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

004263

FEB 5 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of a rabbit teratology with Larvadex (Cyromazine)
EPA # 100-AGR
Accession No. 256348 Caswell No. 167B

TO: Mr. Adam Hayward, PM #17
Registration Division (TS-767)

FROM: Quang Q. Bui, Ph.D. Quang Q. Bui 2/4/85
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Laurence D. Chitlik, DABT
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and

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Registrant:
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RECOMMENDATION

The teratology study with Technical Larvadex in New Zealand Rabbits is presently classified as Core Minimum Data. However, the registrant is requested to provide clarification concerning several issues listed under the "Recommendation" section (page 3 of this memo).

Under the conditions of this study, the maternal NOEL is determined to be 10 mg/kg/day. Reductions in body weight gain and food consumption were noted at 30 mg/kg/day (Maternal LEL). A teratogenic NOEL is demonstrated at 5 mg/kg/day. Apparently, teratogenic responses were demonstrated at 10 mg/kg/day.

STUDY REVIEW

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CHEMICAL: Cyromazine - Larvadex
Tox. Chem. No. 167B

TEST MATERIAL: Technical 95.2%
Label identification FL 841538

STUDY/ACTION TYPE: Teratology - New Zealand Rabbits

STUDY IDENTIFICATION:

Title: A Teratology Study (Segment II) in Albino Rabbits with Cyromazine Technical.
Testing Facility: WIL Research Laboratories, Inc.
Ashland, Ohio 44805
Report No.: WIL 82001
Report Date: 1/23/85
Study Director: Mark D. Nemec
EPA #100-AGR
Accession No. 256348

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CONCLUSIONS

Under the conditions of this study, a maternal NOEL is determined at 10 mg/kg with body weight gain depression and food consumption reduction observed at the 30 mg/kg/day (LEL).

Several malformations were noted in the treated groups but not in the concurrent and historical control data. Cyclopia and diaphragmatic hernia were found in the 10 and 30 mg/kg groups and apparently were compound-related. The limited number of litters (7) and fetuses (45) in the 10 mg/kg group precluded an accurate teratogenic assessment at this dosage level. However, the incidence of anomalies in the 5 mg/kg group was low and comparable to that of the controls and, hence, this dosage level may be considered as the NOEL for fetotoxicity and teratogenic potential of Larvadex (see "Discussion and Conclusions" section on page 11).

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RECOMMENDATION

This study is classified as Core Minimum Data However, the registrant is requested to provide information concerning:

a. The low pregnancy rate in the 10 mg/kg group.

b. Variations in days 0 maternal body weight
Lowest weight recorded: dam #2726 = 2993 grams
Highest weight recorded: dam # 2628 = 5133 grams

c. The low mean values for implantation sites and viable fetuses in all treated groups including the controls

d. Cyclopia was found in two fetuses, one each in the 10 and 30 mg/kg groups. The investigators indicated that this malformation "was possibly of genetic origin as both fetuses were sired by the same male". However, the investigators ' statement could not be justified since semen from that same buck (# 2749) was also used to inseminate 14 females of the vehicle and untreated groups with no similar malformation found.

e. Significant decreases in the number of female fetuses were observed in the 5, 10, and 30 mg/kg groups. The registrant is requested to provide fetal weight data separately for each sex.

f. Historical control data were provided. However, the vehicle used and the method of impregnantation for each study were not mentioned.

BACKGROUND

The teratogenic potential of Technical Cyromazine (Larvadex) in rabbits has been investigated in two studies (IRDC # 382-072 and 382-072a). Both studies were submitted to the Agency by the registrant and were classified as Supplementary Data (see memo of 7/11/84). The Agency has indicated that to adequately assess the teratogenic potential of Technical Cyromazine, a new teratology study in rabbits is requested.

In this action, the registrant has submitted a new teratology study with Technical Cyromazine in New Zealand rabbits. It is noteworthy to indicate that the two previous teratology studies (IRDC #382-072 and 382-072a) were investigated with Dutch Belted rabbits.

