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TXR#: 0051863

DATA EVALUATION RECORD

STUDY TYPE: Oral Developmental Toxicity in Rats
OPPTS Number: 870.3700 OPP Guideline Number: §83-3a

DP BARCODE: D263900 SUBMISSION CODE: S576487
P.C. CODE: 068103 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Methyl isothiocyanate (99.6% a.i.)

SYNONYMS: MITC

CITATION(s): Stump, D., (1998). A Prenatal Developmental Toxicity Study of Methylisothiocyanate (MITC) in Rats. WIL Research Laboratories, Inc., Ashland, OH. Laboratory Study No. WIL-316002, September 2, 1998. MRID 44733602. Unpublished.

Stump, D., (1998). A Dose Range-Finding Prenatal Developmental Toxicity Study of Methylisothiocyanate (MITC) in Rats. WIL Research Laboratories, Inc., Ashland, OH. Laboratory Study No. WIL-316001, September 2, 1998. MRID 44733601. Unpublished.

SPONSOR(s): MLPC International, 40370 Rion Des Landes, France
Osmose Wood Preserving, Inc., 980 Elliott St., Buffalo, NY

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44733602), methyl isothiocyanate (99.6% a.i.; Lot #: WIL Log No. 3518A) was administered in corn oil by gavage twice daily from gestation day (GDs) 6 through 19 to Crl:CD®(SD)BR rats (25/dose level) at concentrations of 0, 3, 10, or 30 mg/kg/day. It was stated that the twice-daily treatment regimen was used because of the irritative properties of the test substance. Dams were sacrificed on GD 20. No treatment-related findings were noted at 3 mg/kg/day.

No premature deaths occurred during the study interval. Many treatment-related clinical signs of toxicity (Table 2) were observed in the 30 mg/kg group at daily examination, at the time of the first and second daily doses, and 1 hour post-dose of the first and second doses. Treatment related salivation was also noted at 10 mg/kg.

At 30 mg/kg/day, decreased ($p<0.05$ or 0.01) body weight gains were observed as follows: during daily measurements on GDs 15-17 (↓22-33%) and GDs 18-20 (↓36-47%); for the GDs 12-20 interval (↓29%); the overall treatment interval (↓27%, GDs 6-20); and the overall study interval (↓20%, GDs 0-20). At 10 mg/kg/day, body weight gains were statistically decreased during GD 15-16 and 18-19 (↓20% and ↓32%, respectively) compared to controls. At 30 mg/kg/day, a decrease in gravid uterine weight was observed (↓13%), along with a reduction in corrected (for gravid uterine weight) body weight gain (↓31%, $p<0.01$). Absolute (g/animal/day) and/or relative (g/kg/day) food consumption was reduced in the 30 mg/kg/day group ($p<0.05$ or 0.01) during the daily measurements on GDs 15-20 (↓11-24%), during the GDs 6-9 (↓12-15%) and 12-20 (↓10-11%) intervals, and for the overall treatment interval (↓6-12%, GDs 6-20).

Females of the highest dose group (8/25 treated) exhibited stomach adhesions and one of these eight also had a stomach abscess; neither of these observations were noted in control animals.

The number of implantations/dam, number of resorptions/dam, percent male, and the extent of pre-and post-implantation losses were similar between control and treated groups.

The maternal LOAEL is 10 mg/kg/day, based on salivation and decreased body weight gain.
The maternal NOAEL is 3 mg/kg/day.

At 30 mg/kg/day the mean fetal weight was decreased by 8% compared to controls ($p< 0.01$). The reduced fetal weight is associated with decreased body weight gain of the maternal animals at the same dose level. Upon skeletal examination, increased incidence of unossified sternebra(e) #1, #2, #3, and/or #4 was observed at 30 mg/kg [2.6 (27.3)] vs controls [0.28 (4.5)]; the litter incidence of this finding was beyond the historical control range (0.0-22.73). No treatment related effects were observed in the 3 or 10 mg/kg/day groups.

The developmental LOAEL is 30 mg/kg/day, based on reduced fetal weight and an increased incidence of the skeletal variation of unossified sternebra(e).
The developmental NOAEL is 10 mg/kg/day.

