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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Methylisothiocyanate (MITC)
Project No. 9-0798, TOX Chem No. 573

FROM: Ray Landolt 4/17/89
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THRU: Mike Ioannou, Acting Section Head
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and

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Toxicology Branch II - Herbicide, Fungicide, and
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Health Effects Division (H7509C) Marcia van Gemert 4/24/89

Registrant: Nor-Am Chemical Company, letter of January 17, 1989

Use: Methylisothiocyanate (MITC) - a soil fumigant.

Action Requested: Review a two-generation rat reproduction study (83-4) submitted in support of the Data Call-In Notice for MITC.

Reviewed By: Ray Landolt 4/17/89
Section I, Toxicology Branch II-HFAS (H7509C)
Secondary Reviewer: Mike Ioannou 4-18-89
Section I, Toxicology Branch II-HFAS (H7509C)

DATA EVALUATION REPORT

Study: Two-Generation Rat Reproduction Study (83-4)

Study Number: TOX 85003 TOX Chem No.: 573

Testing Laboratory: Hazleton UK Accession No.: 409746-01
409746-02

Author: L. Barker Study Date: December 23, 1987

Sponsor: Nor-Am Chemical Company

Test Material: Methylisothiocyanate of batch number 340178 with a purity of 96.5% was used in this study.

Synonyms: MITC

Study Title: T 98 Technical Methylisothiocyanate: 2-Generation Oral (Drinking Water) Reproduction Study in the Rat.

Conclusions:

1. Classification of Data - Supplementary
   a. Deficiency
      i. The report did not provide a tabulation of the clinical signs of toxicity for the adult animals.

      ii. The data (in tabular form) used to derive the Group Mean Test Article Intake in Tables 5 and 15 should be submitted.

      iii. The test levels of MITC administered in this study should be reported as the actual concentration of the working dilution at discard, rather than the nominal concentration, due to the instability of the test material in the drinking water.

      iv. The registrant should address the great fluctuations in the percent of MITC concentrations of the stock solutions, working dilutions and bottle samples reported at discard.

2. Systemic NOEL-Mid Level

3. Systemic LEL- High Level with a significant decrease in parental body weight gain (29%) of the F_1 males.

4. Reproductive NOEL-High Level with no adverse effects on reproductive indices or development of the pups.

Reviewers Note: MITC, when administered in the drinking water for two generations to rats, reduced water intake at the mid and high levels due to the likely unpalatability of the test material. The incidence of systemic effects at high level are marginal, however, the reduced water and likely unpalatability of the test material limit further testing at higher dosage levels.

A. Materials

1. Animals- Weanling male and female Sprague Dawley CD rats were obtained from Charles River for the parental (P) generation.

2. Treatment- Methylisothiocyanate was available ad libitum in the drinking water at the nominal concentrations of 0, 2, 10, and 50 ppm throughtout two successive generations to assess the effect of MITC on reproductive and postnatal development of rats. The water solutions were available from glass bottles fitted with stainless steel ball valve spouts.

   Previously conducted studies with MITC demonstrated the water preparations of the test material to be relatively unstable. For this reason, particular emphasis was placed on the preparation, dispensing, and analysis of the stock, and use dilutions in this study. This study was conducted with stock solutions of MITC prepared at a theoretical concentration of 1200 ppm (w/v) weekly on Friday for dilution with tap water on Friday, Monday, and Wednesday for preparation of the respective use concentrations. To compensate for the anticipated loss of MITC in tap water, the working (use) dilutions were over-formulated by 20%. Theoretical concentrations of 2.4, 12, and 60 ppm were prepared from the stock solutions to allow for a nominal use concentration of 2, 10, and 50 ppm. The working (use) concentrations prepared on Friday were available to the test animals on Saturday, Sunday and Monday, then discarded. The use concentrations prepared on Monday were available to the test animals on Tuesday and Wednesday, then discarded. The use concentrations prepared on Wednesday were available to the test animals on Thursday and Friday, then discarded.
   "The drinking water in the cage bottles was replaced daily with the more recently prepared working dilutions".

