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CASWELL FILE

Tox R 005414

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of teratology studies with Methyl Isothiocyanate
EPA Accession Nos. 257764 and 257765 . Caswell No. 573

TO: Winnie Teeters, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)

FROM: Quang Q. Bui, Ph.D. Quang Q Bui
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Laurence D. Chitlik, D.A.B.T. LDC 1/31/85
Section Head, Section V
Toxicology Branch/HED (TS-769C)

As per your request, the two teratology studies with Methyl-Isothiocyanate in rabbits (Hazleton Lab., Europe, #3686-14/30) and rats (Hazleton Lab., Europe, #3193-14/10) were evaluated.

It is recommended that the rat teratology study be classified as Core Supplementary Data. To fully assess the developmental toxicity NOEL, the registrant is requested to submit additional data pertaining to the issues listed on pages 4 and 8 of this review. This study may potentially be upgraded pending the submission and adequacy of the extensive additional data requested.

The rabbit teratology study is classified as Core Supplementary Data. A list of study deficiencies is given on pages 10 and 16 of this review. Although, additional data must be requested from the registrant to provide some explanation relative to several issues (#, 5, 6, 7, 8, and 9) listed on page 10 of this memo, it is this reviewer's opinion that this study cannot be upgraded.

Reviewer's note

Methyl-isothiocyanate, batch # R27/S 6350, was used in this investigation. This batch was produced on 8/27/81 with an expiration date of 9/30/83. Analysis of the test material was performed on 9/2/81 (certificate of analysis # T 22/81) and revealed a 94.9% of active ingredient.

The rat teratology was conducted from 4/29/82 to 6/30/82 and analytical determinations of the dosing solutions were conducted at several intervals during this period. All analytical results were within the acceptable range limits (>90% of the nominal concentration).

This same batch of methyl isothiocyanate was also used in the rabbit teratology study which was performed from 6/14/83 to 8/4/83. The test material was prepared on 3 occasions (July 4, 11, and 18 of 1983). Low analytical determinations were obtained from the 3 mg/kg dosing solutions prepared on 7/4/83 and from all dosing solutions (1, 3, and 5 mg/kg) prepared on 7/18/83. Re-formulation of these dosing solutions was necessary. However, the treated groups were dosed with these "unacceptable" solutions for at least 1 day. The investigators suggested that the low analytical results were due to "some uneven decomposition". No further explanations were provided. It is this reviewer's opinion that using of the test material at a date (7/83) so close to the expiration date (9/83) may account for the "uneven decomposition observed" and restricts the scientific integrity of the rabbit teratology study.

STUDY REVIEW

Chemical: Methyl Isothiocyanate - SN 32866
Test Material: Technical 94.9%

STUDY IDENTIFICATION:

"Methyl Isothiocyanate (MITC) Oral (Gavage) Teratology Study in the Rat with Amendment to the Final Report"

Testing Facility: Hazleton Lab. Europe
North Yorkshire, England
Final Report No.: 3193-14/10
Final Report Date: 2/83
Addendum: 1/84
EPA Accession No.: 257765

Study Reviewed by: Quang Q. Bui, Ph.D.
Section V, Toxicology Branch
Hazard Evaluation Division

Approved by: Laurence D. Chitlik, D.A.B.T.
Section Head, Section V
Toxicology Branch/HED

CONCLUSIONS

Adminstration of MITC at 25 mg/kg/day (highest dose tested) during the period of major organogenesis in rats resulted in significant decreases in maternal weight gain and food consumption but without any noticeable clinical adverse manifestations. Based upon these findings, it is concluded that the maternal NOEL be established at 5 mg/kg/day.

The developmental toxicity NOEL (including assessment of the teratogenic potential) could not be determined due to the lack of individual litter data as well as disparity relative to the classification of lens opacity. Lens opacity was classified as a variation in this study but as a malformation in both the rat positive control data and rabbit teratology study (Hazleton Europe #3686-14/30) conducted by the same testing facility.

Submission of additional data and explanation relative to several issues listed in the "Discussion" section of this review (page 8) are required prior to final assessment of the study by Toxicology Branch.

RECOMMENDATION

It is recommended that this study be classified as Core Supplementary Data with a maternal NOEL established at 5 mg/kg/day and an undetermined developmental toxicity NOEL. This study will be re-evaluated pending the submission and adequacy of additional data requested as listed on page 8 of this memo.

