

US EPA ARCHIVE DOCUMENT

METHYL ISOTHIOCYANATE

Subchronic Inhalation Toxicity Study- Rat (82-4)

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TXR # 0052720

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Inhalation Toxicity - Rat;
OPPTS 870.3465 (82-4)

DP BARCODE:D272434

SUBMISSION CODE: S592046

P.C. CODE:035602

[068103 correct]

TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Methyl isothiocyanate 96.9%

SYNONYMS: MITC

CITATION: Klimisch, H. J. (1987). Study of the Subchronic Inhalation Toxicity of Methyl Isothiocyanate in Wistar Rats (4 week study). Department of Toxicology, BASF Aktiengesellschaft, D-W6700 Ludwigshafen, Federal Republic of Germany, Project No 40I0231/8539, BASF Reg. document Number 87/0244, January 29, 1987. MRID 45314802. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, 26 Davis Drive, P.O. Box 13528, Research Triangle Park, NC 27709-3528.

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/m³ equivalent to 0, 5.0, 20, or 100 ug/L (measured concentrations 0, 5.1, 19.9 or 100 ug/L) 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 onward. In the high dose rats, the signs persisted during the non-exposure periods. 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 was an increase in serum bilirubin that was statistically significant (p<0.01) in the high dose males. The biological significance of the increase is unknown. There was increased lung weight, accompanied by bronchopneumonia, as well as other gross and microscopic changes in the respiratory tract of high dose male and female rats including, but not limited to, atrophy of the olfactory epithelium; tracheal cell necrosis, and focal squamous cell metaplasia in the respiratory epithelium.

The LOAEL is 100 mg/m³, based on persistent clinical signs, body weight changes, and

gross and histopathological lesions observed in the high dose rats. The NOAEL is 20 mg/m³.

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.

COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, and Flagging statements were provided. The GLP statement indicated that the study was not conducted in accordance with EPA CFR 40 Part 160, but was instead conducted in accordance with the "OECD Principles of Good Laboratory Practice". Flagging statements were not provided..

I. MATERIALS AND METHODS

A. MATERIALS:1. Test Material: Methyl isothiocyanate

Description: Solid brown crystals at room temperature

Lot/Batch #: 6205 MK (supplied by Aldrich)

Purity: 96.9 % a.i.

Stability of compound: Not provided in this document. The registrant referenced Report ARE/RF, January 24, 1986

CAS #: 556-61-6

Structure:



2. Vehicle and/or positive control: There was no positive control. The control animals were exposed to clean air only.

3. Test animals

Species: rat

Strain: SPF-Wistar/Chbb:THOM

Age and weight at study initiation: approximately 8 weeks old; males, 274-280 g; females, 170-173g

Source: Dr. K. Thomae, GmbH, Biberach, FRG

Housing: individually in Makrolon wire cages (type MD III of Becker, Castrop-Rauxel, FRG), except during exposure

Diet: KLIBA rat/mouse/maintenance laboratory diet 24-343-4, 10 mm pellets, Klingentalmühle AG, Kaiseraugst, Switzerland, *ad libitum*, except during exposure (6 hours/day, 5 days/week) Feed Batches: 33-85, and 34-85.

Water: potable water was supplied *ad libitum*

Environmental conditions:

Temperature: 20-24°C

Relative Humidity: 30-70%

Air changes: not specified, rooms were fully air-conditioned

Photoperiod: 12 hours of light and 12 hours of dark

Acclimation period: 7 days

B. STUDY DESIGN:

1. In life dates - start: 11/21/1985 - end: 12/17/1985

2. Animal assignment

Animals were randomly assigned (using the randomization program WTALOC of Instem) to the test groups in Table 1. Animals were identified by ear tattoo, and were exposed by whole body exposure to the test compound 6 hours/day for 20 exposures over a 28 day period.

TABLE 1: STUDY DESIGN

Test Group	No. of Animals M/F	Target Concentration mg/m ³	Analytical Concentration mg/m ³
Control (air only)	5/5	0	0
1 (LCT)	5/5	5.0	5.1 ± 0.53
2 (MCT)	5/5	20.0	19.9 ± 1.27
3 (HCT)	5/5	100	100 ± 5.33

LCT=Low concentration Treatment
MCT=Medium concentration Treatment
HCT=High concentration Treatment

2. Concentration Selection and Rationale

Concentrations were selected based on the results of a one week preliminary study, (Proj No. 30I0231/8113, which demonstrated minimal toxicity at 30 mg/m³ (body weight, clinical chemistry, hematology unaffected; however pathological lesions were observed in the lungs. These data were not submitted to the Agency for review. Based on the information obtained from this preliminary study, the high concentration was selected to be 100 mg/m³.