3. Analysis- "The samples were analyzed by dilution of the aqueous formulations with acetone and measurement of the MITC concentrations by gas liquid chromatography using a nitrogen-selective dectector".

4. Stability- Samples for analysis were taken at the time of preparation and discard of the stock and use concentrations(Chemistry Report 85003).

   a. "The stock solutions (stored at 4 °C for 5 days) generally showed some loss of MITC over the storage period. The average decline over the 5 days was 0.98 % (for weeks 1 to 42)". The concentration of the stock solutions analysed weekly at discard were reported to be in the range of 41.6% to 103.5 % of the theoretical concentration with a mean of 81.3% at discard, during the 42 week period.

   b. "The working dilutions analyzed from each week of the study gave values in the range of 23.7 to 104.5 percent (except for week 13 at 60 ppm, where a result of 0.0 was recorded." A summary of the mean analyses of the working dilutions over the 42-weeks are presented in the following table from the report.

Summary of the mean analyses of working dilutions

Theoretical Concentration	Percent Theoretical as Discarded on Days Monday	Wednesday	Friday
2.4 ppm	60.8	66.5	70.4
12.0 ppm	60.5	65.9	70.8
60.0 ppm	64.7	71.9	75.4

c. Water bottles were analysed at the time of discard on Sunday, Monday, and Tuesday of each week. "The cage bottle samples analyzed gave values in the range of 10.0 to 87.0 percent". A summary of the mean analyses of water bottles over the 42-week period are presented in the following table from the report.

Summary of the Mean Analyses of the Water Bottles

Theoretical Concentration	Percent Theoretical as Discarded on Days Sunday	Monday	Tuesday
2.4 ppm	53.1	55.5	58.9
12.0 ppm	56.1	56.7	62.3
60.0 ppm	62.9	64.2	67.8

From a cursory review of the mean analyses values of the working dilutions and the water bottle concentrations, the test animals received approximately 60 - 65.0 % of the theoretical concentration of MITC in their drinking water over the 42-week period.

4. The animals had free access to pelleted laboratory animal feeding diet (SQC Rat and Mouse Breeder Diet No. 3).

B. Study Design:

1. Aninals were assigned to the following groups:

Generation	Group Number	Dose Level (ppm) Nominal Concentration	Number of Animals Male	Female
P	1- Control	0	30	30
	2- Low	2	30	30
	3- Mid	10	30	30
	4- High	50	30	30
F_1	1- Control	0	25	25
	2- Low	2	25	25
	3- Mid	10	25	25
	4- High	50	25	25

2. The animals allocated to the P generation were approximately

5 weeks old and in the weight range of 133 to 183 g for males and 97 to 136 g for females. The duration of the premating period for the P generation was 70 days and for the F_1 generation, 77 days.

3. The breeding schedule consisted of a mating phase of 20 days, a gestation phase of 21 to 23 days, and a lactation phase of 21 days. Treated females were mated one on one with treated males to produce the P and F_1 generations. During the mating phase, vaginal smears were taken daily until sperm was seen in the smear (0-day of gestation). At the end of the mating period (20 days), the females and males were again housed individually. The test material was available ad libitum in the drinking water throughtout the duration of this study.

4. Statistical Evaluation : Data were analyzed as appropriate using analysis of variance, t-tests, Krushal-Wallis test, and Wilcoxon rank sum test. All tests were carried out at 1 and 5 percent significance levels for a two-sided risk.

C. Method and Results

1. Parents of the P and F_1 generation were examined once daily for signs of toxicity and subjected to a detailed clinical examination once weekly.

   a. No mortality or signs of toxicity were observed relative to the dosage levels administered to the males and females of the P and F_1 generations.

   b. A significant ($p<0.05$) decrease (29%) in the mean body weight gain was reported at the high level for the F_1 males during weeks 14 to 21.

      During the premating phase a significant reduction in body weight gain was reported for P generation females of the mid level ($p< 0.01$) and of the high level ($p<0.05$) by 9 and 7 percent, respectively, without a relationship to dose. Body weight changes during gestation and lactation were comparable between control and test animals.

   c. Food intake for males of the high level was reduced (not statistically significant) during the 10th week for the P and for the F_1 generation males by 4 and 6 percent respectively. No significant effect on mean food conversion efficiency was reported.