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Materials

Cyromazine Technical, 95.2%
Yellow cristalline powder
Lot #FL 841538

Vehicle: 0.5% aqueous Carboxymethylcellulose (Sodium salt)
Sigma Chemical Co. Lot # 123F-0532

Dosage levels: Vehicle, 0, 5, 10, 30, and 60 mg/kg/day

Methods

This study was designed to investigate the teratogenic and embryotoxic effects of Technical Larvadex in New Zealand rabbits (Buckshire Corporation, Pennsylvania) and was conducted in compliance with the EPA Proposed Guidelines of 1978 and the FIFRA Guidelines of 1982.

A copy of the procedures used is appended. However, the following comments are noted:

a. Artificially inseminated females were used in this study. The insemination procedure was adequately described by the investigators.

b. Historical control data were provided and consisted of 23 teratology studies conducted with New Zealand rabbits supplied by 3 different commercial sources:

i. Dutchland: 7 studies (from 7/80 to 4/84)
ii. Langshaw: 14 studies (from 10/81 to 7/83)
iii. Buckshire: 2 studies (from 6/83 to 11/83)
(Note: this study was conducted with rabbits from Buckshire)

Each of the above historical control study was properly identified with the study date, fetal and litter data, fetal and litter incidence for each malformation and variation, maternal reproductive data, and source of animals. However, it is unclear as to what vehicle was used in each study. The method of impregnantation for each study was not indicated.

d. Crown-rump length was measured for non-viable fetuses only.

e. Two groups of control were used: vehicle and untreated control. The vehicle group received 1 ml/kg/day of 0.5% aqueous carboxymethyl cellulose from days 7-19 of gestation.

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RESULTS

1. Clinical Observation Data

Three animals died in this study, one each in the vehicle control (# 2714), 30 mg/kg (# 2721), and 60 mg/kg (#2710) groups. The investigators listed the cause of death for the two treated animals (# 2721 and 2710) as "intubation errors".

Decreased urination and fecal output were noted in the 30 and 60 mg/kg groups and apparently were compound-related. Decreased body weight gain and food consumptions were also associated with these two dosage levels.

2. Maternal Loss

During this investigation, 4 animals aborted. One each in the 0 (#2699) and 30 mg/kg (#2672) groups and 2 in the 60 mg/kg (# 2640 and 2730) group.

The incidences of maternal loss (death, abortion, and non-gravid) are summarized as follows:

Table 1: Incidences of Maternal Loss

	Vehicle	0 mg/kg	5 mg/kg	10 mg/kg	30 mg/kg	60 mg/kg
# females	18	18	18	18	18	18
# dead	1(a)	0	0	0	1	1(a)
# aborted	0	1	0	0	1	2
# non-gravid	4	3	5	11	3	4
# maternal loss	5	4	5	11	5	7
(%)	27.7	22.2	27.7	61.1	27.7	38.9
# gravid	14	15	13	7	14	14
Pregnancy rate (b)	77.8	83.3	72.2	38.9	77.8	77.8

(a) female pregnant and died
(b) ratio of # pregnant/ # mated

An increase in the number of non-gravid females was observed in the 10 mg/kg group resulting in reduction of the pregnancy rate at this dosage level. The biological significance of this finding is unclear since the pregnancy rates of the other treated groups were statistically and biologically comparable to those of the vehicle and untreated control groups.

3. Maternal Body Weight Data

Maternal body weights and body weight gains were recorded at different intervals througout the investigation Non-gravid females were excluded from the data analyses.

