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DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; OPPTS 870.3700a [§83-3a]; OECD 414.

PC CODE:068103 **DP BARCODE:** D289780
SUBMISSION NO.: S634271

TEST MATERIAL (PURITY): MITC (96.9% a.i.)
SYNONYMS: Methylisothiocyanate

CITATION: Hellwig, J. Hildebrand, B.(1987) Report on the study of the prenatal toxicity of MITC in rats after oral administration. BASF Aktiengesellschaft, Department of Toxicology, D-6700 Ludwigshafen/Rhien, Federal Republic of Germany. Report NO. 87/0326, September 2, 1987. MRID 45919417. Unpublished.

SPONSOR: Taminco, Pantserschipstraat 207, B-9000 Gent (Belgium).

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 45919417) MITC (96.9% a.i., batch# 6205 MK) was administered to 25 female Wistar rats/dose by gavage at dose levels of 0, 3, 10 or 30 mg/kg bw/day from days 6 through 15 of gestation (GD). On GD 20, all dams were sacrificed and necropsied, and all fetuses were weighed, sexed, and examined externally. Homogenecity, stability and concentration analysis of the test article was provided in German.

Maternal toxicity as evidenced by reduced body weights and body weight gain was observed at the high-dose level (30 mg/kg bw/day). In addition, statistically significant decrease in the high-dose group maternal mean body weights was noted on days 6-8 (76%), 8-10 (64%), 10-13 (21%), and 15-17 (23%). A statistically significant decrease in body weight was noted in the mid- dose group (10 mg/kg bw/day) only on days 8-10 (39%). A reduction in food consumption was noted in the dams at the high- dose level (30 mg/kg bw/day) during the dosing period. Food consumption was reduced 21% for days 10-13, 15% for days 13-15, and 18% for days 15-17 of dosing. There were no treatment- related effects on mortality. **The maternal LOAEL is 30 mg/kg bw/day, based on reduced body weight gain and food consumption. The maternal NOAEL is 10 mg/kg bw/day.**

The numbers of corpora lutea, implantation, live fetuses, resorptions, and fetal weights were similar among treated and untreated animals. There were no treatment- related effects found upon visceral and skeletal examinations of the fetuses. However, there was a decrease in the placental weight and an increase in the number of runts at the high dose level. **The developmental LOAEL is 30 mg/kg bw/day, based on the higher number of runts and reduced placental weights. The developmental NOAEL is 10 mg/kg bw/day.**

The developmental toxicity study in the rat is classified **unacceptable /guideline (upgradeable)** and **does not satisfies** the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rat. The homogeneity, stability and concentration analyses were provided in German instead of English. Therefore, the concentration of the administrated article could not be verified; these data need to be provided to the Agency.

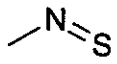
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: MITC; methylisothiocyanate

Description:	Solid, crystalline, brown-yellowish
Lot/Batch #:	6205 MK
Purity:	≥ 96.9 % a.i.
Compound Stability:	stable in storage at +4°C
CAS #of TGAI:	556-61-6
Structure:	



2. Vehicle and/or positive control: The vehicle control article was olive oil.

3. Test animals:

Species:	Rat	
Strain:	Wistar	
Age/weight at study initiation:	11 to 12 weeks old; mean weight 233g at the start of dosing	
Source:	Karl THOMAE Biberach an der FRG	
Housing:	individually in stainless steel wire mesh cages	
Diet:	ground kliba 343 feed rat/mouse/hamster "A", supplied by Klingenthalmuhle, Switzerland *ad libitum*	
Water:	local tap water *ad libitum*	
Environmental conditions:	**Temperature:**	20-24 °C
	Humidity:	30-70 %
	Air changes:	not reported
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	5 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: October 17, 1985 End: November 13, 1985.

2. Mating: Females were paired on a 4:1 basis with stock males of the same strain. Day 0 was designated as the day sperm was detected microscopically in the vaginal smear.