This developmental toxicity study is classified **acceptable-guideline (§83-3[a])** and does satisfy the guideline requirement for a developmental toxicity study in the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Methyl isothiocyanate
   Description: Solid pale yellow to brown nonuniform appearance
   Lot/Batch #: WIL Log No. 3518A
   Purity: 99.6% a.i.
   Storage stability: Dose formulations were stable at room temperature for up to 8 days.
   CAS #: 556-61-6
   Structure: Not provided

2. Vehicle: Corn oil

3. Test animals: Species: Rat
   Strain: Crl:CD®(SD)BR
   Age and weight of females: Approximately 14 weeks old at mating, 236-343 g on GD 0
   Source: Charles River Laboratories, Inc., Portage, MI
   Housing: Individually in suspended wire-mesh cages, except during mating
   Diet: Certified Rodent LabDiet© 5002 (PMI Feeds, Inc.), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 72.2-72.7° F
   Humidity: 32-50%
   Air changes: Approximately 10/hr
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period: 4 weeks

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 12/16/97 end: 1/8/98

2. Mating: Males, of the same source and strain, and females were housed together (1 male and 1 female/cage) for breeding. Mating was confirmed by the presence of a copulatory plug or sperm in a vaginal smear and the day evidence of mating was identified was designated as gestation day (GD) 0.

3. Animal assignment: Animals were randomly assigned (stratified by body weight) to dose groups as indicated in Table 1.

Table 1. Animal assignment [a]

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

a Data obtained from study report, page 16.

4. Dose selection rationale: Doses were based on a range-finding developmental study of Crl:CD®(SD) BR rats (8/dose) in which the test substance in corn oil was administered orally by gavage twice daily (approximately 4 hours apart) at concentrations of 0, 1, 5, 15, 30, or 45 mg/kg/day from GDs 6-19. On GD 20, a laparohysterectomy was performed on all dams. No premature deaths occurred. Various incidences of salivation at time of dosing, brown staining around nose and mouth, clear matting around the mouth and on the thoracic area, and yellow staining on the anogenital area were observed at 1 hour post-dose in the 30 and 45 mg/kg animals; additionally, sporadic observations of salivation and brown staining around the nose and mouth were noted at 15 mg/kg. All previously noted clinical signs were considered to be responses to the irritative properties of the test substance, rather than systemic toxicity. At the time of the first dosing, 45 mg/kg animals were observed rubbing their faces along the cage floor (8 incidences in 4/8 animals); by the second daily dose, animals in the 15 (1 incidence in 1/8 animals), 30 (1 incidence in 1/8 animals), and 45 mg/kg (4 incidences in 3/8 animals) groups were observed to be rubbing their faces on the cage floor. Decreased ($p<0.05$ or 0.01) body weights were observed at 45 mg/kg on GDs 18, 19, and 20 (↓9-13%). Decreased ($p<0.05$ or 0.01) body weight gains were observed at 45 mg/kg on GDs 16-20 (↓40-86%), the GDs 6-9 (↓375%) and 12-20 (↓46%) intervals, the overall treatment interval (↓41%, GDs 6-20), and the overall study interval (↓33%, GDs 0-20); additionally, an isolated increase was observed during the GDs 9-12 interval (↑65%). Reductions ($p<0.05$ or 0.01) were noted at 45 mg/kg in terminal body weights (↓13%), corrected (for gravid uterine weight) body weights (↓9%), and corrected body weight gains (↓39%). Decreases ($p<0.05$ or 0.01) in absolute (g/rat/day) and/or relative (g/kg/day) food consumption were observed at 45 mg/kg as follows: decreased GDs 6-9 (↓36-50%); GDs 17-20 (absolute only,↓23-35%); GDs 17-18 (relative only, ↓30%); GDs 6-9 (↓42-43%); and GDs 6-20 (↓14-18%).

