SECTION HEAD 2

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

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MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Review of a breeding study in rabbits and a developmental toxicity study with Cyromazine in rabbits.
EPA No. 100-631
Tox. Proj. No. 1857 Caswell No. 167 B

TO: Timothy Gardner, PM #17
Registration Division (TS-769C)

FROM: Quang Q. Bui, Ph.D. Quang Q. Bui 11/17/86
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Laurence D. Chitlik, D.A.B.T. 11/18/86
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Toxicology Branch/HED (TS-769C)
and
Theodore M. Farber, Ph.D. 11/21/86
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Ciba Geigy Corporation
Greensboro, N.C. 27419

Action Requested:

Review a breeding study in the control population of BUK rabbits (WIL No. 82005) and a developmental toxicity study with Technical Cyromazine in rabbits (WIL No. 82008).

BACKGROUND INFORMATION

The developmental toxicity potential of Technical Cyromazine (Larvadex) was previously investigated in three rabbit studies (IRDC #382-072, IRDC #382-072a, and WIL #82001). The two IRDC studies, using the Dutch Belted rabbits, were classified as Supplementary Data (questionable suitability and/or health status of animals used; memo of Q. Bui to T. Gardner, 7/11/1984). The WIL study, using the New Zealand (BUK) rabbits, was classified as Core Minimum Data (memo of Q. Bui to A. Heyward, 2/5/1985) with a maternal NOEL determined at 10 mg/kg/day and the developmental toxicity NOEL at 5 mg/kg/day. However, several malformations (cyclopia, diaphragmatic hernia, hydrocephaly,...) noted in the WIL study were found in dams sired by the same buck #2749. To clarify the developmental toxicity potential of Larvadex as well as to investigate the possibility of a genetically-related effect, a proposal to study the spontaneous incidence of malformations in the control population of BUK rabbits was submitted by the registrant on June 4, 1985 and reviewed by the Agency on July 12, 1985 (memo of Q. Bui to A. Heyward).

In this action, the registrant submitted (1) a study on the incidence of fetal malformations in the control population of BUK rabbits and (2) a developmental toxicity study with a postnatal phase with Cyromazine in rabbits.

A data evaluation record (DER) for each of the two studies submitted along with a re-assessment of the scientific validity of study WIL #82001 was performed in this action.

RECOMMENDATION

1. It is recommended that the study of the incidence of fetal malformations in the control population of Buckshire New Zealand rabbits (WIL-82005) is classified as Acceptable Data. The study was initiated by the registrant to demonstrate that the malformations noted in study WIL #82001 were "genetically-related" to buck #2749. Based upon the submitted data, there is no conclusive association between malformations and "genetic defects" with buck #2749 (see study review).

2. It is recommended that study WIL #82008 be classified as Core Supplementary Data pending the submission of additional data to clarify the comments listed on pages 30 and 31 of this memorandum. Under the conditions of this study, a maternal NOEL may tentatively be established at 10 mg/kg with decreased maternal weight gains and food consumption noted at 30 mg/kg during the period of treatment (days 6-19 of gestation).

3. It should be noted that a new developmental toxicity study with Larvadex in rabbits was not required since the registrant has fulfilled the regulatory requirements for rabbit teratogenicity: Study WIL #82001 was classified as Core Minimum Data (memo of Q. Bui to A. Heyward, 2/5/1985) with a developmental toxicity NOEL of 5 mg/kg/day.

4. An overview and risk assessment of the developmental toxicity potential, as well as discussion of the regulatory requirements for teratogenicity testing with Technical Larvadex, are presented in a separate memorandum.

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DATA EVALUATION REPORT

Study Action Type: Developmental Toxicity

STUDY IDENTIFICATION:

"A study of the incidence of fetal malformations in the control population of BUK:(CRL)NZWfBR rabbits"

Testing Facility:	WIL Research Lab. Inc., Ashland, Ohio 44805
Report No.:	WIL-82005
Final Report Date:	2/9/86
Study Authors:	M. D. Nemec et al.,
EPA Accession No.	262638

Reviewed by:	Quang Q. Bui, PhD., DABT. Toxicologist Toxicology Branch/HED (TS-769C)
Review Approved by:	Laurence D. Chitlik, DABT. Section Head Toxicology Branch/HED (TS-769C)

CONCLUSIONS AND RECOMMENDATION

It is recommended that the study of the incidence of fetal malformations in the control population of Buckshire New Zealand rabbits is classified as Acceptable Data.