PROCEDURES

Test Material:	Methyl-Isothiocyanate 94.9% Batch No. R27/S 6350
Dose Levels:	0, 1, 5, and 25 mg/kg/day from days 6-15 of gestation
Vehicle:	Corn Oil, Volume = 10 ml/kg
Species:	Sprague-Dawley SD-CD rats (Charles River, Kent, England)

A copy of the procedures used is appended. In general, the protocol used was not exceptionally different from that of the 1982 FIFRA Guidelines. However, the following comments are noted:

1. Upon arrival, all animals were vaccinated against Sendai virus and acclimatized for 12 days. The authors stated that vaccination would not interfere with achievement of the study objectives.
2. 2/3 of the fetuses in each litter were dissected and the viscera examined. The carcasses were then stained using routine staining procedure (Alizarin Red S). The remaining 1/3 of the fetuses were fixed in Bouin's solution. Free-hand transverse sections were conducted only on the head. The authors indicated that the trunk of the 1/3 fixed fetuses was examined by dissection.
3. The uteri of non-pregnant animals were not fixed with 10% ammonium sulfide for detection of possible early implantation loss.
4. Individual litter data were not submitted.
5. Historical control data for litter incidence were not provided.
6. Historical control data for variations were not submitted.
7. Age and weight ranges of the males used were not indicated.
8. Fetuses were killed by an intracardiac injection of Euthatal (unknown volume). This technique may produce perforation and anatomical distortion of the cardiac structures and, hence, may interfere with the assessment of the cardiovascular and pulmonary findings.

RESULTS

1. Test Material

A certificate of analysis (#T 22/81, 9/2/81) was submitted by the sponsor and revealed that the batch used (R 27/S 6350) contained 94.9% of methyl isothiocyanate. Analytical determinations of the dosing solutions were conducted at several intervals during the investigation (4/29/82 - 6/30/82). All analytical results were within the acceptable range limits (> 90% of the nominal concentration).

2. Mortality and Clinical Observations

No maternal deaths were observed during the entire investigation. There were no apparent clinical signs which could be attributed to administration of the test material.

3. Abortion and Pregnancy Index

No dams aborted in this investigation. The pregnancy index (ratio of # dams pregnant/# dams mated) was similar among all groups being 89%, 88%, 82%, and 89% for the vehicle control, 1, 5, and 25 mg/kg groups, respectively.

4. Maternal Body Weight

Significant decreases in maternal weight gain during the dosing period were found at the 25 mg/kg dosage level. However, compensatory increases in weight gains were noted in this group at cessation of test material administration (days 16-20 of gestation).

The net weight gain (absolute weight gain minus gravid uterine weight) of the 25 mg/kg group was also statistically different from control values. These findings collectively suggest that the body weight reductions noted in the 25 mg/kg group were treatment-related.

Body Weight Data (grams)

	Control	1 mg/kg	5 mg/kg	25 mg/kg
# of dams pregnant	23	21	23	24
Mean Weight Gain				
Days 0-6	20	20	18	21
Days 6-15	36	36	33	24*
Days 15-20	51	55	52	57
Days 0-20	107	111	103	102
Mean Uterine Weight	60.2	64.8	62.9	66.6
Net Weight Gain°	46.8	46.2	40.1	35.4*

(°) Net weight gain = Absolute weight gain - gravid uterine weight
(*) Significantly different from controls, $P < 0.05$

5. Food Consumption

Significant decreases in food consumption (g/animal/day) were observed only in the 25 mg/kg group during the dosing period. No treatment-related effects with respect to food consumption were noted in the 1 and 5 mg/kg groups.

6. Reproductive Data

The reproductive status at laparotomy is presented in the next table:

Reproductive Data at Necropsy

	Control	1 mg/kg	5 mg/kg	25 mg/kg
# dams mated	26	24	28	27
# dams pregnant (%)	23 (89)	21 (88)	23 (82)	24 (89)
$\bar{X}$ corpora lutea/dam	14.4	14.6	14.0	15.1
$\bar{X}$ implantations/dam	12.4	13.5	13.0	14.7
Pre-implantation loss(%)	13.6	7.5	7.1	2.8
$\bar{X}$ resorptions	0.7	0.8	0.8	0.6
$\bar{X}$ viable fetuses/dam	11.7	12.8	12.3	14.0
Post-implantation loss(%)	5.6	5.6	6.0	4.3
Implantation Efficiency(%)	94.4	94.4	94.0	95.7

No compound-related effects were observed relative to the mean numbers of corpora lutea, implantations, resorptions, or viable fetuses. The indices of pre- and post-implantation loss as well as implantation efficiency were similar among all groups. All control values were comparable to those of the historical control data provided.