When compared to concurrent controls, the number of early resorptions was increased (not statistically significant [NS]) at 5, 15, 30, and 45 mg/kg (8, 11, 11, and 28, respectively, vs 4 in controls); additionally, the percent early resorptions was increased at

5, 15, 30, and 45 mg/kg (↑28, 49, 70, and 308%, respectively, vs controls). The number of viable fetuses was decreased (NS) at 45 mg/kg (↓18%). Mean fetal weights were decreased ($p<0.01$) at 30 and 45 mg/kg (↓11 and 24%, respectively); male fetal weights were decreased ($p<0.01$) at 30 and 45 mg/kg (↓13 and 23%, respectively), while female fetal weights were reduced at 45 mg/kg only (↓22%, $p<0.01$). No external malformations or variations were observed in the fetuses.

Based on the results of this range finding study, the doses presented in Table 1 were selected for the subsequent full developmental toxicity study.

5. Dosage preparation and analysis - Test formulations were prepared weekly during the study by heating the test substance until liquified, then mixing the appropriate amount of test substance with corn oil, and diluting with additional vehicle to achieve the desired concentration. Formulations were stored at room temperature. Prior to the initiation of dosing, dose formulations of 3, 10, and 30 mg/mL were evaluated for homogeneity (top, middle, bottom). These dose levels were also analyzed for stability when stored at room temperature for 8 days (mid- and high-dose levels were analyzed in the range finding study). Concentration analyses were performed on all concentrations three times during the study.

   Results - Homogeneity (range as mean % of nominal): 92.6-105% with relative standard deviations of 0.61-6.0%.

   Stability analysis: 95.6-103% of time 0.

   Concentration analysis (range as mean % of nominal): 86.9-104%

   The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: All doses were administered twice daily by gavage on GDs 6 through 19 in a volume of 5 mL/kg body weight. It was stated that the twice-daily treatment regimen was used because of the irritative properties of the test substance. Dosing was based on the most recent body weight. Control animals received the vehicle only.

C. OBSERVATIONS

1. Maternal observations and evaluations - The animals were checked for moribundity and mortality twice daily. Detailed clinical signs were recorded daily from GDs 0-20. Clinical observations were conducted approximately one hour following each dosing. Body weight and food consumption (g/animal/day and g/kg/day) were recorded on GD 0 and daily on GDs 6-20. Dams were sacrificed on GD 20. Examinations at sacrifice

consisted of a gross exam of the pelvic, thoracic, and abdominal cavities. The reproductive tract was removed, examined, and the following were recorded:

- gravid uterine weight
- number and distribution of corpora lutea
- number and distribution of implantation sites
- number and distribution of fetuses (live and dead)
- number and distribution of resorptions (early and late)

The uteri of non-pregnant females were opened and placed in 10% ammonium sulfide for detection of implantations.

2. Fetal evaluations - All fetuses were weighed, sexed, and received a detailed external examination. Crown-rump measurements were recorded. Visceral exams were performed on all fetuses and included dissection of the heart and major vessels and examination of the kidneys (graded for renal papillae development). Heads from approximately one-half of the fetuses were fixed in Bouin's solution for free-hand sectioning and examination using WILSON's technique; the heads of the remaining fetuses were examined by a mid-coronal slice. All fetal carcasses were eviscerated, fixed in alcohol, macerated (KOH), stained with Alizarin Red S and Alcian Blue, and examined for skeletal abnormalities. Abnormalities were classified as malformations or variations.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures.

2. Indices: The following indices were calculated by the sponsor:

For cesarean section findings:

Postimplantation loss/litter = # dead fetuses and/or resorptions per group /# pregnant females per group

Summation per group (%) = postimplantation loss per litter (%)^a /# litters per group
a = # dead fetuses and or resorptions per litter /# implantation sites per litter X 100

For fetal developmental findings:

Summation per group (%) = viable fetuses affected per litter (%)^a /# litters per group
a = # viable fetuses affected per litter/ # viable fetuses per litter X 100

3. Historical control data: Historical control data were provided to allow comparison with treated groups.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical observations: No premature deaths occurred during the study interval. Many treatment-related clinical signs of toxicity (Table 2) were observed in the 30 mg/kg group at daily examination, at the time of the first and second daily doses, and 1 hour post-dose of the first and second doses. Treatment related salivation was noted at 10 mg/kg.