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Table 2: Maternal Body Weight Data (grams)

	Vehicle	0 mg/kg	5 mg/kg	10 mg/kg	30 mg/kg†	60 mg/kg
Mean Body Weight						
Days 0	3636	3700	3800	3529	3613	3836
Days 7	3809	3803	3899	3640	3755	3934
Days 20	4038	3975	4116	3885	3914	3788
Days 29	4052	4077	4230	3961	4029	4174
Body Weight Changes						
Days 0-7	178	103	99	110	142	98
Days 7-20	229	173	216	246	159	-113*
Days 20-29	10	106	114	76	99	306*
Days 0-29	419	405	430	432	395	361
Uterine wt.	280	260	322	391	346	330
Net body wt. change (a)	139	145	108	41	49	32

* : Significantly different from controls, $P < 0.01$
† : Excluding animal # 2629
(a): Body weight gain (days 0-29) minus uterine weight

Prior to treatment (days 0-7), the body weight gains of the treated groups were similar to controls. However, during the dosing period (days 7-20), reductions in body weight gain were observed in the 30 and 60 mg/kg levels with statistical significances ($P<0.01$) found at the 60 mg/kg. In the 30 mg/kg group, female #2629 was not used in data analysis since this animal had an initial body weight of 5.1 kg which was well beyond the mean and SD for this group (3.7 +/- 0.54 kg) After the treatment period (days 20-29), compensated increases in body weight gain were found in the 60 mg/kg group.

Increased mean uterine weights were noted in the treated groups as compared to the controls. However, this apparent increase may be associated with a larger litter size found in the treated groups.

Decreases in net body weight change (body weight gain from days 0-29 minus uterine weight) were observed in the 10, 30, and 60 mg/kg groups.

4. Food Consumption Data

The mean food consumption is tabulated as follows:

Table 3: Food Consumption (grams/animal/day)

	Vehicle	0 mg/kg	5 mg/kg	10 mg/kg	30 mg/kg†	60 mg/kg
Days 0-7	163	159	154	146	166	158
Days 7-20	165	162	152	156	139	91**
Days 20-29	102	144	120	115	120	169**
Days 0-29	144	156	143	141	140	136

**: Significantly different from controls, $P < 0.01$
†: Excluding female #2629

During the entire investigation (days 0-29), no apparent changes in food consumption were noted among the test groups. However, during the dosing period (days 7-20), decreases in mean food consumption were found in the 30 and 60 mg/kg groups with significant differences found at the 60 mg/kg level.

Significant increases in food consumed were noted in the 60 mg/kg after the treatment period (days 20-29) and reflected a compensatory effect after withdrawal of the test chemical administration.

5. Reproduction Parameters

The following table represents the reproduction data of animals sacrificed on day 29 of gestation.

Table 4: Reproductive Data

	Vehicle	0 mg/kg	5 mg/kg	10 mg/kg	30 mg/kg	60 mg/kg
# sacrificed	17	17	18	18	16	15
# non-gravid	4	3	5	11	3	4
# dams with resorptions only	1	1	1	0	3	0
# dams with viable fetuses	12	13	12	7	10	11
Mean corpora lutea	8.5	8.3	9.9	11.0	8.6	9.5
Mean implantation sites	5.4	4.6	5.6	7.3	6.7	7.3
Mean Preimplantation loss (a)	3.2	3.6	4.3	3.7	1.9	2.2
Mean viable fetuses	4.5	3.8	4.8	6.4	5.5	4.9
Mean dead fetuses	0.0	0.0	0.0	0.0	0.0	0.0
Mean Postimplantation loss	0.9	0.9	0.8	0.9	1.2	2.4

(a): Calculated by this reviewer

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Three dams with only resorptions were found at the 30 mg/kg level and one each in the vehicle, 0, and 5 mg/kg groups. The number of corpora lutea was similar between the treated and control groups except for 10 mg/kg group which had a slightly higher number of corpora lutea.

In this study, all groups including the controls had lower means implantation sites than both sets of historical control data provided.

Mean implantation sites, historical control data, all suppliers:	8.2
Mean implantation sites, historical control data, Buckshire only:	8.1

Similarly, the mean viable fetuses per dam in this study for both control and treated animals (3.8 - 6.4) was relatively low when compared to either the historical data collected from all suppliers (mean = 7.2) or from Buckshire (mean = 7.3).