3. Animal Assignment: Animals were assigned randomly to dose groups as indicated in Table 1.

TABLE 1. Animal Assignment

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	25
Low (LDT)	3	25
Mid (MDT)	10	25
High (HDT)	30	25

4. Dose selection rationale: The dose levels were selected based on the approximative LD_{50} value after single oral administration, which was about 150 mg/kg body weight. One fifth of this value (30 mg/kg/ bw) was taken as the highest dose level at which signs of maternal toxicity were expected. Ten and 3 mg/kg/bw were selected as further doses.

5. Dosage preparation and analysis

Test material-vehicle\ mixture was prepared daily by mixing appropriate amounts of test substance that was weighed and stored in a refrigerator. Each day a portion of cooled test substance was warmed in a water bath of about 37°C and subsequently dissolved in olive oil. Prior to the start of the study, stability of the test substance in olive oil was evaluated. The mean content of active ingredient was 98.1% before the study began and 96.9% after termination of the study.

Results - Homogenecity and concentration analysis of the test article was provided in German.

6. Dosage administration: All doses were administered once daily by gavage, on gestation days 6 through 15 in a volume of 5 mL/kg of body weight/day. Dosing was based on the body weight on the most recent body weight on gestation day #6.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked daily for mortality and clinical signs. Body weight and food consumption data were recorded on days 0, 1, 3, 5, 8, 10, 13, 15, 17 and 20 of gestation days. Dams were sacrificed on day 20 of gestation. Animals were examined at sacrifice in the following manner. A gross external examination of each animal was performed. Uterine contents were examined for numbers of corpora lutea, implantation sites, resorption sites (classified as early SALEWSKI, early, intermediate, or late), and distribution of live and dead fetuses in each uterine horn. Resorption sites classification are described as follow: early resorption according to SALEWSKI from uteri from apparently non-pregnant animals and the empty uterus horn in the case of single- horn pregnancy; early resorptions (dead embryos visible to the naked eye in the form of yellowish-brown spots); intermediate resorptions (dead and resorbed embryos in which individual parts of the body could be differentiated

macroscopically); late resorptions (dead and resorbed embryos in which individual parts of the body could be differentiated macroscopically; dead fetuses (hypoxemic fetuses which did not breathe spontaneously after the uterus had been opened.

2. Fetal Evaluations - The fetuses were examined in the following manner: Each fetus was weighted, sexed, and examined for external abnormalities. Individual placental weights and placental abnormalities were recorded. Approximately two third of the fetuses were dissected and examined. The eviscerated fetuses were processed and stained according to a modified method of Kimmel, and the skeletons were examined. The remaining fetuses were placed in Bouin's fixative and examined according to the method of Barrow and Taylor.

D. DATA ANALYSIS

1. Statistical analyses: The following data were analyzed using the Fisher Test: conception rate, mortality, percentage of litters with anomalous fetuses and of fetuses with variations and/ or retardation. The Williams Test was used for statistical evaluation of body weight, uterus weight, and weight of placentae. In addition, the Krauth Test was used for statistical evaluation of corpora lutea, implantations, percentage of live and dead implantations per pregnant animal, and percentage of live fetuses with anomalies and with variations and /or retardation per litter.

2. Indices: The following indices were calculated from cesarean section records of animals in the study:

Preimplantation loss (%) = (total number of corpora lutea minus total number of implantations/ total number of corpora lutea) x 100

Postimplantation loss (%) = (total number of dead implantations/total number of implantations) x 100

3. Historical control data: Historical control data were not provided to allow comparison with concurrent controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: There were no maternal deaths or abortions during the study.

2. Body Weight - Body weight data are summarized in Table 2. Mean absolute body weights throughout gestation and the estimated corrected mean terminal body weight and corrected body weight gains of the treated and vehicle control groups were similar except at the high dose. Decreased body weight gains in the high dose dams during the treatment interval followed by decrease body weight gain by the group post treatment were considered treatment-related.