Table 2. Selected clinical signs (total occurrence/# of animals with clinical sign) at daily examination, at time of dosing, and 1 hour post-dosing [a]

Observation	Dose in mg/kg/day			
	0	3	10	30
Daily examinations				
Dried yellow matting, anogenital area	0/0	0/0	0/0	20/8
At time of dosing–first daily dose				
Wet clear matting, ventral neck	0/0	0/0	0/0	29/17
Wet clear matting, right forelimb	0/0	0/0	0/0	38/20
Wet clear matting, left forelimb	0/0	0/0	0/0	39/20
Salivation	0/0	0/0	16/11	64/22
Wet clear matting around mouth	0/0	0/0	2/2	71/25
Salivation prior to dose administration	0/0	0/0	1/1	63/21
1 hour post-dose–first daily dose				
Wet tan matting, ventral neck	0/0	0/0	0/0	42/18
Wet tan matting, right forelimb	0/0	0/0	0/0	30/15
Wet tan matting, left forelimb	0/0	0/0	0/0	20/15
Wet yellow matting, urogenital area	0/0	0/0	0/0	14/10
Wet clear matting around mouth	0/0	0/0	0/0	14/11
Wet tan matting around mouth	0/0	0/0	0/0	43/18
Dried tan material around mouth	0/0	0/0	0/0	12/9
At time of dosing–second daily dose				
Wet clear matting, ventral neck	0/0	0/0	0/0	72/24
Wet clear matting, right forelimb	0/0	0/0	0/0	64/23
Wet clear matting, left forelimb	0/0	0/0	0/0	63/23
Salivation	0/0	1/1	12/9	76/23
Wet clear matting around mouth	0/0	0/0	0/0	108/25
Salivation prior to dose administration	0/0	0/0	5/3	123/25
1 hour post-dose–second daily dose				
Wet clear matting, ventral neck	0/0	0/0	1/1	20/13
Wet tan matting, ventral neck	0/0	0/0	0/0	56/16
Wet clear matting, right forelimb	0/0	0/0	0/0	21/12
Wet clear matting, left forelimb	0/0	0/0	0/0	22/12
Wet tan matting, right forelimb	0/0	0/0	0/0	51/15
Wet tan matting, left forelimb	0/0	0/0	0/0	51/15
Wet yellow matting, urogenital area	0/0	0/0	0/0	24/12
Wet clear matting around mouth	0/0	0/0	0/0	25/18
Wet tan matting around mouth	0/0	0/0	0/0	60/17

a Data extracted from study report, Tables 2 through 6, pages 37 though 44; n=25 animals/dose group.

2. Body weight: Decreased ($p<0.05$ or 0.01) body weight gains (Table 3) were observed at 30 mg/kg as follows: during daily measurements on GDs 15-17 (↓22-33%) and GDs 18-20 (↓36-47%); for the GDs 12-20 interval (↓29%); the overall treatment interval (↓27%, GDs 6-20); and the overall study interval (↓20%, GDs 0-20). Reductions ($p<0.05$ or 0.01) were observed at 10 mg/kg on GDs 15-16 (↓20%) and 18-19 (↓32%). When compared to concurrent controls, dose-dependent decreases were observed in gravid uterine weight at 3, 10, and 30 mg/kg (↓9, 10, and 13%, respectively), but the decreases at 3 and 10 mg/kg were minor and considered not to be of toxicological concern. A reduction in corrected (for gravid uterine weight) body weight gain was noted at 30 mg/kg (↓31%, $p<0.01$).

Table 3. Selected mean maternal body weight gains (g) [a]

Interval	Dose in mg/kg/day			
	0 n=22	3 n=25	10 n=23	30 n=22
Pretreatment: Days 0-6	31	32	31	33
Treatment:				
Days 13-14	6	7	6	6
Days 15-16	15	13	12* (↓20%)	10** (↓33%)
Days 16-17	18	16	15	14** (↓22%)
Days 18-19	19	18	13** (↓32%)	10** (↓47%)
Days 19-20	14	15	17	9* (↓36%)
Treatment interval: Days 12-20	105	99	95	75** (↓29%)
Overall treatment: Days 6-20	121	117	113	88** (↓27%)
Overall: Days 0-20	152	149	144	121** (↓20%)
Gravid uterine weight (g)	92.1	83.4* (↓9%)	82.8* (↓10%)	80.0** (↓13%)
Corrected body weight gain: [b] Day 20	59.7	65.8	60.4	41.1** (↓31%)

a Data extracted from the study report, Tables 8 and 9, pages 47 through 49. Nonpregnant females were excluded from the mean by the sponsor. Percent difference from controls is presented parenthetically.
b Corrected for gravid uterine weight.
* or ** Significantly different from controls at $p<0.05$ or 0.01, respectively.