This study (WIL 82005) was initiated by the registrant to demonstrate that the malformations noted in study WIL #82001 were "genetically-related" to buck #2749. Based upon the submitted data, there is no conclusive association between malformations and "genetic defects" with buck #2749 for the following reasons:

1. Insemination of does with semen from buck #2749 (groups I and III) did not result in significant alterations in reproductive performance as characterized by similar pregnancy rate, fecundity, litter size, postimplantation loss, fetal weight, and fetal sex ratio as compared to does in the control group II.
2. No statistical and biological differences in sperm count, motility, and morphology were noted between buck #2749 and control bucks #2272 and 1195.
3. Although several malformations (acrania, gastroschisis, anophthalmia, thoracogastroschisis, cleft palate, and malpositioned kidneys) were noted only in litters sired by buck #2749, they occurred at a relatively low incidence and, hence, may well be of spontaneous origin.
4. The malformation rates in groups sired by buck #2749 (groups I and III) were not biologically higher than those of the concurrent control (group II) and historical control data.

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MATERIALS AND METHODS

This study was designed to investigate:

i. the possibility of genetic-related findings in dams sired by buck 2749.
ii. the rate of spontaneous malformations in this strain.
iii. the effects of stress on the spontaneous malformation rate within the colony.

In general, the study was designed to comply with the 1982 FIFRA Guidelines except for the omission of the test material. A quality assurance statement was appended with the final report. The following highlights and comments are noted:

1. Study Group Arrangement:

Groups	Treatment	# Buckshire New Zealand assigned	Sire
I	Control (untreated)	56	2749
II	Control-Control (untreated)	59	1195, 2272
III	Control-sham °	56	2749

(°) sham-gavaged daily from days 7-19 of gestation

2. Artificially inseminated females were used. The insemination procedure was adequately described by the authors.
3. Historical control data were provided for both Buckshire and Hazleton strains of New Zealand rabbits.
4. Semen evaluation and sperm morphology data were submitted.
5. Each fetus was examined viscerally by a modified Staples' technique to include the heart and major vessels as well as serial sectioning of the brain. The Dawson's technique was used for skeletal staining.

CLINICAL OBSERVATIONS

Clinical observations were performed daily for appearance, abnormal behavior, moribundity, and mortality.

Results: Sixteen (16) animals died during this investigation: 9, 5, and 2 in the groups I, II, and III, respectively. The authors indicated that an "apparent bacterial infection" was probably the cause of death for 13 dams, uterine hemorrhage for 1 dam, and cause unknown for 2 dams. Necropsy observations indicated "intestines, reddened and loss of epithelium" as the common finding in those dams affected with bacterial infection. One animal in group III (#3091) was sacrificed due to broken hind limb and one dam in group II (#1605) aborted on gestational day 27. Clinical signs of alopecia, soft stool, and ocular discharge were comparable among all groups.

BODY WEIGHTS

Individual body weights were recorded on days 0, 7, 10, 14, 20, 24, and 29 of gestation. The mean gravid uterine weights, body weight changes, and net

body weight changes were calculated. Non-gravid animals were excluded from data analysis.

Results:

Body Weight Gains (grams)

	Control I	Control-Control II	Control-Sham III
Days 0- 7	88	136	58
Days 7-20	128	106	44*
Days 20-29	33	43	116*
Days 0-29	221	295	223
Gravid uterine weight	312	311	272
Net body weight change†	- 95	- 12	- 54

(†) Net body weight change = Body weight gain - gravid uterine weight
(*) Significantly different from control group I at $p < 0.05$

A statistically significant decrease in body weight gains was found in the sham control III during gestational days 7-20 with a rebound increase noted after cessation of sham gavage (days 20-29). These findings suggested that gavage, by itself, may be stressful to the pregnant animals. Although the control-control group II apparently gained more weight during the entire gestational period (days 0-29) as compared to groups I and III, the difference was not considered as biologically significant in light of the erratic nature of rabbit body weight during pregnancy. No significant differences were found relative to net body weight changes.

FOOD CONSUMPTION

Individual food consumption was recorded daily and food intake was computed as g/animal/day and g/kg body weight/day. Data from non-gravid animals were excluded from calculation.

Results:

No biologically or statistically significant differences were found among all groups. Throughout the entire gestational period, dams from groups I, II, and III consumed an average of 31, 33, and 33 g/kg/day, respectively.

PREGNANCY RATE

Diluted semen from buck #2749 was used to inseminate females in groups I and III whereas semen from bucks #1195 and #2272 were used for females in group II. Following insemination, all does received an intravenous administration of human chorionic gonadotropin (100 USP Units) via the marginal ear vein to ensure ovulation. Semen was evaluated for sperm motility, morphology, and count.