7. Developmental Toxicity

a. Fetal Weight and Crown-Rump Length

Fetal weight and crown-rump length data are summarized as follows:

Fetal Weight and Crown-Rump Length

	Control	1 mg/kg	5 mg/kg	25 mg/kg
Mean fetal weight (g)	3.4	3.3	3.4	3.1*
Mean litter weight (g)	39.6	42.5	41.2	43.5
Mean crown-rump length (milimeter)	37.5	37.5	37.8	36.2*

(*) Statistically significant from controls, $P < 0.05$

Significant decreases in mean fetal weight and mean crown-rump length were found at the 25 mg/kg dosage level. The larger mean litter size associated with this group (14.0 vs. 11.7 of control) may account in part for these differences. However, these 25 mg/kg values were lower than those of the historical control (fetal weight = 3.3 - 3.9 grams, fetal length = 37.8 - 40.1 mm) which had comparable litter size (13.2) as the 25 mg/kg group (14.0). Therefore, the decreases in body weight and length observed at the 25 mg/kg dose level may be attributed to the administration of MITC. No differences in the fetal sex-ratio were observed.

b. Malformations

In this study, the authors distinguished between major and minor malformations and variations. From a regulatory standpoint, assessment should be placed on either malformations or variations without emphasis on differentiation between minor and major malformations. However, it should be noted that calculation of litter incidence for each malformation was not permissible since individual litter data was not submitted. Further, fetuses with multiple malformations may be reported more than once in the next table.

Scattered incidences of external, visceral, and skeletal malformations were noted in both control and treated groups. Anophthalmia was found in one fetus of the 1 mg/kg group whereas micropthalmia was noted only in one fetus of the 25 mg/kg group. A slight increase in bilateral ureter dilation was found in the treated groups but the increase was still within the historical control range (3.2%). When the incidences of unilateral and bilateral ureter dilation were combined, respective percentages of 5.9, 5.2, 7.4, and 9.2 were found for the 0, 1, 5, and 25 mg/kg dosage levels. Findings in the 5 and 25 mg/kg group (7.4% and 9.5%) were higher than those of the historical control provided (6.6%) and may be indicative of compound-related effects.

MALFORMATIONS AND VARIATIONS

	Control	1 mg/kg	5 mg/kg	25 mg/kg
Total fetuses examined	270	268	282	337
# fetuses examined, skeletal	187	185	196	232
# fetuses, fixed in Bouin's solution	83	83	86	105
Unilateral anophthalmia	0	1(0.4)°	0	0
Unilateral microphthalmia	0	0	0	1(0.3)
Brain, enlarged ventricles	1(0.4)†	0	0	0
Unilateral hydronephrosis	1(0.4)	1(0.4)	0	1(0.3)
Bilateral hydronephrosis	1(0.4)	0	0	0
Unilateral ureter dilation	14(5.2)	8(3.0)	17(6.0)	20(5.9)
Bilateral ureter dilation	2(0.7)	6(2.2)	4(1.4)	11(3.3)
Unilateral testis agenesis	0	0	1(0.4)	0
Parietal bones inc. ossified *	2(1.1)	1(0.5)	6(3.1)	8(3.4)
Interparietal bones inc. ossified *	13(7.0)	14(7.6)	28(14.3)	42(18.1)
Occipital bones inc. ossified *	13(7.0)	2(1.1)	15(7.7)	57(24.6)
Sternebrae 1-4 inc.ossified *	1(0.5)	4(2.2)	4(2.0)	11(4.7)
Pubis not ossified *	0	1(0.5)	1(0.5)	6(2.6)
Sternebrae #5, incomplete or non ossif.	64(34.2)	87(47.0)	74(37.8)	131(56.5)
Sternebrae #6, incomplete or non ossif.	56(29.9)	64(34.6)	67(34.2)	103(44.4)
Unilateral lens opacity **	10(5.3)†	19(10.3)†	24(12.2)†	25(10.8)†
Bilateral lens opacity **	117(62.6)†	97(52.4)†	85(43.4)†	99(42.7)†