TABLE 2. Mean (±SD) Maternal Body Weight Gain (g) [a]

Interval	Dose in mg/kg bw/day (# of Dams)			
	Control (25)	3 (22)	10 (18)	30 (23)
Pretreatment: Days 3-6	11.32 ± 5.06	11.18 ± 4.68	9.44 ± 1.03	11.04 ± 5.15
Treatment: Days 6-8	1.88 ± 5.09	1.95± 3.98	2.50 ± 4.08	-1.43 ± 4.8 (76)[b]
Days 8-10	10.72 ± 4.05	9.68 ± 3.47	6.56 ± 5.06 (39)[b]	3.87 ± 3.6 (64)[b]
Days 10-13	13.64 ± 3.64	13.91± 4.28	12.83 ± 3.17	10.83 ± 3.48 (21)[b]
Days 13-15	12.00 ± 3.54	9.36 ± 4.94	11.44 ± 4.77	9.87 ± 4.12
Posttreatment: Days 15-17	23.44 ± 4.18	20.14 ± 6.37	21.94 ± 3.67	17.96 ± 5.22 (23)[b]
Days 17-20	43.80 ± 7.30	40.05 ± 13.19	43.44 ± 8.38	39.61 ± 9.12

a Data obtained from pages 43-46 in the study report.
b Indicate the (%) decrease in body weight
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

3. <u>Food Consumption</u> - There was treatment- related effect on maternal food consumption. Decreased in mean food consumption in the high- dose group correlated with the previously mentioned decreased body weight gains by this group.

TABLE 3. Mean (±SD) Food Consumption (g) [a]

Interval	Food in g/day (# of Dams)			
	Control (25)	3 (22)	10 (18)	30 (23)
Pretreatment: Days 3-6	23.7 ± 2.5	23.1 ± 1.5	24.7± 2.2	24.1± 2.5
Treatment: Days 6-8	20.8 ± 1.8	19.3 ± 1.7	19.5 ± 1.8	16.0 ± 2.8
Days 8-10	22 ± 2.2	20.7 ± 2.0	20.7 ± 2.8	15.3 ± 2.7
Days 13-15	23.4 ± 1.7	21.5 ± 1.7	23.4 ± 4.3	19.8 ± 2.7
Posttreatment: Days 15-17	27.7 ± 2.0	24.9 ± 2.7	26 ± 2.5	22.6 ± 2.2

Days 17-20	28.8 ± 2.5	27.6 ± 3.5	28.2 ± 1.8	27.1 ± 2.5

a Data obtained from pages 43-46 in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

4. Gross Pathology - There were no treatment related gross pathology findings for the maternal animals.

5. Cesarean Section Data - Data collected at the scheduled cesarean section are summarized in Table 3. There were no abortions, early deliveries, or total litter resorptions. There were no significant differences between the treated and control groups in mean numbers of corpora lutea, implantation sites, and live fetuses per litter, pre- and postimplantation losses, early or late deaths, fetal death and fetal weights. However, there was a decrease in the placental weight and an increase in the number of runts at the high dose level.

TABLE 4 Cesarean Section Observations [a]

Observation	Dose (mg/kg bw/day)			
	0	3	10	30
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant	25	22	18	23
Pregnancy Rate (%)	100%	88%	72%	92%
# Nonpregnant	0	3	7	2
Maternal Wastage				
# Died	0	0	0	0
# Died Pregnant	0	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	374	332	267	338
Corpora Lutea/Dam	14.96	15.09	14.83	14.78
Total # Implantations	331	287	243	305
(Implantations/Dam)	13.24	13.05	13.5	13.26
Total # Live Fetuses	300	246	223	263
(Live Fetuses/Dam)	90.42	82.77	92.44	86.60
Total # Dead Fetuses	31	41	20	43
(Dead Fetuses/Dam)	9.58	17.23	7.56	13.40
Total # Dead Implantation [b]				
Early (Salewski)	0	1	0	0
Early	30	38	19	42
Intermediate	1	1	1	1
Late	0	1	0	0
Total # Resorptions/Dam	1.24	1.86	1.11	1.87