      Food intake for females of the high level was reduced (not statistically significant) by 6 to 9 percent for the P females during weeks 4 to 10 and by 5 to 11 percent for the F_1 females during weeks 7 to 10. No significant effect on mean food conversion efficiency was reported.

d. A significant decrease ($p < 0.01$) in water intake was reported at the high level by 15 to 25 percent for the P males and by 17 to 28 percent for the F_1 males during the 10-week premating period.

A significant decrease ($p < 0.05$) in water intake was reported at the mid level by 3 to 12 percent for the P males during the 10-week premating period.

A significant decrease ($p < 0.01$) in water intake was reported at the high level by 7 to 25 percent for the P females and by 19 to 37 percent for the F_1 females during the 10-week premating period.

A significant decrease ($p < 0.01$) in water intake was reported at the mid level by 4 to 24 percent for the F_1 females during the 10-week premating period.

During the lactation period, a significant decrease ($p < 0.01$) in the mean daily water intake was reported for the P generation females at the mid and high levels by 11 and 19 percent, respectively.

During the lactation period, a significant decrease ($p < 0.01$) in the mean daily water intake was reported for the F_1 generation females by 27 percent at the high level.

e. A summary of the group mean intake of the test material in mg/kg/day during the 10-week premating period is presented from Tables 5 and 15 of the report

Group Mean (mg/kg/day) Intake of MITC

		Level		
Generation	Sex	Low	Mid	High
P	Male	0.16	0.76	3.58
	Female	0.21	1.01	4.76
F_1	Male	0.15	0.71	3.40
	Female	0.19	0.87	4.22

It is not clear in this report whether the calculated mean intake (mg/kg/day) values reported in Tables 5 and 15 were derived from the analyses of the working solutions discarded on Monday, Wednesday, and Friday or based on the theoretical concentrations of the respective dose levels. The data used to derive the Group Mean Test Article Intake in Tables 5 and 15 should be submitted in tabular form.

f. Mating performance and fertility indices for the P and F_1 generation are presented in the following tables, from the report.

Group Indices - P Generation

	Group 1	Group 2*	Group 3	Group 4
Total number of females	30	30	30	30
Total number of mated females	30	28	30	30
Percent females mated	100.0	93.3	100.0	100.0
Mating index percent	93.8	92.9	90.9	100.0
Total number of pregnant females	30**	28**	29	27
Fertility index percent	100.0	93.3	96.7	90.0
Fecundity index percent	100.0	100.0	96.7	90.0

*Day of mating of two animals not determined.
**Includes females presumed remated but subsequently killed.

Group Indices - F_1 Generation

	Group 1	Group 2	Group 3*	Group 4
Total number of females	25	23	25	25
Total number of mated females	25	21	24	25
Percent females mated	100.0	91.3	96.0	100.0
Mating index percent	89.3	100.0	82.1	96.0
Total number of pregnant females	24	20	21	25
Fertility index percent	96.0	87.0	84.0	100.0
Fecundity index percent	96.0	95.2	87.5	100.0

*Day of mating of one animal not determined.

Mating performance and fertility indices were not affected by the test material at the dosage levels administered.

g. Terminal Observations

i. Necropsy - All animals were subjected to gross necropsy. "The contents of the uterus of pregnant females, killed or found dead, were examined and the number of corpora lutea on each ovary counted". Implantations were divided into live, dead, and resorbed fetuses. None of the P generation died. Two females of the F_1 generation, group 2 were killed on weeks 3 and 6, respectively.

Gross necropsy findings of the adult P and F_1 generations were without adverse effects from the dose levels administered.

ii. Organ Weights and Histopathology - The following tissues were taken from 10 P and F_1 animals of each sex per group to be weighed (X) and examined histopathologically. The reproductive organs from all P and F_1 animals in control and high-dose group and reproductive organs from nonfertile animals were examined histopathologically.