(NOTE: Fetuses with multiple malformations may be reported more than once in this table)

(°): Number of fetuses affected (%), calculated by the investigators
(†): Percentage calculated by the study authors - see "Discussion" section
(*): Classified as minor malformation by the investigators
(**): Classified as a variation by the investigators

In this study, the authors classified lens opacity as a variation. Disparity relative to this classification is discussed in the next section. Increased incidence of unilateral lens opacity was noted in all treated groups as compared to concurrent control data. Historical data for lens opacity was not reported. Consequently, the high incidences of bilateral and unilateral lens opacity in the control group could not be verified nor confirmed. It should be noted that the frequency of fetuses with lens opacity reported by the authors did not include observations made on fetuses fixed in Bouin's solution.

Overall, the incidence of fetuses with malformations and variations apparently was increased in the 25 mg/kg group as compared to the controls. However, neither calculation of the total number of fetuses with malformations nor the number of affected litters could be assessed in this review due to the lack of individual litter data.

DISCUSSION

Administration of MITC at 1, 5, and 25 mg/kg/day during the period of major organogenesis of rats did not result in noticeable clinical toxicity nor maternal death. However, significant decreases in maternal weight gain and food consumption during the dosing period were found at the highest dose level used (25 mg/kg/day). The net weight gain (absolute weight gain minus gravid uterine weight) was also affected at this dosage level suggesting a compound-related effect to the mothers.

Dosing up to and including a dosage level of 25 mg/kg/day, however, did not interfere with the reproductive performance of the treated animals. No differences relative to the pregnancy index, mean corpora lutea, mean implantation sites, mean resorptions, and mean viable fetuses were detected among all groups.

Based upon these findings, the maternal NOEL was established to be 5 mg/kg/day.

The investigators indicated that 2/3 of the fetuses in each litter were dissected and examined for visceral malformations. The carcasses were then stained for skeletal evaluation. The remaining 1/3 of the fetuses were fixed in Bouin's solution with the head examined by transverse sections and the fixed trunk was evaluated by dissection and preserved with the head sections. Consequently, 83, 83, 86, and 105 heads of the 0, 1, 5, and 25 mg/kg groups, respectively, were sectioned. Enlarged brain lateral ventricles is a finding that can only be observed by sectioning of the head. The authors reported one control animal with this finding and indicated a 0.4% incidence. This percent (0.4%) is misleading since it was calculated using the total number of fetuses into consideration (1/270 control fetuses = 0.4%). It is this reviewer's opinion that this incidence should be properly reported as 1/83 control heads examined = 1.2%. Likewise, the incidences given by the authors relative to lens opacity were also erroneously reported. The incidences reported by the investigators excluded fetuses that were fixed in Bouin's solution. Lens opacity should have been examined on all fetuses.

Bilateral and unilateral lens opacities were reported at a high incidence in both control and treated groups. However, historical control data for this finding were not available. Further, classification of lens opacity as a variation by the investigators was inconsistent due to the following reasons:

a. Lens opacities were classified as malformations in the rabbit study conducted by the same testing facility (Hazleton Europe #3687-14/30)

b. The positive control data with Acetylsalicylic acid in rats submitted with this final report indicated lens opacities as malformations (page C-142).

The registrant is requested to submit and/or provide explanation relative to the following issues prior to our completion of the study evaluation:

i. Disparity relative to the classification of lens opacity.
ii. Detailed description of lens opacities and grading
iii. Historical control data for lens opacities
iv. Distinction between lens opacity and cataract
v. Individual litter data for malformations and variations to fully assess the developmental toxicity NOEL (embryo/fetal toxicity and teratogenic potential)
vi. Impact of Sendai virus vaccination on rat pregnancy

Methyl isothiocyanate toxicology review

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Pages 9 through 24 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

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STUDY REVIEW

Chemical: Methyl Isothiocyanate - SN 32866
Test Material: Technical 94.9%

STUDY IDENTIFICATION:
"Methyl Isothiocyanate (MITC) oral (gavage) teratology study in the New Zealand Rabbit".

