenal weights of males and females at the high dose were significantly ( $p < 0.05$ ) increased.

- c. Histopathological examinations of the low and mid dose animals were without effects from the application of MITC.

The application site of all the high dose animals "showed extended necroses which occurred partly together with sclerosis of the adjacent fatty and connective tissues." "A minimal reactive epidermal hyperplasia of the adjacent area" was observed in seven high dose animals. Moderate lymphatic depletion of the spleen observed in two animals was regarded as stress related.

- D. Discussion: This study was conducted with 10 rats/sex/dose of 1.0, 10.0 and 100 mg/kg of MITC applied dermally for a 5-hour exposure 5-days/week over a 4-week period. The Subdivision F Guidelines of November 1989 recommends a 6-hour exposure over a 3-week period for a 21-day dermal toxicity study. Due to the severe necrosis reported at 100 mg/kg dose in this study a 6-hour daily dermal exposure to MITC could have affected the survival at this dose and it is doubtful if additional information would have been provided by an additional one hour exposure.

Toxicology review (DER 003991) August 16, 1984 determined that this study (No. 4411) was deficient and was not reviewed. The reviewer requested the registrant "to submit a complete study with all data representing all parameters investigated, Individual data should also be included to validate the reported findings." The report was revised by the performing laboratory July 16, 1986, but not submitted for review.

The affect on dermal exposure to MITC is severe dermal irritation at the 100 mg/kg dose accompanied by signs of severe pain, death (1/20), decreased body weight gain and food intake, increased water intake, increased blood glucose and decreased albumin values associated with severe necrosis at this dose.

In addition, at the 100 mg/kg dose, increased globulin values, increased blood and urinary lactate dehydrogenase values and increased absolute and relative liver and adnenal weights were reported.

E. Conclusion: Systemic NOEL = 1.0 mg/kg

LOEL = 10.0 mg/kg with decreased serum albumin and increased globulin values and increased relative liver weight reported at this level.

Dermal NOEL = 1.0 mg/kg

LOEL = 10.0 mg/kg with erythema and desquamation observed at this level.

#### Classification of Data - Minimum

This study is acceptable and satisfies the guideline data requirement (82-2) for a 21-day Dermal Toxicity Study.

Primary Review by Ray Landolt *9/8/94*  
Review Section I, TB II/HED (7509C)  
Secondary Review by Mike Ioannou *9/8/94*  
Review Section I, TB II/HED (7509C)

## DATA EVALUATION RECORD

Study Type: Dermal Sensitization - (81-6)

Test Material: Trapex 40

MRID 412214-04

Chem No. 068103

Chemical Name: Methyl isothiocyanate

Barcode D194652

Submission S447097

Synonym: MITC

Classification: Wood (Timber) Treatment

Study No.: RCC 056395

Date of Study: March 17, 1986

Title of Report: T95-Methyl Isothiocyanate: Test for Delayed  
Contact Hypersensitivity in the Albino Guinea Pig  
with TRAPEX 40 (CQ 070) Maximization Test.

Author: L. Ullmann and K. Sachsse

Testing Facility: Research & Consulting Company AG, Switzerland

Sponsor: Schering AG, Agrochemical Division

Registrant: Degussa Corporation

Conclusion: Trapex 40 is a mild sensitizer when tested by the  
Guinea Pig Maximization Test.

This study is Acceptable and satisfies the guideline  
data requirements (81-6) for a dermal sensitization  
study.

## Experimental Design for the Guinea Pig Maximization Test (GPMT)

**Animals:** Fifteen male and 15 female, 8-9 week old Dunkin-Hartley albino guinea pigs weighing between 432 and 530 g were used in this study.

**Test Materials:** Trapex 40 (CQ 070), of batch number 310 8210 was applied as a 1.0% solution in corn oil. The purity of the Trapex 40 formulation tested was not available in this report. However, FIFRA 88 Phase 3 Summary of this study declared "Trapex 40 to be a commercial formulation containing 40% MITC in a solvent"

Freund's Complete Adjuvant (FCA) was administered in 50:50 with corn oil for injection. A positive control group was not determined in this study, but referenced with a 71% positive response reported for this strain of guinea pigs when treated with dinitrochlorobenzene during March 1985.

**Procedure:** The study protocol follows that of Magnusson, B. and Kligmann, A.M., The Identification of Contact Allergens by Animal Assay., J. Invest. Dermatol. 52.:268-276, 1969.

A range finding study was conducted to determine the irritancy of the test concentration to be applied during the induction and challenge phase. Intradermal injections (0.1 ml) of 0.1, 0.5 or 1.0% of the test material were administered to two animals. Four guinea pigs received a topical application 0.1, 0.5 or 1.0% in corn oil for a 24 hour exposure. A 1.0% concentration was selected for the induction and challenge phase.

For the induction phase three paired intradermal injections of FCA in corn oil, test material (1.0%) and FCA plus test material were made to the shaven left and right shoulder area of 10 guinea pigs/sex. After 7-days, a topical application of the test material (1.0%) on filter paper was applied to the test sites, covered with aluminum foil, wrapped with elastic bandage and secured with adhesive tape for a 48 hour exposure. The first challenge was applied 2-weeks after the topical application and in the same manner as the topical application for a 24 hour exposure. The second challenge was applied two weeks after the first challenge similar to the first challenge for a 24 hour exposure. The challenge sites were scored according to the Draize method immediately after exposure then at 24 and 48

hours after removal of the test material.  
The control group of 5 animals/sex were treated in  
the same manner without the test material.

Results: No signs of toxicity or mortality were reported in  
the test or vehicle control groups. Body weight gains  
between the test and vehicle control groups were  
comparable.

All animals of the control and test groups showed erythema  
and edema of the three paired injection sites during days  
2 to 4, followed by a crusty area at the injection sites  
during days 5 to 9, exfoliation during days 10 to 34 and  
a healing trend reported for the remainder of the study.

Incidence Of Positive Erythema

	<u>24 hours</u>	<u>48 hours</u>
First Challenge	4/20 (20%)	3/20 (15%)
Second Challenge	2/20 (10%)	1/20 (5%)

Slight erythema was observed on 4 animals at 24 hours  
and on 3 animals at 48 hours following the first  
challenge. After the second challenge slight erythema  
was observed on 2 animals at 24 hours and on 1 animal  
at 48 hours.

The vehicle controls were negative for erythema  
following the first and second challenge.

Conclusion: Trapex 40 is classified a mild sensitizer  
according to the allergenicity rating of  
Kligman, 1966\*.

---

\* Kligman, A.M. 1966. The identification of contact  
allergens by human assay. The maximization test. A  
procedure for screening and rating contact sensitizers.  
J. Invest. Dermatol. 47:393-409.