(X) Adrenals	(X) Ovaries
Gastrointestinal tract	(X) Prostate/seminal vesicle
(X) Heart	(X) Pituitary
(X) Kidneys	(X) Testes/epididymides
(X) Liver	(X) Uterus/vagina
	All gross lesions

The relative pituitary weight of adult females administered the high level was increased significantly ($p < 0.01$) by 60 percent for the P females.

No significant changes in the relative organ weights were reported for the F_1 adults.

No histopathological findings (including pituitary and gonadal) were reported for the P and F_1 parental generation relative to the dosage levels of MITC administered.

2. Litter Data for F_{1a} and F_{2a}

a. Indices

Group Mean Litter Data for All Litters of P Generation

	Group 1	Group 2	Group 3	Group 4
Number of mated females surviving to Day 1L	29	24	30	30
Number of pregnant females surviving to day 1L	29	24	29	27
Percent of pregnancies	100.0	100.0	96.7	90.0
Mean duration of gestation (Days) 0G-1L	22.6	22.6	22.5	22.8
Number of pups born	424	352	400	369
Mean number per female	14.6	14.7	13.8	13.7
Sex ratio males : females	1:0.94	1:1.16	1:0.95	1:1.17
Number of pups alive Day 1	416	334	389	364
Mean number per female	14.3	13.9	13.4	13.5
Number of pups alive Day 4	412	299	361	355
Mean number per female	14.2	12.5	12.4	13.1
Number of pups alive Day 7	412	298	356	352
Mean number per female	14.2	12.4	12.3	13.0
Number of pups alive Day 14	401	283	317	327
Mean number per female	13.8	11.8	10.9	12.1
Number of pups alive Day 21	396	278	306	316
Mean number per female	13.7	11.6	10.6	11.7
Sex ratio males : females	1:0.95	1:1.16	1:1.00	1:1.09
Gestation index percent	100.0	100.0	100.0	100.0
Live birth index percent	98.1	94.9	97.1	98.6
Viability index 1 percent	99.0	89.5	92.8	97.5
Viability index 2 percent	100.0	99.7	98.6	99.2
Viability index 3 percent	97.3	95.0	89.0	93.9
Viability index 4 percent	98.8	98.2	96.5	96.6
Viability index 5 percent	95.2	83.2	78.7	86.8
Preweaning loss percent	6.6	21.0	23.5	14.4

L = Lactation
G= Gestation

A decrease (not statistically significant) in the mean number of pups born per female of the P generation in groups 3 and 4 was not dose related and may be incidental to the administration of the test material. In test groups 2, 3, and 4 the mean number of pups alive on days 1, 4, 7, 14, and 21 was less than (not statistically significant) the control values for these respective intervals. An increased preweaning loss was reported for groups 2, 3, and 4, as compared to the control values was reported, but was not dose related.

Group Mean Litter Data for All Litters of F_1 Generation

	Group 1	Group 2	Group 3	Group 4
Number of mated females surviving to Day 1L	24	21	23	24
Number of pregnant females surviving to day 1L	23	20	20	24
Percent of pregnancies	95.8	95.2	87.0	100.0
Mean duration of gestation (Days) 0G-1L	22.6	22.2	22.3	22.0
Number of pups born	254	227	246	263
Mean number per female	11.0	11.4	12.3	11.0
Sex ratio males : females	1:1.00	1:1.15	1:1.11	1:0.89
Number of pups alive Day 1	248	219	224	254
Mean number per female	10.8	11.0	11.2	10.6
Number of pups alive Day 4	239	213	207	236
Mean number per female	10.4	10.7	10.4	9.8
Number of pups alive Day 7	234	210	206	235
Mean number per female	10.2	10.5	10.3	9.8
Number of pups alive Day 14	234	208	205	233
Mean number per female	10.2	10.4	10.3	9.7
Number of pups alive Day 21	233	206	205	232
Mean number per female	10.1	10.3	10.3	9.7
Sex ratio males : females	1:1.03	1:1.08	1:1.09	1:0.83
Gestation index percent	100.0	100.0	100.0	100.0
Live birth index percent	97.6	96.5	91.1	96.6
Viability index 1 percent	96.4	97.3	92.4	92.9
Viability index 2 percent	97.9	98.6	99.5	99.6
Viability index 3 percent	100.0	90.0	99.5	99.1
Viability index 4 percent	99.6	99.0	100.0	99.6
Viability index 5 percent	94.0	94.1	91.5	91.3
Preweaning loss percent	8.3	9.3	16.7	11.8