3. Food consumption - When compared to concurrent controls, absolute (g/animal/day) food consumption was reduced ($p<0.05$ or 0.01) in the high-dose dams during the daily measurements on GDs 15-20 (↓13-24%), during the GDs 6-9 (↓15%) and 12-20 (↓11%) intervals, and for the overall treatment interval (↓12%, GDs 6-20). Relative (g/kg/day)

food consumption (Table 4) was reduced ($p<0.05$ or 0.01) in the high-dose dams during the daily measurements on GDs 15-19 (↓11-18%), during the GDs 6-9 (↓12%) and 12-20 (↓10%) intervals, and for the overall treatment interval (↓6%, GDs 6-20).

Table 4. Selected mean maternal relative food consumption (g/kg/day) [a]

Interval	Dose (mg/kg/day)			
	0 n=22	3 n=25	10 n=23	30 n=22
Pretreatment:				
Days 0-6	71	72	73	74
Treatment:				
Days 13-14	47	49	50	49
Days 15-16	55	55	52	47** (↓15%)
Days 16-17	58	56	55	50** (↓14%)
Days 17-18	54	54	53	48** (↓11%)
Days 18-19	51	50	48	42** (↓18%)
Treatment intervals:				
Days 6-9	42	44	42	37* (↓12%)
Days 12-20	51	51	50	46** (↓10%)
Overall treatment:				
Days 6-20	48	49	48	45** (↓6%)

a Data extracted from the study report, Table 11, pages 52 and 53.
* or ** Significantly different from controls at $p<0.05$ or 0.01, respectively.

4. Gross pathology - At 30 mg/kg, 8/25 females showed stomach adhesions and one of these eight also had a stomach abscess; neither of these observations were noted in control animals. No other treatment-related pathological findings were noted.

5. Cesarean section data - Fetal weights (Table 5) were reduced at the high-dose level in males and females when analyzed both separately and combined (↓8% each, $p<0.01$). The number of implantations/dam, number of resorptions/dam, percent male, and the extent of pre-and post-implantation losses were similar between control and treated groups.

Table 5. Cesarean section observations [a]

Observation	Dose (mg/kg/day)			
	0	3	10	30
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant Pregnancy Rate (%)	22 (88)	25 (100)	23 (92)	22 (88)
# Nonpregnant	3	0	2	3
# Total Dams Died # Died Pregnant # Died Nonpregnant # Aborted # Premature Delivery	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0
Total # Corpora Lutea Corpora Lutea/Dam	395 18.0±1.96	441 17.6±1.47	390 17.0±2.36	391 17.8±2.49
Total # Implantations Implantations/Dam	373 17.0±2.32	403 16.1±1.36	358 15.6±3.63	361 16.4±1.59
Total # Litters Examined	22	25	23	22
Total # Live Fetuses Live Fetuses/Dam	359 16.3±2.71	371 14.8±1.55	337 14.7±3.50	345 15.7±1.49
Total # Dead Fetuses Dead Fetuses/Dam	0 0	0 0	0 0	0 0
Total # Resorptions Early Late Total resorptions/Dam Early Late Litters with Total Resorptions	14 13 1 0.6±0.79 0.6±0.80 0.0±0.21 0	32 32 0 1.3±1.06 1.3±1.06 0 0	21 21 0 0.9±1.04 0.9±1.04 0 0	16 15 1 0.7±0.94 0.7±0.95 0.0±0.21 0
Mean Fetal Weight (g) Males Females	3.7±0.23 3.8±0.23 3.6±0.22	3.7±0.16 3.8±0.16 3.6±0.18	3.9±0.54 3.9±0.53 3.7±0.22	3.4±0.20** (↓8%) 3.5±0.20** (↓8%) 3.3±0.18** (↓8%)
Sex Ratio (% Male)	52.4	53.3	55.6	46.0
Preimplantation Loss (%)	5.5	8.4	9.4	7.0
Postimplantation Loss (%)	4.1	7.9	5.6	4.3

a Data extracted from the study report, Tables 1, 12, and 13, pages 36, 54, and 55 through 57. Percent difference from controls is presented parenthetically.