No explanations were given by the investigators.

No compound-related changes in preimplantation loss were found. However, the incidences of postimplantation loss were slightly increased in the two highest dosage levels and exceeding the historical control data ranges.

6. Fetal Data

	Vehicle	0 mg/kg	5 mg/kg	10 mg/kg	30 mg/kg	60 mg/kg
# viable fet.	58	53	62	45	71	54
# of live fetuses/dam	4.5	3.8	4.8	6.4	5.5	4.9
# males/dam	1.7	2.0	3.2	4.0	3.5	2.5
# females/dam	2.8	1.8	1.6*	2.4*	1.9*	2.5
Fetal wt (g)†	44.3	47.8	46.7	41.5	42.6	45.7

*: Significantly different from controls, $P < 0.05$
†: male and female fetuses combined

Significant decreases in the number of female fetuses/dam were noted in the 5, 10, and 30 mg/kg groups. No explanations were given by the investigators.

No significant changes in fetal weight were found between the the treated and control groups. Slight decreases in fetal weight were noted in the 10 and 30 mg/kg groups but apparently resulted from a larger litter size associated with these two dosage levels. Since significant decreases in female fetuses were found, the registrant is requested to provide fetal weight separately for each sex.

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7. Fetal Malformations

a. External Malformations

	Vehicle	0 mg/kg	5 mg/kg	10 mg/kg	30 mg/kg	60 mg/kg
# fetuses examined (litters)	58(12)	53(13)	62(12)	45(7)	71(10)	54(11)
Open eyelid	0	0	0	0	0	4(1)
Micrognathia	0	0	0	0	1(1)	0
Umbilical hernia	0	0	0	0	°1(1)	1(1)
Cyclopia	0	0	0	1(1)	°1(1)	0
Spina bifida	0	0	0	0	°1(1)	0
Skull anomaly	0	0	0	0	0	1(1)
Short tail	0	0	0	0	0	3(1)
Absent tail	0	0	1(1)	0	0	0
Bent tail	0	0	0	0	0	1(1)
Total	0(0)	0(0)	1(1)	1(1)	2(2)	10(5)*
Percentage	0(0)	0(0)	1.6(8.3)	2.2(14.3)	2.8(20.0)	27.8(54.5)

°: malformations found in same fetus (# 6 of dam 2678)
*: Significantly different from controls, P <0.05

No external malformations were found in either control groups. "Open eyelid", "short tail", and "skull anomaly" (small nostrils and cleft palate) occurred only in the 60 mg/kg group with significant increases in the number of litters with external malformations found at this dosage level. Several findings were noted at the 30 mg/kg level but most of them were found in the same fetus. Cyclopia was found in 2 fetuses, one each in the 10 and 30 mg/kg groups.

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b. Visceral Malformations

	Vehicle	0 mg/kg	5 mg/kg	10 mg/kg	30 mg/kg	60 mg/kg
# fetuses examined (litters)	58(12)	53(13)	62(12)	45(7)	71(10)	54(11)
Hydrocephaly	0	0	0	1(1)	2(2)	1(1)
Diaphragmatic hernia	0	0	0	1(1)	3(2)	0
Coarctation of left carotid	0	0	1(1)	0	0	0
Kidney/ureter absent	1(1)	0	0	0	0	0
Kidney/ureter anomaly	0	0	0	0	1(1)	0
Other	0	0	0	0	0	2(2)
Total	1(1)	0	1(1)	2(2)	6(4)	3(3)
Percentage	1.7(8.3)	0	1.6(8.3)	4.4(28.6)	8.5(40.0)	5.6(27.3)

Hydrocephaly was noted in the three highest dose groups. The percentages of fetuses (litters) with hydrocephaly were 2.2 (14.3), 2.8 (20.0), and 1.8 (9.1) for the groups receiving 10, 30, and 60 mg/kg, respectively. The incidences of hydrocephaly provided by the historical control data were as follows:

	Fetuses	Litters
Historical, all suppliers	2/1926= 0.10%	2/330= 0.61%
Historical, Buckshire only	1/218= 0.46%	1/42= 2.38%

The percentages of fetuses and litters with hydrocephaly for all three groups of this study were higher than those provided by either sets of historical control data.