3. Generation of the test atmosphere and description of the chamber:

The test chemical was placed in a glass container in a heated water bath and the vapor was carried in a nitrogen stream to a mixing device, diluted to the target concentration from an air supply (8000 L/hr, relative humidity 50%, temperature 22°C) before being introduced into a 500 liter glass and steel inhalation chamber connected to an exhaust air system.

The water bath temperature was maintained at approximately 40° C, and the system was maintained with a slightly positive pressure (exhaust air flow 8110-8300 l/hr) to prevent contamination of the laboratory from possible leakages from the inhalation chamber. Time to equilibrium was not stated.

The temperature, air flow, the supply air, and the exhaust air were continuously

monitored for all test groups, using a digital thermometer and radiometer and recorded 3 times during each exposure. The pressure in the chambers was measured continuously by an inclined tube manometer and recorded daily. Humidity was measured twice during each exposure, using a humidity measuring probe.

Analytical Chemistry

The exposure concentrations were determined by gas chromatography after absorption of methyl isothiocyanate in 2-propanol. The gas chromatograph was fitted with a flame ionization detector and was calibrated daily with weighed amounts of methyl isothiocyanate. Six vapor samples per concentration group were taken from the breathing zones of the treated animals daily. The samples were collected in two absorption vessel connected in series with a downstream glass flask, each filled with 15-20 ml 2-propanol. 0.5 ml of internal standard was added to the samples and filled with 2-propanol in 50 ml graduated flasks. Two ml of the samples were filled in tubes for the automatic sampler and analyzed. The injection volume was 2.5 ul. The calibration graph was plotted to give a straight line from 0.20 mg to 0.60 mg in 50 ml.

Table 2. Exposure Chamber Conditions in MITC Study

Test Group (5 rats/sex/group)	MITC Concentration mg/m ³	Nitrogen flow L/hr	Water Bath temperature C°	Supply Air L/hr	Exhaust Air L/hr
Control	0	0	-	8000	7800
LCT	5	0.28	30.7	8000	8300
MCT	20	1.91	41	8000	8300
HCT	100	7.98	42.8	8000	8110

a = Data extracted from Study Report page 18; MRID 45314802.

3. Statistics

Statistical analysis of body weight and body weight changes were carried out using ANOVA and Dunnett's Test. Clinical chemistry, hematology data and organ weights were carried out by analysis of means and standard deviations followed by Williams "t" test for comparison of differences between means of several doses with a zero dose control. The levels of significance used were $p < 0.05$ and $p < 0.01$.

C. METHODS:

1. Observations:

Animals were inspected daily for signs of toxicity and mortality.

2. Body weight

Individual body weights were measured prior to the initiation of exposure (preflow period), at the beginning of exposure, and weekly thereafter. Body weight change was calculated relative to the beginning of exposure.

3. Food consumption

Food consumption for each animal was not determined in this study.

4. Ophthalmoscopic examination

No Ophthalmoscopic examinations were performed.

5. Blood was collected for hematology and clinical chemistry analysis from the treated rats, 27 days after study start. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*		Reticulocyte count
x	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

X	ELECTROLYTES	X	OTHER
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*	x	Total Cholesterol
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
	ENZYMES	x	Total bilirubin
x	Alkaline phosphatase (ALK)	x	Total serum protein (TP)*
	Cholinesterase (ChE)	x	Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urinalysis was not performed in this study.

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue		Aorta*		Brain*Periph. nerve*
	Salivary glands*	x	Heart*		Spinal cord (3 levels) ^T
	Esophagus*		Bone marrow*		Pituitary*
	Stomach*		Lymph nodes*		Eyes (optic n.) ^T
	Duodenum*	x	Spleen*		
	Jejunum*		Thymus*		
	Ileum*				
	Cecum*		UROGENITAL	xx	GLANDULAR
	Colon*	xx	Kidneys*+		Adrenal gland*
	Rectum*		Urinary bladder*		Lacrimal gland ^T
x	Liver**		Testes**		Mammary gland ^T
x	Gall bladder*		Epididymides		Parathyroids* ^{**}
	Pancreas*		Prostate		Thyroids* ^{**}
	RESPIRATORY		Seminal vesicle		OTHER
	Trachea*		Ovaries		Bone
x	Lung**		Uterus*		Skeletal muscle
x	Nose				Skin
x	Pharynx				All gross lesions and masses*
x	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines.