** Significantly different from controls at $p<0.01$.

B. DEVELOPMENTAL TOXICITY: Fetal examinations included external, visceral, and skeletal observations at necropsy.

1. External examination - No treatment-related external malformations were observed in any treatment group; additionally, no external variations were detected at any dose level. The only finding is shown in Table 6a.

Table 6a. External examinations [a]

Observations	Dose (mg/kg/day)			
	0	3	10	30
#Fetuses (#litters) examined	359 (22)	371 (25)	337 (23)	345 (22)
Malformations				
Microphthalmia and/or anophthalmia	0.28 (4.5)	0 (0)	0 (0)	0 (0)

a Data extracted from the study report, Table 14, page 58. For individual observations, data are presented as % fetal incidence (% litter incidence) and were calculated by reviewers.

2. Visceral examination - There were no visceral malformations or variations observed at any dose level.

3. Skeletal examination - Increased incidence of unossified sternebra(e) #5 and/or #6 was noted in the 3 [17.0 (64.0)], 10 [16.9 (65.2)], and 30 mg/kg [37.4 (86.4)] groups vs controls [10.0 (59.1)]. This finding was within the historical control ranges--although at the extreme high end [0.27-37.11 (4.17-100)]. Increased incidence of unossified sternebra(e) #1, #2, #3, and/or #4 was observed in the 3 [1.1 (12.0)], 10 [0.29 (4.3)], and 30 mg/kg [2.6 (27.3)] animals vs controls [0.28 (4.5)]; the litter incidence of this finding was beyond the historical control range (0.0-22.73) at 30 mg/kg.

Table 6b. Skeletal examinations [a]

Observations	Dose (mg/kg/day)				
	0	3	10	30	Historical control ranges [b]
#Fetuses (#litters) examined	359 (22)	371 (25)	337 (23)	345 (22)	40685 (3573)
Malformations					
Rib anomaly	0.28 (4.5)	0 (0)	0 (0)	0 (0)	NA
Costal cartilage anomaly	0.28 (4.5)	0 (0)	0 (0)	0 (0)	NA
Cartilaginous centra anomaly	0.28 (4.5)	0 (0)	0 (0)	0 (0)	NA
Variations					
14th rudimentary rib(s)	17.3 (81.8)	16.4 (76.0)	10.1 (52.2)	9.6 (54.5)	NA
Hyoid unossified	1.4 (13.6)	1.3 (16.0)	0.89 (8.7)	1.4 (9.1)	NA
Cervical centrum #1 ossified	9.7 (50.0)	19.4 (72.0)	9.8 (56.5)	9.3 (40.9)	NA
Sternebra(e) #5 and/or #6 unossified	10.0 (59.1)	17.0 (64.0)	16.9 (65.2)	37.4 (86.4)	0.27-37.11 (4.17-100)
Sternebra(e) #1, #2, #3, and/or #4 unossified	0.28 (4.5)	1.1 (12.0)	0.29 (4.3)	2.6 (27.3)	0.0-2.56 (0.0-22.73)

a Data extracted from the study report, Tables 14 and 16, pages 58 and 63, and Appendix C, page 276 and 358 through 406. For individual observations, data are presented as % fetal incidence (% litter incidence) and were calculated by reviewers.

b In the provided historical control data, it was stated that the total number of fetuses examined was 40676 and total number of litters examined was 3574, however when these totals were calculated by reviewers the total number of fetuses examined was 40685, while the number of litters examined was 3573.

NA Not applicable

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS - Administration of the test substance at 30 mg/kg/day resulted in maternal toxicity characterized by reduced body weight gains and food consumption and gross pathological findings including stomach lesions. The maternal LOAEL is 30 mg/kg/day and the NOAEL is 10 mg/kg/day.