Diaphragmatic hernia, a rare visceral malformation, was noted in 1(1) and 3(2) fetuses (litters) of the 10 and 30 mg/kg groups, respectively. This finding was absent in both sets of historical control data provided.

Although the incidences of hydrocephaly and diaphragmatic hernia in this study were not dose-related, their findings at a higher frequency than historical control data suggested that these malformations may possibly be compound-related. Furthermore, with respects to visceral malformations, the percentages of fetuses and litters with malformations in the 10, 30, and 60 mg/kg groups were all higher than those of the control groups.

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c. Skeletal Malformations

	Vehicle	0 mg/kg	5 mg/kg	10 mg/kg	30 mg/kg	60 mg/kg
# fetuses examined (litters)	58(12)	53(13)	62(12)	45(7)	71(10)	54(11)
Rib anomaly	1(1)	0	1(1)	0	1(1)	2(2)
Verte.anomaly(a)	4(4)	1(1)	3(1)	1(1)	3(3)	7(4)
Severe verteb. malaligned	0	1(1)	0	1(1)	0	0
Fused nasal bones	1(1)	0	0	0	0	0
Skull anomaly	0	0	0	0	1(1)	0
Fused sterneb.	0	0	0	0	0	2(1)
Total	6(6)	2(2)	4(2)	2(1)	5(5)	11(6)
Percentage	10.3 (50.0)	3.8 (15.4)	6.5 (16.7)	4.4 (14.3)	7.0 (50.0)	20.4 (54.5)

(a) vertebral anomaly with or without associated rib anomaly

Skeletal malformations occurred in all test groups at a similar frequency and severity. No apparent dose-related increases were found for each individual finding. The number of fetuses with skeletal malformations was slightly increased in the 60 mg/kg group (20.4% compared with 10.3% of the vehicle group).

8. Fetal Variations

Visceral and skeletal variations were found at comparable frequency between the treated and control groups. All findings were adequately graded by the investigators. No discrepancies were noted by this reviewer when the final report was compared with individual litter data.

Findings of interest are summarized in the following table.

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	Vehicle	0 mg/kg	5 mg/kg	10 mg/kg	30 mg/kg	60 mg/kg
# fetuses examined (litters)	58(12)	53(13)	62(12)	45(7)	71(10)	54(11)
Accessory skull bones	1(1)	0	1(1)	1(1)	1(1)	4(3)
Skull, reduced ossification	0	0	0	0	0	2(1)
Hyoid body/arch unossified	0	0	1(1)	2(1)	0	2(1)
13th rudimentary ribs	2(2)	4(3)	5(3)	3(2)	7(4)	10(7)*
14th full ribs	8(4)	12(4)	10(7)	8(6)	7(6)	13(8)
27 presacral vert.	1(1)	5(3)	6(4)	0	2(2)	6(4)
Extra ribs †	10(5)	16(6)	15(7)	11(6)	14(7)	23(8)
% fetuses with extra ribs †	17.2	30.2	24.2	24.4	19.7	42.6
% litters with extra ribs †	41.7	46.2	58.3	85.6	70.0	72.7

*: Significantly different from controls, $P < 0.05$
†: sum of rudimentary and full ribs, calculated by this reviewer

The "accessory skull bones" involved the bones located in either the parietal or nasal sutures. Significant increases in "13th rudimentary ribs" were noted in the 60 mg/kg group. When the incidences of "extra ribs" were calculated by this reviewer, increases in the percentages of litters with extra ribs were found at the 10, 30, and 60 mg/kg levels.