+ Organ weight required in subchronic and chronic studies.

^T = required only when toxicity or target organ.

** Organ weight required for non-rodent studies.

II. RESULTS

A. Observations

1. Toxicity

All control and low concentration treatment group (LCT) appeared normal throughout the study. In the mid concentration treatment group (MCT), during exposure, animals demonstrated eyelid closure, somnolence, and ruffled fur from the third exposure day. The animals appeared normal between exposures. The high concentration treatment group (HCT) demonstrated similar signs during exposure, but in addition, these did not disappear between exposures. As the study duration increased, the high dose group additionally demonstrated reddish nasal discharge, salivation, eye discharge, difficulty breathing, whooping respiration, stretched posture. Some males were observed to be breathing in the upright position during inhalation exposure.

2. Mortality

All study animals survived to termination.

B. Body weight and weight gain

Body weights were significantly depressed in the high dose males from day 7 to study termination (Table 3). Body weight gain was also statistically significantly depressed in this group. These changes were not seen in any other treatment group.

Table 3 Mean Body Weights

Mean Body Weight (g) of Male Rats Administered MITC for 28 Days					
	Day 0	Day 7	Day 14	Day 21	Day 27
Control	307.9	331.4	350.5	370.8	387.6
LCT	311.0	335.8 (101.1)	356.6 (101.7)	378.3 (102.2)	394.6 (101.8)
MCT	302.9	322.3 (97.3)	340.7 (97.2)	358.8 (96.8)	375.7 (96.9)
HCT	310.7	291.5* (88.0)	288.7** (82.4)	282.6** (76.2)	295.8** (76.3)

Taken from Table 2. MRID 45314802, page 64

Values in parenthesis =% of the controls. calculated by the reviewer.

* P<0.05 ** P<0.01

C. Food consumption

No results are available for this parameter..Food consumption was not measured in this study

D. Ophthalmoscopic examination

There were no ophthalmoscopic measurements.

E. Blood work1. Hematology

In HCT males, clotting time was increased (35.9 seconds controls vs 41.8 seconds HCT, which was statistically significant ($p < 0.05$). In HCT females, there was an increase in leucocytes (5.2×10^6 controls vs 6.7×10^6 HCT, which was statistically significant ($p < 0.05$). In both sexes, there was an increase in neutrophilic polymorphonuclear granulocytes at HCT (males: 5.8% controls vs 20% HCT; Females: 10% controls vs 20.8% HCT). In MCT males, there was an increase in neutrophilic polymorphonuclear granulocytes (5.8% controls vs 11.0% MCT). At MCT, a slight increase in neutrophilic polymorphonuclear granulocytes is not considered as biologically significant. No changes in hematological parameters were observed in the low dose animals.

2. Clinical chemistry

Table 4 Selected Clinical Chemistry Measurements.

Dose	Urea	Glucose	Triglyceride	Albumin	Bilirubin
Male Rats					
Controls	8.45±0.36	7.80±0.25	3.25±0.26	39.88±0.89	1.30±0.11
LCT	7.91±0.16	7.68±0.27	2.72±0.28	40.20±0.32	1.35±0.16
MCT	7.02±0.64	7.44±0.37	3.14±0.64	37.90±0.98	1.83±0.70
HCT	6.64±0.59*	6.49±0.19**	1.35±0.32**	36.52±0.96*	2.50±0.32**
Female Rats					
Controls	8.74±0.97	7.09±0.30	2.04±0.34	42.04±1.67	2.14±0.12
LCT	8.58±0.85	7.38±0.31	2.08±0.22	40.44±1.40	1.71±0.33
MCT	8.11±0.19	7.12±0.46	2.08±0.44	41.30±0.60	1.72±0.20
HCT	6.14±0.53*	6.08±0.22*	1.13±0.21	36.95±1.65	1.79±0.19

Taken from Tables B001-B026, MRID 45314802, page 117

Values in parenthesis =% of the controls, calculated by the reviewer.

* $P < 0.05$ ** $P < 0.01$

As shown in Table 4, in HCT male and female rats, the following clinical chemistry changes were seen: statistically significant decreases ($p < 0.05$) in: urea; glucose;

triglyceride and albumin in males and urea and glucose in females: the biological significance of these changes is unknown. Bilirubin concentration was statistically significantly increased ($p < 0.01$) increased in males only.

