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Reregistration Branch 2, Health Effects Division (7509C) **Date** _____
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Template version 11/01

TXR#: 0051394

DATA EVALUATION RECORD

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

PC CODE: 068103

DP BARCODE: D289780
SUBMISSION NO.: S634270

TEST MATERIAL (PURITY): MITC (98%)

SYNONYMS: Methyl isothiocyanate

CITATION: Becker, H. and Sachsse, K. (1986) Report on the study of the embryotoxicity (including teratogenicity) in the rabbit with MITC. RCC, Research & Consulting Company AG, CH-4452 Itingen, Switzerland. RCC Project No. 056687, September 5, 1986. MRID 45919418). Unpublished.

SPONSOR: Taminico

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 45919418) MITC (98% a.i., ZNT-No. 85/231-2)] was administered to 16 female Chinchilla rabbits/dose in corn oil by gavage at dose levels of 0, 1, 3 or 10 mg/kg bw/day from days 6 through 18 of gestation. Insemination was by natural means.

One dam in the high dose group died on GD 11 following weight loss and loss of hindlimb function. No other clinical signs attributed to administration of MITC occurred in this study. No abortions occurred in any group. Variation in body weight and food consumption are large. Because of the large variations, no statistically significant decreases in maternal weight gain and food consumption were noted. However, mean body weight gain and food consumption were decreased compared to the control at the highest dose level.

There were no differences in mean corpora lutea, mean implanatation sites, mean resorptions, and mean viable fetuses were detected among all dose groups.

In the data provided in the report, the mean measured concentration ranged from 67% to 88% of the nominal concentration. This large range provides uncertainty surrounding the amount of MITC the rabbits recieved.

The tentative maternal LOAEL is 10 mg/kg bw/day, based on reduced body weight gain and food consumption. The tentative maternal NOAEL is 3 mg/kg bw/day.

There were no differences in fetal weights or sex ratio among the treatment groups. There were also no treatment related changes in external, skeletal or visceral examinations following *in utero* exposure to MITC.

The developmental LOAEL was not established. The developmental NOAEL is 10 mg/kg bw/day.

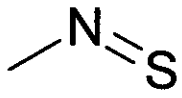
The developmental toxicity study in the rabbit is classified **unacceptable-guideline** and does **not** satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbit. The homogeneity, stability, and concentration information provided in the study report are inadequate. This study could be upgraded if additional data discussed in the DER are provided.

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 #:	Ch. 6205 MK; ZNT-No. 85/231-2
Purity:	98 % a.i.
Compound Stability:	Shown to be at least 90 min in corn oil
CAS #of TGAI:	556-61-6
Structure	



2. Vehicle control: Corn oil

3. Test animals:

Species:	Rabbit	
Strain:	Chinchilla (Kfm: CHIN, hybrid, SPF Quality)	
Age/weight at study initiation:	4-6 months/2721-4080 gm	
Source:	KFM, Kleintierfarm Madderin AG, Switzerland	
Housing:	individual stainless steel cages	
Diet:	pelleted standard Kliba 341 *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	22 +/-3 °C
	Humidity:	40-70%
	Air changes:	10-15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	7 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: January 27, 1986 End:March 12, 1986

2. Mating: After acclimination, the female rabbits were house 1:1 with males until mating was observed. The day of mating was designated as gestation day [0].

3. Animal Assignment: Animals were assigned randomly based on a computer program to dose groups as indicated in Table 1.

TABLE 1: Animal Assignment

Dose (mg/kg bw/day)	0 (vehicle control)	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
# Females	16	16	16	16

4. **Dose selection rationale:** The dose levels were selected based on the results from a preliminary study (RCC Project 056676, 1986). The preliminary study was not provided.

5. **Dosage preparation and analysis**

Test material-vehicle mixture was prepared daily by mixing appropriate amounts of test substance with corn oil using a homogenizer. Prior to the start of the study, stability of the test substance in corn oil was evaluated immediately after preparation and 90 minutes after preparation The temperature was not provided; room temperature is assumed. MITC is a highly volatile compound; the room temperature could impact the amount of chemical which remains in the corn oil.

The study report indicates that the homogeneity of the solution was tested. However, the study report does not provide data from various places in the mixture (e.g. top, middle, bottom) to support this statement.