Reduced fetal body weights and increased incidence of skeletal variations, which included unossified sternebra, were observed at 30 mg/kg. The developmental LOAEL is 30 mg/kg/day and the NOAEL is 10 mg/kg/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Methyl isothiocyanate (99.6% a.i.; Lot #: WIL Log No. 3518A) was administered in corn oil by gavage twice daily from GDs 6 through 19 to Crl:CD®(SD)BR rats (25/dose level) at concentrations of 0, 3, 10, or 30 mg/kg/day. It was stated that the twice-daily treatment regimen was used because of the irritative

properties of the test substance. The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the study animals was acceptable. Dams were sacrificed on GD 20. No treatment-related findings were noted at 3 or 10 mg/kg/day.

No premature deaths occurred during the study interval. Many treatment-related clinical signs of toxicity (Table 2) were observed in the 30 mg/kg group at daily examination, at the time of the first and second daily doses, and 1 hour post-dose of the first and second doses. Treatment related salivation was also noted at 10 mg/kg.

At 30 mg/kg/day, decreased ($p<0.05$ or 0.01) body weight gains were observed as follows: during daily measurements on GDs 15-17 (↓22-33%) and GDs 18-20 (↓36-47%); for the GDs 12-20 interval (↓29%); the overall treatment interval (↓27%, GDs 6-20); and the overall study interval (↓20%, GDs 0-20). At 10 mg/kg/day, body weight gains were statistically decreased during GD 15-16 and 18-19 (↓20% and ↓32%, respectively) compared to controls. At 30 mg/kg/day, a decrease in gravid uterine weight was observed (↓13%), along with a reduction in corrected (for gravid uterine weight) body weight gain (↓31%, $p<0.01$). Absolute (g/animal/day) and/or relative (g/kg/day) food consumption was reduced in the 30 mg/kg/day group ($p<0.05$ or 0.01) during the daily measurements on GDs 15-20 (↓11-24%), during the GDs 6-9 (↓12-15%) and 12-20 (↓10-11%) intervals, and for the overall treatment interval (↓6-12%, GDs 6-20).

Females of the highest dose group (8/25 treated) exhibited stomach adhesions and one of these eight also had a stomach abscess; neither of these observations were noted in control animals.

The number of implantations/dam, number of resorptions/dam, percent male, and the extent of pre-and post-implantation losses were similar between control and treated groups.

The maternal LOAEL is 10 mg/kg/day, based on salivation and decreased body weight gain.
The maternal NOAEL is 3 mg/kg/day.

2. DEVELOPMENTAL TOXICITY:

a. Deaths/Resorptions: The numbers of resorptions/dam and viable fetuses/dam for the treatment groups were not significantly different from the concurrent controls.
b. Altered Growth: Fetal weights were reduced at the high-dose level in males and females when analyzed both separately and combined (↓8% each, $p<0.01$). The reduced fetal weights are associated with reduced maternal weight gain at the same dose level.
c. Developmental Variations: Upon skeletal examination, increased incidence of unossified sternebra(e) #1, #2, #3, and/or #4 was observed at 30 mg/kg [2.6 (27.3)] vs controls [0.28 (4.5)]; the litter incidence of this finding was beyond the historical

control range (0.0-22.73. Increased incidence of unossified sternebra(e) #5 and/or #6 was noted in the 3 [17.0 (64.0)], 10 [16.9 (65.2)], and 30 mg/kg [37.4 (86.4)] groups vs controls [10.0 (59.1)]. This finding was within the historical control ranges--although at the extreme high end [0.27-37.11 (4.17-100)].

d. Malformations: There were no treatment-related malformations detected at any dose level.

Developmental LOAEL = 30 mg/kg/day, based reduced fetal weight and on an increased incidence of the skeletal variation of unossified sternebra(e)
Developmental NOAEL = 10 mg/kg/day

This developmental toxicity study is classified **acceptable (§83-3[a])** and does satisfy the guideline requirement for a developmental toxicity study in the rat.

C. STUDY DEFICIENCIES - No deficiencies were noted.

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
068103	44733602	developmental	rats	14 days	oral	gavage	3-30	0, 3, 10, 30	3	10	body weight, salivation	Maternal
068103	44733602	developmental	rats	14 days	oral	gavage	3-30	0, 3, 10, 30	10	30	fetal weight, unossification	Developmental