Results

The pregnancy rates for the groups I, II, and III were 76.8, 81.4, and 80.4%, respectively.

Semen evaluation data are presented in the next table.

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Semen evaluation and sperm morphology

	Buck 2749	Buck 2272	Buck 1195
Semen Volume (ml)	0.2 - 1.0	0.2 - 1.1	0.7
Sperm motility (%)	55 - 75	65 - 80	65
Sperm count (millions/ml)	6.5 - 182	123	30
Normal sperm (%)	78 - 94	86 - 98	No data
Abnormal sperm (%)	6 - 22	2 - 14	No data

No significant differences relative to semen volume, sperm motility, and sperm count were noted among the three bucks. The percentage of abnormal sperm (criteria for abnormal sperm were not described in the final report) in buck #2749 (6-22%) apparently was not biologically different from buck #2272.

NECROPSY DATA

All does were sacrificed on day 29 of gestation and the uterine contents were examined by routine developmental toxicity procedures. The uterus was excised, examined, and weighed. Uteri with no visible implantation sites were placed in 10% ammonium sulfide for detection of early implantation loss. Necropsy was also performed on all animals which died during the investigation.

Results:

	Control I	Control-Control II	Sham-Control III
No. assigned	56	59	56
No. dead	9	5	3
No. aborted	0	1	0
No. sacrificed	47	53	53
No. non-gravid	11	8	10
No. gravid	36	45	43
No. complete resorption	3	6	6
No. with viable fetuses	33	39	37

The numbers of does with resorptions only were 3, 6, and 6 (representing 8, 13, and 14% of gravid animals) in the groups I, II, and III, respectively. These data indicated that no adverse effects on female fertility could be attributed to the semen of buck #2749.

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Reproductive Status at Laparotomy

	Control I	Control-Control II	Sham-Control III
No. gravid	36	45	43
No. fully resorbed	3	6	6
No. with viable fetuses	33	39	37
$\bar{X}$ corpora lutea	10.1	10.5	10.2
$\bar{X}$ implantation sites	6.1	6.6	5.5
Implantation Efficiency(%)	60.2	62.9	54.0
$\bar{X}$ viable fetuses	5.1	5.2	4.3
$\bar{X}$ postimplantation loss	1.1	1.4	1.2
Postimplantation loss (%)	16.9	21.8	21.2
Total No. fetuses	182	233	186
Males/Females	95/97	119/114	81/105
$\bar{X}$ fetal weight (g)	43.0	41.7	41.9

No significant differences relative to corpora lutea, implantation sites, resorption sites, viable fetuses, implantation efficiency, and postimplantation loss were noted among all groups. The implantation efficiency in the sham-control group III was slightly lower than the groups I and II. However, a biological significance could not be positively attributed to the gavage-inducing stress factor. No differences in fetal sex ratio and fetal weights were detected. The fetal weight of all groups was comparable to that of the historical control data (42.6 g).

DEVELOPMENTAL TOXICITY

Each fetus was examined for external, soft tissue, and skeletal anomalies. A modified Staples' technique was used to include examination of the heart and vessels and serial dissection of the brain. The skeleton was fixed in isopropyl alcohol and stained with Alizarin Red S by the Dawson's method. The authors reported their findings as either malformations or variations.

Results

The following tables represent data on external, soft tissue, and skeletal examinations:

	Control I	Control-Control II	Sham-Control III
External Observations			
No. fetuses (litters)	182(33)	233(39)	186(37)
Conjoined Twins	0	1(1)	0
Acephaly	0	1(1)	0
Spina Bifida	1(1)	1(1)	2(2)[c]
Carpal/Tarsal flexure	0	1(1)	3(3)
Acrania	0	0	1(1)[a]
Head Anomaly	0	0	1(1)[b]
Cleft palate	0	0	1(1)[d]
Gastroschisis	0	0	1(1)[a]
Thoraco-gastroschisis	0	0	1(1)[b]
Brachydactyly	0	0	1(1)
Macroglossia	0	1(1)[e]	0
Short tail	0	1(1)	1(1)[c]
Soft Tissue Examinations			
Hydrocephaly	2(2)	1(1)[e]	1(1)
Retroesophag.aortic arch	0	1(1)	0
Malpositioned kidneys	0	0	1(1)[c]
Great vessel anomaly	1(1)	0	0
Intervent. septal defect	0	0	1(1)[d]
Diaphragmatic hernia	0	1(1)	0
Absent kidney/ureter	0	1(1)[e]	0
Iris bombe	1(1)	0	0
Retrocaval ureter	2(1)	11(8)*	2(2)
Bilobed gallbladder	1(1)	0	0
Gallbladder, absent/small	2(2)	0	1(1)
Iris, hemorrhagic ring	2(2)	0	1(1)
Skeletal Examinations			
Skull anomaly	0	1(1)	0
Accessory skull bones	5(4)	29(18)*	6(5)
Sternebrae fused	2(2)	2(2)	5(5)
Sternebrae, extra site	0	3(3)	1(1)
Sternebrae, malaligned	2(2)	1(1)	1(1)
Vertebrae, centra anomaly	2(2)	2(2)	1(1)
Vertebral anomaly with/ without rib anomaly	3(2)	3(3)	4(3)
27 presacral vertebrae	19(11)	51(22)	16(13)
Rib anomaly	2(2)	0	0
7th cervical ribs	3(1)	1(1)	0
13th full ribs	51(22)	93(28)	52(24)
13th rudimentary ribs	30(17)	27(15)	21(16)
Bent limb bones	0	1(1)	0

(*) Significantly different from control I, $p < 0.05$

(a): Fetus #1, doe 1591 - (b): Fetus #2, doe 1591 - (c): Fetus #6, doe 3072
(d): Fetus #4, doe 1124 - (e): Fetus #7, doe 1119

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DISCUSSION

The objectives of this investigation are three-fold:

1. To assess the possibility of genetic-related findings with buck #2749 (Group I: Control)
2. To assess the combined effects of stress (sham-gavage) and possibility of genetic-related findings with buck #2749 (Group III: Sham-Control)
3. To determine the incidence of spontaneous malformations in the New Zealand Buckshire rabbits (Group II: Control-Control)

1. Assessment of possibly genetic-related findings with buck #2749

Insemination of does with semen from buck #2749 (groups I and III) did not result in significant alterations in reproductive performance as characterized by similar pregnancy rate, fecundity, litter size, postimplantation loss, fetal weight, and fetal sex ratio as compared to does in the control group II. No biological differences in sperm count, motility, and morphology were noted between the semen of buck #2749 and bucks 2272 and 1195.

Several malformations (acrania, gastroschisis, anophthalmia, thoracogastroschisis, cleft palate, and malpositioned kidneys) were observed only in litters inseminated by buck #2749 semen (groups I and III) but at a relatively low incidence: fetus #1, doe 1591 had acrania, gastroschisis - fetus #2, doe 1591 had head anomaly and thoracogastroschisis - fetus #6, doe 3072 had spina bifida, short tail, and malpositioned kidneys - fetus #4, doe 1124 had cleft palate and interventricular septal defect. Cranio-facial defects (acrania, head anomaly, cleft palate, hydrocephaly, acephaly, and macroglossia) were observed in 2(2), 2(2), and 4(4) fetuses (litters) in the groups I, II, and III, respectively. The percentages of fetuses (litters) with cranio-facial defects were, respectively, 1.1 (6.1), 0.9 (5.1), and 2.2 (10.8). These incidences are not significantly different from the Buckshire historical control data provided, which indicated 1.2 (7.7)% for fetuses (litters) with hydrocephaly, macrophthalmia, or microphthalmia.

The following table compares the incidences of malformations between this investigation and the Buckshire historical control data:

	Does Sired by buck #2749 †	Control-Control Group II	Buckshire Histo. Data	Total Control°
No. litters examined	70	39	67	106
Litters with external malformations (%)	6 (8.6)	6 (15.4)	4 (6.0)	10 (9.4)
Litters with visceral malformations (%)	7 (10.0)	3 (7.7)	3 (4.5)	6 (5.7)
Litters with skeletal malformations (%)	18 (25.7)	10 (25.6)	18 (26.8)	28 (26.4)
Total litters with malformations (%)	25 (35.7)	15 (38.5)	22 (32.8)	37 (34.9)

(†) Sum of groups I and III - calculated by this reviewer
(°) Sum of findings in control group II and historical control data - calculated by this reviewer

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The total number of litters with malformations in does sired by buck #2749 (groups I and III) was not significantly different from the concurrent control-control group II as well as the historical incidence for Buckshire rabbits. All groups had approximately 33 - 35% litters with malformations. No differences relative to the number of litters with external, visceral, or skeletal malformations were found among the groups.