Litters with Total Resorptions	0	0	0	0
Mean Fetal Weight (g)				
Males	3.76± 0.20	3.89± 0.23	3.85± 0.19	3.75± 0.29
Females	3.63± 0.24	3.67± 0.19	3.66± 0.20	3.53± 0.23
Sex Ratio (% Male)	50	54	46	53
Preimplantation Loss (%) [c]	11.49	11.9	10.3	10.9
Postimplantation Loss (%) [d]	9.58	17.23	7.56	13.40

a Data obtained from pages 52-53 in the study report.

b Early resorptions according to SALEWSKI from uteri from apparently non- pregnant animals and the empty uterus horn in the case of single- horn pregnancy; Early resorptions (dead embryos visible to the naked eye in the form of yellowish-brown spots; Intermediate resorptions (dead and resorbed embryos in which no parts of the body could be differentiated macroscopically); Late resorptions (dead and resorbed embryos in which individual parts of the body could be differentiated macroscopically.

c Calculated by reviewer as Preimplantation Loss = [(Total corpora lutea- Total implantations)/Total corpora lutea] x 100.

d Calculated by reviewer as Postimplantation Loss = (Total dead implantation/ Total implantations) x 100.

B. DEVELOPMENTAL TOXICITY: The total numbers of live fetuses (litters) in the 0, 3, 10, and 30 mg/kg bw/day groups were 300 (25), 246 (22), 223 (18), and 263 (23), respectively. Selected fetal morphological observations are given in Tables 5a-5d.

1. External Examination - There were no external abnormalities or retardations observed at caesarian section. One fetus (1) in both the 3 and 10 mg/kg bw/ day dose groups exhibited variations of the hindlimb (abnormal position).

2. Visceral Examination - Visceral variations, included enlarged renal pelvises bilateral and unilateral, in treated fetuses were presented in all treated and control groups.

3. Skeletal Examination - No skeleton malformations, variations or retardation were found in the test groups at incidences outside of the controls.

TABLE 5a. External Examinations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	3	10	30
#Fetuses(litters) examined	300 (25)	246 (21)	223 (18)	263 (23)
Variations				
Abnormal position of hindlimb	0 (0) [c]	1 (1)	0 (0)	1 (1)

a Data obtained from pages 55-59 in the study report.
b Some observations may be grouped together.
c Fetal (litter) incidence

TABLE 5b. Visceral Examinations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	3	10	30
#Fetuses(litters) examined	199 (25)	164 (21)	149 (18)	177 (23)
Variations				
Enlarged renal pelvis bilateral	16	9	4	6
Enlarged renal pelvis unilateral	35	14	11	30
Anomalities				
Anophthalmia unilateral	0	0	1	0

a Data obtained from pages 60-70 in the study report.
b Some observations may be grouped together.

TABLE 5c. Skeletal Examinations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	3	10	30
#Fetuses(litters) examined	199 (25)	163 (21)	149 (18)	177 (23)
Thoracic vertebra body bipartited, ossification centers connected w/ cartilage, notch of the cartilage, cranial	3	2	0	0
Thoracic vertebra body dumbell-shaped, notch of the cartilage, caudal	0	1	0	0
Thoracic vertebra body dumbell-shaped, notch of the cartilage, cranial	2	3	3	0

Thoracic vertebra body dumbell-shaped, notch of the cartilage, cranial/ caudal	2	3	2	0
Thoracic vertebra body bipartited, ossification centers connected w/ cartilage, notches of the cartilage, cranial/ caudal	12	7	4	0
Thoracic vertebrae bodies dumbbell-shaped, notches of the cartilage, cranial/caudal	1	1	1	0
Thoracic vertebrae bodies bipartited, ossification centers not connected with cartilage	0	1	0	0
Fetus with multiple anomalies	1	0	0	1
Mandibulae fused	1	0	0	1
Sternebra bipartited, ossification centers not connected with cartilage	0	0	0	1
Sternebrae ossificartion centers dislocated, ventral segments of ribs asymmetrically fused with the sternum	4	0	2	0
Sternebra ossification centers dislocated, ventral segments of the ribs asymmetrically fused with the sternum	2	0	0	0
Rib-13 shortened, cartilages absent bilateral	12	10	1	18
Rib-13 shortened, cartilages present bilateral	0	0	0	1
Rib-13 shortened, cartilages present unilateral	19	7	8	8
Rib-13 shortened, cartilages absent unilateral	1	0	1	1
Sternebrae of irregular shape	1	8	2	0
Sternebrae ossification centers dislocated	17	13	10	7

a Data obtained from pages 71-78 in the study report.
b Some observations may be grouped together.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