Concentration of the test mixture were evaluated twice. The mean measured concentration ranged from 67% to 88% of the nominal concentration. This range of measured concentrations is considered large and provides uncertainty to the study.

6. **Dosage administration:** All doses were administered once daily by gavage, on gestation days 6 through 18, in a volume of 2 mL/kg of body weight/day. Dosing was based on the body weight on the daily body weight determination.

C. OBSERVATIONS

1. **Maternal Observations and Evaluations** - The animals were checked for mortality or clinical signs twice a day. Body weight was recorded daily and food consumption was recorded on gestation days 6, 11, 15, 19, 24, and 28. Dams were sacrificed on day 28 of gestation. Examinations at sacrifice consisted of gross macroscopic examination of all internal organs, with emphasis on the uterus, position of fetuses in the uterus, # of corpora lutea. Uteri of females found not to be pregnant were placed in ammonium sulfate.

2. **Fetal Evaluations** - The fetuses were examined for external abnormalities, dissected for examination of body cavities and organs, and ossification abnormalities. The heads were fixed in trichloroacetic acid and formaldehyde followed by cross sectioning and examination of the cephalic viscera. Sections were perserved in ethanol and glycerine. The trunks were put in KOH and stained with alizarin red followed by skeletal examination.

D. DATA ANALYSIS

1. Statistical analyses: Only females with live fetuses were included in the calculations of body weight gain and mean food consumption. All data collected were subjected to routine appropriate statistical procedures. ANOVA was used to assess intergroup differences if the variable could be assumed to be normal. Dunnett t-test was used for intergroup comparisons. Wilcoxon ranks with the Kruskall-Wallis test were applied to the reproduction data. Fisher's exact text for 2x2 tables for dichotomous data.

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

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 provided to allow comparison with concurrent controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: One control animal did not have any live fetuses. One high dose female was found dead on GD 11. This high dose animal exhibited body weight loss begining the first day of treatment until day 10. On day 10, this female was unable to stand on its hind limbs.

2. Body Weight - Body weight data are summarized in Table 2. The control, mid and high doses groups exhibited weight loss during the first 6 days of treatment. For the entire treatment period, the highest dose group exhibited a (not s.s.) decreased body weight gain. The standard deviations were not provided in the study report. However the study report admits the body weight means had 'wide range of deviations.'

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

Interval	Dose in mg/kg bw/day (# of Dams)			
	0 (vehicle, n=15)	1 mg/kg/day (n=16)	3 mg/kg/day (n=16)	10 mg/kg/day (n=15)
Pretreatment: Days 0-6	137 ±55[b]	147 ±95	151 ±65	180 ±52
Treatment: Days 6-11	-55 ±105	12 ±68	-40 ±113	-120 ±123
Treatment: Days 11-15	61 ±60	23 ±56	60 ±41	70 ±61
Treatment: Days 15-19	30 ±43	64 ±56	53 ±52	54 ±59
Treatment: Days 6-19	36 ±118	99 ±102	73 ±107	4 ±140
Posttreatment: Days 19-28	135 ± 85	175 ± 77	147 ±81	212 ±63

a Data obtained from page 54 in the study report.
b. Standard deviations calculated by the reviewer.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

3. **Food Consumption** - Food consumption data are summarized in Table 3. During GD 6-11 and 11-15, the highest dose consumed less (not s.s.) food than the control or the other treatment groups. The standard deviations were not provided in the study report. However the study report admits the mean food consumption data had 'wide range of deviations.'

TABLE 3 Mean Food Consumption (g) [a]

Interval	Dose in mg/kg bw/day (# of Dams)			
	0 (vehicle, n=15)	1 mg/kg/day (n=16)	3 mg/kg/day (n=16)	10 mg/kg/day (n=15)
Pretreatment: Days 0-6	192 ±39[b]	188 ±28	201 ±22	198 ± 27
Treatment: Days 6-11	101 ± 49	140 ±29	96 ±41	71 ±52
Treatment: Days 11-15	142 ±39	141 ± 35	137 ±32	120 ±42
Treatment: Days 15-19	135 ±56	144 ±40	143 ±41	126 ± 34
Treatment: Days 19-24	148 ±31	164 ±25	176 ±38	188 ±19
Posttreatment: Days 24-28	136 ± 43	143 ±38	132 ± 40	151 ± 29

a Data obtained from page 55 in the study report.
b. Standard deviations calculated by the reviewer.
* 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 - There were no treatment related changes in corpora lutea, implantations, pre- or post-implantation loss, fetal weight or number of live fetuses.