Testing Facility: Hazleton Lab. Europe
North Yorkshire, England
Final Report No.: 3687-14/30
Report Date: 6/84
Study Director: L.F.H. Irvine
EPA Accession No. 257764

Study Reviewed by: Quang Q. Bui, Ph.D.
Section V, Toxicology Branch
Hazard Evaluation Division

Approved by: Laurence D. Chitlik, D.A.B.T.
Section Head, Section V
Toxicology Branch/HED

CONCLUSIONS

Apparently, a maximum maternal tolerated dose was not used in this study. The slight decreases in maternal weight gain and food consumption noted at the 5 mg/kg/day dosage level (highest dose tested) were not statistically different from control values. The clinical signs attributed as treatment-related effects by the investigators (peri-anal staining and nasal discharge) were non-specific and did not follow a dose-response relationship. Further, the adjusted maternal weight gains during gestation (maternal weight gain minus gravid uterine weight) were comparable among all groups suggesting that administration of MITC at the dose levels selected did not induce any noticeable maternal systemic effects. The maternal NOEL was determined to be > 5 mg/kg/day (highest dose tested). If submitted, information pertaining to the dose-range finding study (3377-14/29) may provide some support relative to the selection of 5 mg/kg/day level as the highest dose tested.

The statistically significant decreases in fetal weight and length observed at the 5 mg/kg dosage level were considered by this reviewer as of questionable biological significance. The large mean litter size observed in the 5 mg/kg group may account for the decreased fetal weight and length noted at this dose level as compared to those of the concurrent controls. Further, the fetal length and weight of the 5 mg/kg group were greater than those of the historical control data provided (see page 6 of this review). Therefore, the purported fetal effects noted in the 5 mg/kg/group were not considered MITC-related toxic effects by this reviewer.

Slight increases in the incidences of malformations and variations were found

at the 5 mg/kg/day dosage level (highest dose tested). However, an accurate assessment of these potential developmental toxicity effects (including teratology) could not be completed in the absence of individual litter data and due to the questionable validity of the concurrent control data (see "Discussion" section)

RECOMMENDATION

It is recommended that this study be classified as Supplementary Data for the following reasons:

1. A maximum maternal tolerated dose apparently was not tested.

2. Poor reproductive performance of the control group. Indices of pre-implantation loss, post-implantation loss, implantation efficiency, and mean viable fetuses in the concurrent control group were not biologically comparable to those of the historical control data provided.

3. Significant differences in external and skeletal findings were noted between the concurrent and historical control data (see "Discussion" section)

4. Problems in dosing preparation: Re-formulation was necessary on two occassions due to unusual variation in test material concentration (unacceptable low analytical concentrations) in the original formulation. However, all groups were dosed with the original formulation for at least one day.

5. Individual litter data was not provided. Hence, the litter incidence for each finding could not be calculated and precludes an accurate assessment of developmental toxicity.

6. Fetuses with multiple defects may be reported more than once in the final report tabulation. In the absence of individual litter incidence, distinction of these findings and re-tabulation of the data were not permissible.

7. The appended historical control data did not provide litter incidence for each finding.

8. Group mean body weight was calculated using data from animals with live fetuses in utero and not based upon all pregnant animals.

9. The method used for visceral examination was not described nor referenced.

10. The method described for brain examination (slicing through the line of the fronto-parietal suture) would not permit an adequate examination of all brain structures to be performed.

11. Pups were euthanized by an intracardiac injection of Euthatal. This approach may produce perforation and anatomical distortion of the cardiac structures (depending on the volume used) and, hence, may affect the assessment of all cardiovascular and pulmonary findings.

PROCEDURES

1. Test Material:	Yellowish solidified melt, Methyl-isothiocyanate 94.9% a.i., batch # R27/S 6350 Expiration Date: 9/30/83
2. Dose levels:	0 (corn oil), 1, 3, and 5 mg/kg/day
3. Dosing period:	days 7-19 of gestation (inclusive) July 10-22, 1983
4. Species:	New Zealand Rabbits (Essex, England)

A copy of the procedures used is appended. In general, the procedures are not exceptionally different from the 1982 FIFRA Guidelines except for the following comments:

a. Age and weight ranges of the bucks used for natural mating were not indicated.
b. The method used for visceral examination was not described nor referenced.
c. 24-hour old pups were sacrificed by an intracardiac injection of Euthatal (unknown volume) prior to visceral examination. This approach may produce perforation and anatomical distortion of the cardiac structures (depending on the volume used) and, hence, may affect the assessment of all cardiovascular and pulmonary findings.
d. The head was examined by slicing "through the line of the fronto-parietal suture and the brain was examined for visible abnormalities". The slicing technique described was vague and apparently was not appropriate to examine all possible intracranial structures. The registrant is requested to provide a detailed description of the slicing technique used.
e. Fetal survival data was recorded during a 24-hour incubation period following removal of the fetuses from the mothers.
f. Group mean body weight was calculated using data from animals with live fetuses in-utero and not based upon all pregnant animals including those with complete resorptions.