In MCT males, urea only was statistically significantly decreased ($p < 0.01$): No other changes in clinical chemistry were reported at this dose level, and no clinical chemistry changes were observed in the low dose animals.

F. Urinalysis

No results are available. This parameter was not measured.

G. Sacrifice and pathology

1. Organ weight

As shown in Table 5 the lung weight of both male ($p < 0.05$) and female ($p < 0.01$) high dose rats was significantly increased. Significantly decreased liver and kidney weights were reported for high dose males. The biological significance of the decreases in these organ weights is unknown.

Table 5. Organ Weights (g) of Rats Exposed to MITC (mg/m^3) for 28 days.

Males				
Organ	Control	LCT(5)	MCT(20)	HCT(100)
Liver	12.028	11.734	10.694	8.882**
Kidneys	2.328	2.274	2.172	1.944**
Lungs	0.962	1.028	0.930	1.650**
Females				
Organ	Control	LCT	MCT	HCT
Lungs	0.624	0.706	0.686	1.062*

Data taken from Tables 14 and 15 of the Study Report

* $p < 0.05$, ** $p < 0.01$

2. Gross pathology

Grossly, the lungs of all male and 3/5 high concentration females appeared pale, puffy and rigid. There were no other gross lesions reported.

3. Microscopic pathology

Microscopically, the lungs of all high concentration males and 2/5 females demonstrated bronchopneumonia accompanied by widened alveolar septa and activated alveolar cover cells. There was epithelial cell proliferation of the bronchi, bronchioli as well the trachea. Single cell necrosis of the trachea also occurred in all male and female high dose rats. Rhinitis was reported in the nasal cavity of all high dose animals. Also, the olfactory epithelium was atrophic. There was also focal squamous metaplasia in the respiratory epithelium in 3/5 males and all females in this group.

III. DISCUSSION

- A. In a 28 day inhalation (20 exposures) toxicity study 5 rats/sex/dose Wistar rats were exposed 6 hours/day to MITC analytical concentrations of 5.1, 19.9, or 100 mg/m³ or to clean air.

All animals survived to study termination. From the third exposure period onward, all mid and high concentration treatment group animals demonstrated dose related eyelid closure, somnolence, ruffled fur, reddish nasal discharge, salivation, eye discharge, excessive grooming, and respiratory difficulty during the exposure periods. In the mid but not the high dose animals, these signs were reversible during the non exposure periods. There were no clinical signs reported in the low concentration treatment group.

In HCT males, but not females body weight was significantly depressed ($p < 0.01$) at all reporting periods. Urinalysis and ophthalmoscopic data were not provided. Clinical chemistry analysis revealed significantly reduced ($p < 0.05$) urea, glucose, triglyceride and albumin values in the HCT group. The biological significance of these changes is unknown, and these changes are not considered to be adverse effects. Additionally, bilirubin values ($p < 0.01$) and thromboplastin time ($p < 0.05$) were increased at the HCT in male rats only. Random enzyme changes were seen in the high dose female rats, which were unlikely to be related to exposure to MITC.

The respiratory tract was clearly the target. This was demonstrated primarily by the increase in lung weights, severe irritation (bronchopneumonia, epithelial proliferation in the bronchi, bronchioles, and trachea); purulent rhinitis in the nasal cavity; focal metaplasia, and secondarily by the changes in leucocytes in females and the increase in neutrophilic polymorphonuclear granulocytes in both sexes of the HCT group rats. The clinical signs related to the respiratory tract at the MCT were mild and represented transitory irritation, which disappeared upon withdrawal of the animals from the test substance. In addition, there were no systemic toxicity in the MCT group rats.

The data presented demonstrate a NOAEL of 20 mg/m³ and a LOAEL of 100 mg/m³ based on the persistent clinical signs observed in both sexes, the body weight changes, and the gross and microscopic changes observed at the highest dose tested. This NOAEL may not be protective of potential irritation effects in humans because

irritation effects were observed in the mid concentration treatment group during the exposure period.

This 28 day study is classified **Acceptable-Non-Guideline**. It does not fulfill the requirements of Guideline 82-4 (subchronic inhalation study). It was not conducted for 90 days, used insufficient numbers of animals, and the details of the inhalation chamber and procedure were inadequately reported.

B. Study deficiencies

No flagging statements were provided. The time to equilibrium was not reported for the exposure chamber. This study was not conducted for 90 days, used insufficient numbers of animals, and the details of the inhalation chamber and procedure were inadequately reported.