Table 5d. Mean (±SD) Litter Data [a]

Observations	Dose (mg/kg)			
	0	3	10	30
Placental Weight				
Males	0.45 ± 0.04	0.45 ± 0.04	0.46 ± 0.07	0.42 ± 0.04 (7)[b]
Females	0.45 ± 0.06	0.43 ± 0.03	0.44 ± 0.04	0.41 ± 0.04 (9)[b]
Runts				
Total (#)	1	2	1	5 (80)[c]

a Data obtained from pages 53-54 in the study report.
b % decrease in observations.
c % increase in observations.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study report concluded that oral administration of MITC to pregnant female Wistar rats during organogenesis elicited clear signs of maternal toxicity (e.g., reduced food consumption and body weight gain) at a dose level of 30 mg/kg/day, and to a much lesser degree, at a dose of 10 mg/kg bw/day. There were only discrete signs of embryo-/ fetotoxicity (higher number of runts and reduced placental weights) in the highest dose group (30 mg/kg bw/ day). Oral administration of MITC at 3 mg/kg bw/day produced no maternal or developmental adverse effects.

B. REVIEWER COMMENTS:

1. Maternal toxicity: Following oral administration of the test substance, MITC (96.9% a.i.), to pregnant rats on days 6-15 of gestation, maternal toxicity was evidenced by reduced body weight gains was observed at the high- dose level (30 mg/kg bw/day). Maternal body weight gains were reduced 5-7.5% during the dosing period in the high-dose group relative to the controls. In addition, statistically significant decrease in the high-dose maternal mean body weights was noted on days 6-8 (76%), 8-10 (64%), 10-13 (21%), and 15-17 (23%). A statistically significant decrease in body weight was also noted in the mid- dose group (10 mg/kg bw/day) only on days 8-10 (39%). A reduction in food consumption was noted in the dams at the high- dose level (30 m/kg bw/day) during the dosing period. Food consumption was reduced 21% for days 10-13, 15% for days 13-15, and 18% for days 15-17 of dosing. There were no treatment- related effects on mortality.

Therefore, the maternal toxicity LOAEL is 30 mg/kg bw/day, based on reduced body weight gain and food consumption. The maternal NOAEL is 10 mg/kg bw/day.

2. Developmental toxicity:

a. Deaths/Resorptions: The numbers of resorption/dam for the treatment groups were

not significantly different from the concurrent controls.

b. Altered Growth: There was no significant reduction or increase in fetal body weight in any of the treatment groups. There was a decrease in the placental weight and an increase in the number of runts (5 (23)) at the high dose level (30 mg/ kg bw/day).

c. Developmental Variations: Treatment with the test article did not result in an increased incidence of fetal structural alterations.

d. Malformations: Treatment with the test article did not result in an increased incidence of fetal malformations.

Therefore, the developmental toxicity LOAEL is 30 mg/kg bw day, based on the increased number of runts and reduced placental weight. The developmental NOAEL is 10 mg/kg bw/ day.

C. STUDY DEFICIENCIES: The toxicity findings in the fetuses were reported grouped together which make it difficult to tell which animal (litters) showed specific clinical signs. The homogeneity, stability and concentration analyses were provided in German instead of English. Therefore, the concentration of the administrated article could not be verified; these data need to be provided to the Agency.

DATA FOR ENTRY INTO ISIS

Developmental Study - rats (870.3700a)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
068130	45919417	developmental	rats	GD 6-15	oral	gavage	0-30	0, 3, 10, 30	3	30	body weight decr	Maternal
068130	45919417	developmental	rats	GD 6-15	oral	gavage	0-30	0, 3, 10, 30	10	30	incr. # of runts	Developmental