TABLE 3 Cesarean Section Observations [a]

Observation	Dose (mg/kg bw/day)			
	0 (vehicle)	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
# Animals Assigned (Mated)	16	16	16	16
# Animals Pregnant	16	16	16	16
Pregnancy Rate (%)	100%	100%	100%	100%
# Nonpregnant	0	0	0	0
Maternal Wastage	0	0	0	
# Died	0	0	0	1
# Died Pregnant	0	0	0	1
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	140	159	153	138
Corpora Lutea/Dam	9.3±1.6	9.9±2.2	9.6±1.5	9.2±1.5
Total # Implantations	134	155	136	132
(Implantations/Dam)	8.9±1.6	9.7±2.2	8.8±1.5	8.8±2.2
Total # Litters	15	16	16	16
Total # Live Fetuses	120	134	116	118
(Live Fetuses/Dam)	8.0±2.3	8.4±2.9	7.3±2.1	7.9±2.4
Total # Dead Fetuses	0	0	0	0
(Dead Fetuses/Dam)				
Total # Resorptions	14	21	20	14
Early	9	16	16	10
Late	5	5	4	4
Resorptions/Dam	0.9	1.3	1.3	0.9
Early	0.6	1.0	1.0	0.7
Late	0.3	0.3	0.3	0.3
Litters with Total Resorptions	1	0	0	0
Mean Fetal Weight (g)	31.5±5.2	33.0±5.1	33.9±5.1	31.9±4.2
Sex Ratio (% Male)	48.3	47.8	50.0	45.8
Preimplantation Loss (%)	4.3	2.5	11.1	4.3

a Data obtained from pages 30-31 in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

B. DEVELOPMENTAL TOXICITY

There were no treatment related changes in external, skeletal or visceral examinations following *in utero* exposure to MITC.

1. External Examination -There were no treatment related changes in external examinations following *in utero* exposure to MITC.

TABLE 4a. External Examinations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0 (vehicle)	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
#Fetuses(litters) examined	120 (15)	134 (16)	116 (16)	118 (16)
#Fetuses(litters) affected	0 (0)	1 (1)	2 (1)	0 (0)
Omphalocele and microphalmia	0 (0) [c]	1 (1)	0 (0)	0 (0)
Hydrocephalus, microphthalmia	0 (0)	0 (0)	1 (1)	0 (0)
Hydrocephalus, coelioschisis from the sternum to the genitals organs	0 (0)	0 (0)	1 (1)	0 (0)

a Data obtained from pages pg. 36 in the study report.
b Some observations may be grouped together.
c Fetal (litter) incidence
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

2. Visceral Examination - There were no treatment related changes in visceral examinations following *in utero* exposure to MITC.

TABLE 4b. Visceral Examinations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0 (vehicle)	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
#Fetuses(litters) examined	120 (15)	134 (16)	116 (16)	118 (16)
#Fetuses(litters) affected	0 (0)	1 (1)	1 (1)	1 (1)
Hemidiaphragm	0 (0) [c]	1 (1)	0 (0)	1 (1)
Kidneys grown together and adhered to intestine	0 (0)	0 (0)	1 (1)	0 (0)

a Data obtained from pages 37 in the study report.
b Some observations may be grouped together.
c Fetal (litter) incidence
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

3. Skeletal Examination - There were no treatment related changes in skeletal examinations following *in utero* exposure to MITC.