L = Lactation
G = Gestation

The duration of gestation of groups 2, 3, and 4 is reduced (not statistically significant) as compared to the control value. An increased preweaning loss for groups 2, 3, and 4 was reported, but was not dose related.

1/

b. Litter weights were recorded on Days 1, 4, 7, 14, and 21.

Group Mean Pup Weight (g) P Generation

	Group 1	Group 2	Group 3	Group 4
Mean Weight (g) Day 1				
Male	6.4	6.2	6.4	6.4
Female	6.0	5.8	6.0	6.0
Combined	6.2	6.0	6.2	6.2
Mean Weight (g) Day 4				
Male	8.6	8.4	8.6	8.7
Female	8.2	8.0	8.2	8.3
Combined	8.4	8.2	8.4	8.5
Mean Weight (g) Day 7				
Male	12.1	12.1	12.1	12.2
Female	11.6	11.6	11.6	11.8
Combined	11.9	11.9	11.8	12.0
Mean Weight (g) Day 14				
Male	23.8	24.6	24.7	23.5
Female	22.7	24.2	23.4	23.2
Combined	23.2	24.5	24.3	23.4
Mean Weight (g) Day 21				
Male	38.5	41.3	41.8	39.7
Female	36.9	40.0	39.2	38.6
Combined	37.7	40.7	40.6	39.1

Group Mean Pup Weight (g) F_1 Generation

	Group 1	Group 2	Group 3	Group 4
Mean Weight (g) Day 1				
Male	6.5	6.4	6.3	6.2
Female	6.1	6.1	6.0	5.8
Combined	6.3	6.3	6.2	6.1
Mean Weight (g) Day 4				
Male	8.8	8.6	8.8	8.7
Female	8.4	8.2	8.4	8.1
Combined	8.6	8.4	8.6	8.5
Mean Weight (g) Day 7				
Male	13.0	12.6	12.9	12.7
Female	12.4	11.9	12.4	11.9
Combined	12.7	12.3	12.6	12.4
Mean Weight (g) Day 14				
Male	26.3	25.4	25.7	25.2
Female	25.3	24.4	25.0	23.7
Combined	25.8	24.9	25.5	24.7
Mean Weight (g) Day 21				
Male	42.2	41.3	41.7	40.3
Female	40.9	40.2	40.9	38.1
Combined	41.6	40.7	41.6	39.6

The group mean pup weight of the P generation was comparable between the control and test levels through day 21. A slight decrease (not statistically significant) of 4 to 7 percent in the F_1 generation group mean pup weight was reported at the high level during the 14- to 21-day period.

c. Developmental parameters, pinna unfolding, tooth eruption, and eye opening were measured for each litter of the P and F_1 generation.

Pup developmental parameters were comparable between test and control groups.

d. Functional tests performed on each pup from the F_1a and F_2a control and high-dose groups during postpartum day-1 - tail pinch, day-17 - air righting, and day-21 - grip strength, papillary reflex, visual placing responses, and auditory startle response.

The results of the functional tests performed on days 1, 17, and 21 were comparable between the controls and group 4 pups of the F_1a and F_2a generations.

e. Terminal Observations
For the F_1a and F_2a litters the tissues listed previously, were taken from one male and one female weaned pup from each of the selected litters per dose group (except for group 2 of the F_2a where tissues were taken from 8 litters) for organ weights.

i. Organ Weights

The relative gonadal weights of F_1a female pups were increased significantly ($p < 0.01$) for females of the mid and high level by 64 and 80%, respectively.

No gross necropsy findings were reported for the F_1a and F_2a pups relative to the administration of MITC. Tissues of the F_1a and F_2a litters were not subjected to histopathological examination.