RESULTS

1. Test Material

The test material was prepared on 3 occasions (July 4, 11, and 18 of 1983). The dosing solutions for the 3 mg/kg group prepared on 7/4/83 were discarded and re-formulated on 7/5/83 due to unusual variation in test material concentration (69% of nominal concentration). All dosing solutions prepared on 7/18 were discarded (unacceptable low analytical concentrations). Re-formulation of these solutions was conducted on 7/19/83. However, all treated groups were dosed with the original formulations for at least 1 day (7/18/83). Analytical determinations of the repeat formulations (7/19) indicated the MITC concentration was within acceptable limits (>90% of nominal concentration) except for the 3 mg/kg group formulation which was relatively low (86-88% of theoretical concentration).

The investigators suggested that the low analytical results obtained from the solutions prepared on 7/18 (50, 82, and 86% of nominal concentrations were determined from the 1, 3, and 5 mg/kg dosing solutions, respectively) were due to "some uneven decomposition". A direct assay of the original batch used indicated a purity of 86% of nominal concentration.

It should be noted that the batch used in this study was produced on 8/27/81 with an expiration date of 9/30/83 (Appendix 13) and was given to the test animals from 7/10 to 7/22/83. Using of the test material at a date so close to the expiration date may account for the "uneven decomposition" observed.

2. Mortality and Clinical Observations

Animal # 7213 of the control group died prior to the dosing period and was replaced. No other deaths were noted during the course of the study.

Nasal exudate was observed in 0/16, 0/16, 3/16, and 3/16 and peri-anal staining was found in 2/16, 1/16, 2/16, and 3/16 animals of the 0, 1, 3, and 5 mg/kg groups, respectively. The investigators indicated that the higher incidence of animals with clinical signs observed at the 3 and 5 mg/kg dosage levels was possibly treatment-related. This reviewer disagrees with their opinion since peri-anal staining and nasal discharge were common in rabbits and did not increase in a dose-response relationship in this study.

3. Maternal Body Weight Data

The group mean body weight gain data is presented as follows:

	Body Weight Gain (kg)			
	Control	1 mg/kg	3 mg/kg	5 mg/kg
Days 0-6	0.14	0.14	0.17	0.14
Days 7-19	0.26	0.21	0.21	0.16
Days 20-29	0.16	0.26	0.26	0.28
Weight Gain (days 0-29)	0.56	0.61	0.64	0.58
Uterine weight	0.39	0.46	0.44	0.46
Adjusted Weight Gain°	0.17	0.15	0.20	0.12

(°) Weight gain - gravid uterine weight

Prior to the dosing period (days 0-6), no differences in weight gains were noted among the groups. During the dosing period (days 7-19), a slight decrease but of questionable biological importance in maternal weight gain was noted at all dosage levels tested. After cessation of dosing (days 20-29), all treated groups apparently gained more weight than controls, however, no statistical differences were detected.

The investigators stated that although the differences observed in the 5 mg/kg group were not statistically different from controls, it still should be considered as compound-related effects. This reviewer disagrees with their opinion due to the well recognized erratic nature and, hence, limited utility of rabbit weight gain and to the lack of statistical differences in adjusted weight gains (body weight gain minus gravid uterine weight) found among the control and treated groups.

4. Food Consumption

During the dosing period (days 7-19), the mean food consumption of the 5 mg/kg group was lower than control mean (119 g/animal/day vs. 140 g/animal/day)

but did not attain statistical significance. When the food consumption was calculated for the whole gestation period, no differences were found. The mean values of food consumption for the 0, 1, 3, and 5 mg/kg groups during the entire gestation period were respectively 140, 151, 147, and 144 g/animal/day.