TABLE 4c. Skeletal Examinations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0 (vehicle)	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
#Fetuses(litters) examined	120 (15)	134 (16)	116 (16)	118 (16)
#Fetuses(litters) affected	5 (4)	4 (4)	5 (3)	4 (6)
Abnormally ossified sternbrae nos 2, 3, 4, 5, and/or 6	2 (2) [c]	1 (1)	5 (5)	0 (0)
Incompletely ossified sternbrae no 2	0 (0)	0 (0)	2 (2)	0 (0)
Incompletely ossified sternbrae no 5	0 (0)	0 (0)	1 (1)	0 (0)
Abnormally ossified and fused sternbrae nos 3, 4 and/or 5	0 (0)	0 (0)	2 (2)	1 (1)
Fused ribs nos 3, 4, 5, and/or 6	0 (0)	0 (0)	0 (0)	2 (2)
Fused ribs nos 10 and 11, right side	1 (1)	0 (0)	0 (0)	0 (0)
Bipartite sternebrae no. 5	1 (1)	0 (0)	1 (1)	0 (0)
Bipartite sternebrae no. 2	0 (0)	1 (1)	0 (0)	0 (0)
Fused thoracic vertebral bodies, nos 2 and 3 (resulted in different # of ribs on the right side)	0 (0)	0 (0)	1 (1)	0 (0)
Thoracic vertebrae no. 4, partial absent (right side); corresponding ribs and vertebral arches, absent	1 (1)	0 (0)	0 (0)	0 (0)
Thoracic vertebrae no. 11, absent (right side); corresponding ribs and vertebral arches, absent	0 (0)	1 (1)	0 (0)	0 (0)
Supernumerary ossification center between vertebral bodies nos 11 and 12; absence of thoracic vertebral arch and rib	0 (0)	1 (1)	0 (0)	0 (0)

a Data obtained from pages pp 23-25 and 32-35 in the study report.
b Some observations may be grouped together.
c Fetal (litter) incidence
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS:

The study author indicated that there was a "slightly higher reduction in body weight at 10 mg/kg/day during the first third of the application period" and there was a "reduction in food consumption over the entire treatment period at 10 mg/kg". The author also indicates that "MITC did not show any embryotoxic or teratogenic effects up to the highest dose level of 10 mg/kg body weight."

B. REVIEWER COMMENTS:

One dam in the high dose group died on GD 11 following weight loss and loss of hindlimb function. No other clinical signs attributed to administration of MITC occurred in this study. No abortions occurred in any group. Variation in body weight and food consumption are large. Because of the large variations, no statistically significant decreases in maternal weight gain and food consumption were noted. However, mean body weight gain and food consumption were decreased compared to the control at the highest dose level.

There were no differences in mean corpora lutea, mean implanatation sites, mean resorptions, and mean viable fetuses were detected among all dose groups.

In the data provided in the report, the mean measured concentration ranged from 67% to 88% of the nominal concentration. This large range provides uncertainty surrounding the amount of MITC the rabbits recieved.

The tentative maternal LOAEL is 10 mg/kg bw/day, based on reduced body weight gain and food consumption. The tentative maternal NOAEL is 3 mg/kg bw/day.

There were no differences in fetal weights or sex ratio among the treatment groups. There were also no treatment related changes in external, skeletal or visceral examinations following *in utero* exposure to MITC.

The developmental LOAEL was not established. The developmental NOAEL is 10 mg/kg bw/day.

C. STUDY DEFICIENCIES

There are critical deficiencies in homogeneity, stability, and concentration information provided in the report.

Test material-vehicle mixture was prepared daily by mixing appropriate amounts of test substance with corn oil using a homogenizer. Prior to the start of the study, stability of the test substance in corn oil was evaluated immediately after preparation and 90 minutes after preparation. The temperature was not provided; room temperature is assumed. MITC is a highly volatile compound; the room temperature could impact the amount of chemical which remains in

the corn oil.

The study report indicates that the homogeneity of the solution was tested. However, the study report does not provide data from various places in the mixture (e.g. top, middle, bottom) to support this statement.

Concentration of the test mixture were evaluated twice. The mean measured concentration ranged from 67% to 88% of the nominal concentration. This range of measured concentrations is considered large and provides uncertainty to the study.

DATA FOR ENTRY INTO ISIS

Developmental Study - rabbits (870.3700b)

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
068103	45919418	developmental	rabbits	GD 6-18	oral	gavage	1-10	1, 3, 10	3	10	body weight, food consumption	Maternal
068103	45919418	developmental	rabbits	GD 6-18	oral	gavage	1-10	1, 3, 10	10	NA	none identified	Developmental