DISCUSSION AND CONCLUSIONS

Background information regarding the historical control pregnancy rate of artificially inseminated rabbits performed at this testing facility was not indicated in this report. Although the provided historical control data reported the pregnancy rate of animals in those studies, it is unclear to this reviewer as to whether all animals in the historical control data were artificially inseminated or naturally mated.

A conception rate of 80% and greater in rabbits is usually obtained by artificial insemination (Adams, 1961; Gibson et al., 1966; Woo, 1984). The low pregnancy rate noted in the 10 mg/kg group (38.9%) restricts the extend of information that could have been obtained from this group. Only 7 litters with viable fetuses were obtained from this group with a limited number of fetuses (45).

Dosing of pregnant rabbits with 5 and 10 mg/kg/day of Technical Larvadex did not produce toxic symptoms or any significant changes in food consumption, body weight, and reproductive status.

The maternal NOEL appears to be 10 mg/kg/day with depression of body weight gain and reduction in food consumption observed at 30 mg/kg/day (maternal LEL).

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Several malformations were noted in this study. Cyclopia and diaphragmatic hernia were found in the 10 and 30 mg/kg groups but not the 60 mg/kg groups. Hydrocephaly was noted in all treated groups except the 5 mg/kg group.

Cyclopia was found in 1(1) fetus (litter) each in the 10 and 30 mg/kg groups. This finding was not found in either sets of historical control data provided. The investigators indicated that this malformation "was possibly of genetic origin as both fetuses were sired by the same male". However, this statement cannot be justified since semen from that same buck (#2749) was also used to inseminate 14 rabbits of the control groups with no similar malformation found.

Diaphragmatic hernia was observed in 1(1) and 3(2) fetuses (litters) in the 10 and 30 mg/kg groups. Respective percentages of fetuses (litters) with diaphragmatic hernia were 2.2 (14.3) and 4.2 (20.0). Diaphragmatic hernia was not found in the historical control data.

The presence of diaphragmatic hernia and cyclopia should be of concern since the historical control data provided by the registrant listed a zero incidence of cyclopia and diaphragmatic hernia out of 1926 fetuses/330 litters examined.

Although a dose response increase for each malformation was not evident due to the absence of similar findings in the 60 mg/kg group, the incidences of these findings at the two mid doses cannot be neglected. The lack of similar findings in the 60 mg/kg group may be questionable due to abortion and the high incidence of postimplantation loss in this dosage group (mean of 2.4 vs. 0.9 of vehicle and untreated control) which may have masked these effects.

Diaphragmatic hernia and cyclopia are malformations which were not observed in both concurrent and historical control data. The findings of these malformations even within a restricted number of litters (7) and fetuses (45) examined in the 10 mg/kg group suggested that these malformations apparently were compound related. Their presence in the 10 mg/kg group cannot be attributed to random occurrence since both malformations were observed at the next highest dose (30 mg/kg).

The investigators mentioned that the incidences of "hydrocephaly" were comparable to the historical controls. However, calculations by this reviewer indicated that the fetal and litter incidences of hydrocephaly in all three treated groups (10, 30, and 60 mg/kg) exceeded both the concurrent and historical control values.

The malformations in the 5 mg/kg group were similar in frequency and severity to the controls.

Under the conditions of this study, a teratogenic NOEL is determined at 5 mg/kg/day. Evidence of a teratogenic effect is demonstrated by the findings of unusual malformations at the 10 mg/kg/day dosage level and above.

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CORE CLASSIFICATION

This study is presently classified as Core Minimum Data. However, the registrant is requested to provide clarification concerning the issues listed under the "Recommendation" section (page 3 of this memo).

REFERENCES

Adams, C.E., 1961. Artificial insemination. In: The semen of animals and artificial insemination, J.P. Maude ed., England

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Woo, D.C., 1984. Control data of teratological studies in New Zealand rabbits (A joint study by MARTA), in Principles in Teratology, Teratology Society.