5. Reproductive Data

	Control	1 mg/kg	3 mg/kg	5 mg/kg
# animals mated	16	16	16	16
# dams pregnant	14	15	16	16
Fertility Index (%)	88	94	100	100
# dams aborted	0	0	1	0
# dams with complete resorptions	2	0	1	0
# dams with live fetuses	12	15	14	16
$\bar{X}$ corpora lutea/dam	8.2	9.1	8.3	9.1
$\bar{X}$ implantations/dam	6.9	8.7	7.9	8.8
Pre-implantation loss (%)	16.5	3.7	4.8	3.4
$\bar{X}$ resorptions/dam	1.2	0.5	0.8	0.3
$\bar{X}$ fetuses/dam	5.6	8.3	7.1	8.5
Post-implantation loss (%)	17.7	5.3	10.9	3.5
Implantation Efficiency(%)	82.3	94.7	89.1	96.5
$\bar{X}$ fetal wt/dam[a] (g)	44.9	41.7	41.5	39.3*
$\bar{X}$ litter weight (g)	275.4[a]	344.1	308.6	330.7
$\bar{X}$ crown rump length (mm)	107.6	105.2	105.0	103.6*

(*) Significantly different from controls, $P < 0.05$
(a) dams with live fetuses only

No compound-related effects were noted relative to the fertility index, the number of dams which aborted as well as for the number of dams with complete resorptions. It is interesting to note that all treated groups had a lower pre-implantation loss and a higher implantation efficiency than controls. A comparison of this study control data and the historical control data is illustrated as follows:

	Concurrent Control	Historical Control
Pre-implantation loss	16.5%	9.9%
Post-implantation loss	17.7%	10.1%
Implantation Efficiency	82.3%	89.9%
$\bar{X}$ viable fetuses/dam	5.6	8.2
$\bar{X}$ fetal weight (g)	44.9	36.1
$\bar{X}$ crown rump length (mm)	107.6	97.2

These data suggest that the performance of the concurrent control animals was less than desirable. Higher incidences of pre- and post-implantation loss were noted in the concurrent control group resulting in a lower implantation efficiency as compared to historical control data. Further, the reduced mean viable fetuses/dam of the concurrent control as compared with the historical control may well account for the increased fetal weight and length noted in the concurrent control group. Consequently, the statistically significant differences observed in the 5 mg/kg group with respect to fetal weight and crown-rump length as compared to concurrent control values could not be regarded as of biological significance since the large litter size in this group may account for the smaller fetal weight and length and, further, these fetal values are biologically better than those of the historical control data provided.

6. Fetal Observations

In this investigation, fetal observation data was separated into major malformations, minor malformations and variants. From a regulatory standpoint, findings are considered as either variations or malformations and will be discussed as such in this review. It should be noted that in the absence of individual litter data, this reviewer is unable to calculate the litter incidence for each finding as well as to distinguish fetuses with multiple malformations.

a. Malformations

	Control	1 mg/kg	3 mg/kg	5 mg/kg
Total # fetuses examined	79	123	106	136
External/Visceral				
Cleft palate	0	0	1(0.9)°	0
Internal hydrocephaly	0	0	0	1(0.7)
Persistent truncus arteriosus	1(1.3)	0	2(1.9)	0
Pulmonary valvular atresia	0	0	0	1(0.7)
Stenosis of descending aorta	0	0	0	1(0.7)
Bilateral hydronephrosis	1(1.3)	0	0	0
Talipes, both hindlimbs	0	1(0.8)	0	0
Bilateral lens opacity	29(36.7)	23(18.7)	19(17.9)	59(43.4)
Unilateral lens opacity	0	0	2(1.9)	0
Persistent ductus arteriosus	0	0	0	5(3.7)
Abnormal common carotid	2(2.5)	1(0.8)	3(2.8)	6(4.4)
Atrium/atria reduced	0	2(1.6)	1(0.9)	1(0.7)
Skeletal				
Cleft palate	0	0	1(0.9)	0
Frontals, fused	0	0	1(0.9)	0
Frontals, Incomplete ossified	15(19.0)	11(8.9)	25(23.6)	25(18.4)
Cleft in frontals	32(40.5)	55(44.7)	39(36.8)	40(29.4)
Sternebrae, fused	0	0	1(0.9)	5(3.7)

(°) Percent of fetuses affected

Scattered incidences of cleft palate, hydrocephaly, persistent truncus arteriosus, pulmonary valvular atresia, bilateral hydronephrosis, and talipes were noted in both control and treated fetuses. Slight increases in the number of fetuses with bilateral lens opacity, persistent ductus arteriosus, abnormal common carotid, and fusion of sternebrae were observed at the 5 mg/kg dosage level.

It is interesting to note that bilateral lens opacity was found in 29 control fetuses (36.7%) as compared to 39/1986 (1.95%) fetuses of the historical control data. Similarly, the incidences of frontals incompletely ossified and cleft in frontals in the concurrent control group were statistically different from those of the historical control. Percent fetal frequencies of 19.0 and 40.5 were noted in the concurrent control for frontals incompletely ossified and cleft in frontals, respectively, as compared to 1.6% and 1.5% of the historical control data. These unexpectedly high values in the concurrent control group negate any possible statistical significance that may be associated with findings in the treatment groups. They also raise serious questions as to the utility of the control group data as well as on the general conduct of this investigation.

b. Variants

Extra ribs were slightly increased in the treated groups being 27(34.2%), 48(39.0%), 40(37.7%) and 70(51.5%) for the 0, 1, 3, and 5 mg/kg groups, respectively. No other significant increases in variants were found. The authors reported that 51 (64.6%), 86 (69.9%), 70 (66.0%), and 93 (68.4%) fetuses of the 0, 1, 3, and 5 mg/kg groups had variants. The litter incidence for each variant could not be determined in the absence of individual litter data.

7. Survival Data

In this investigation, survivability of the fetuses were recorded for a 24-hour period. The 24-hour survival index for the 0, 1, 3, and 5 mg/kg group was 93.7%, 91.5%, 87.6%, and 89.0%, respectively. Administration of MITC did not adversely affect fetal survivability at 24-hours post cesarean delivery.

DISCUSSION AND CONCLUSIONS

The authors stated that compound-related effects were apparent in the dams at 5 mg/kg/day as evidenced by decreased body weight gain and food consumption as well as by increases in the number of dams with clinical observations. This reviewer disagrees with their conclusions since:

1. It is well recognized that maternal weight gain in rabbits is erratic in nature and, hence, limited the sensitivity of this parameter. Further, statistical differences in maternal weight gains and food consumption during the dosing period as well as throughout gestation were not detected between the control and treated groups.

2. The adjusted body weight gain (weight gain minus gravid uterine weight) was identical among all groups suggesting that the dams were not affected by the administration of MITC.

3. The clinical signs of peri-anal staining and nasal discharge are common in rabbits, non-specific, and did not increase in a dose-response relationship.

Therefore, it is this reviewer's opinion that a maximum maternal tolerated dose was not used in this study. Information pertaining to the dose-range finding study (3377-14/29) may provide some usefulness relative to the selection of 5 mg/kg/day as the highest dose tested.

With respect to developmental toxicity, the authors implied that the 5 mg/kg dosage level was associated with significant decreases in fetal weight and length as well as increases in malformations and variants. It is this reviewer's opinion that a developmental toxicity NOEL including teratogenic potential could not be properly assessed for the following reasons:

1. Findings in the concurrent control group were in many cases significantly different from those of the historical control data and, hence, restricted the validity of the concurrent control data. A comparison of some selected findings is tabulated:

	Control	Historical Control
Fetal weight (grams)	44.9	36.1
Fetal length (mm)	107.6	97.2
Pre-implantation loss (%)	16.5	9.9
Bilateral lens opacity (%)	36.7	1.95
Frontal bones, incompletely ossified (%)	19.0	1.6
Frontal bones, cleft (%)	40.5	1.5

The registrant is requested to provide explanation relative to:

a. Unexpected high incidences of lens opacity and frontal bones incompletely ossified and cleft in the concurrent control group.
b. Distinction between frontal bones incomplete ossified and cleft.
c. Detailed description of lens opacity

2. Although the decreases in fetal weight and length at the 5 mg/kg dose level were statistically different from controls, they should not be considered as indicative of toxicity. The 5 mg/kg group had a larger litter size (8.5 vs. 5.6 for control) which may account for the lower fetal weight and length observed. Further, the 5 mg/kg fetal weight and length were greater than those of the historical control data provided and thus could not be attributed to treatment with MITC.

3. Individual litter incidences were not provided. Hence, litter relationships for each malformation or variation could not be determined.

4. Historical control litter incidence data were not submitted.

5. Raw data of the dosing records were not reported.

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