From the data submitted, there is no conclusive evidence to attribute the findings as "genetically-related" for the following reasons:

1. No significant differences relative to semen volume, sperm motility, sperm count, and sperm morphology were noted between buck #2749 and the control bucks #2272 and #1195 (see page 6 of this memo).
2. No significant alterations in reproductive parameters at laparotomy between does sired by buck #2749 and those sired by control bucks (see page 7 of this memo).
3. The malformations found in litters sired by buck # 2749 were at a very low incidence and could occur spontaneously in this strain and species (see pages 8 and 9 of this memo).
4. No significant increases in the litter incidences of skeletal, visceral, external, or total malformations were found between does sired by buck #2749 and those sired by other bucks (see table on page 9 of this memo).
5. The incidences of malformations observed in does sired by buck 2749 were neither biologically nor statistically different from the historical control data (see table on page 9 of this memo).

2. Assessment of the combined effects of stress (sham-gavage) and genetic-related findings.

In this investigation, does from both groups I and III were inseminated with semen from buck #2749 but does from group III also received a daily sham-gavage treatment from days 7-19 of gestation.

No differences in the reproductive performance (fertility, fecundity, litter size, postimplantation loss, fetal weight, and fetal sex ratio) were noted between the two groups. The total number of litters with malformations was comparable between the two groups, being 39.4 and 32.4%, respectively. The number of litters with cranio-facial defects was biologically similar between the two groups affecting 1.1 (6.1) and 0.9 (5.1)% fetuses (litters), respectively.

From the data submitted, there is evidence that gavaging the animals during the period of major organogenesis may have a maternal effect as characterized by a decrease in body weight gain. However, no definitive statement could be made concerning the effect of gavage-related-stress on the fetal development.

3. Determination of the incidence of spontanous malformations in the New Zealand Buckshire rabbits.

The fetal and litter incidences of malformations were compiled by the testing facility for the Buckshire New Zealand rabbits from 6/28/83 to 5/8/85. Litter incidences of 6.0, 4.5, 26.8, and 32.8% were found for the number of litters with external, visceral, skeletal, and total malformations, respectively. Respective values of 15.4, 7.7, 25.6, and 38.5% were found in the concurrent control-control group II. When these data are combined, respective values of 9.4, 5.7, 26.4, and 34.9% were obtained. In Dutchland New Zealand rabbits, the litter incidences were, respectively, 5.5, 6.6, 13.5, and 21.5%.

Malformation Rates in Different Strains of New Zealand Rabbits

	Buckshire Control-control group II	Buckshire Historical Control Data	Buckshire Combined (°)	Dutchland Historical Control Data
% litters with external malformations	15.4	6.0	9.4	5.5
% litters with soft tissue malformations	7.7	4.5	5.7	6.6
% litters with skeletal malformations	25.6	26.8	26.4	13.5
% total litters with malformations	38.5	32.8	34.9	21.5

(°) Sum of control group II and historical control - calculated by this reviewer

These data demonstrate that the spontaneous incidence of malformations in the Buckshire rabbit is higher than that of the Dutchland strain.

REASSESSMENT OF THE SCIENTIFIC MERIT OF STUDY WIL 82001

A developmental toxicity investigation with Larvadex was conducted in Buckshire rabbits (WIL 82001) at 0, 5, 10, 30, and 60 mg/kg/day. The study was reviewed by the Agency (Q. Bui's memo to A. Heyward, 2/5/85) and classified as Core Minimum Data. Diaphragmatic hernia was observed in 1(1) and 3(2) fetuses (litters) in the 10 and 30 mg/kg groups. Cyclopia was found in 1(1) fetus (litter) each in the 10 and 30 mg/kg groups. Hydrocephaly was noted in 1(1), 2(2) and 1(1) fetuses (litters) in the 10, 30, and 60 mg/kg groups, respectively. Umbilical hernia was observed in 1(1) fetus (litter) each in the 30 and 60 mg/kg groups. A single case of spina bifida and micrognathia was found in the 30 mg/kg group and four fetuses with short or bent tail were observed in the 60 mg/kg group. No malformations were observed in the concurrent control group. This reviewer concluded that although a dose response increase for each malformation was not evident, the findings of diaphragmatic hernia and cyclopia at the 10 and 30 mg/kg groups should be of concern since the historical control data listed a zero incidence for these findings. The other abnormalities (hydrocephaly, umbilical hernia, spina bifida, and micrognathia) were within historical control range.

The registrant suggested that all these abnormalities were genetically related since they were found in litters sired by the same buck (#2749) and to confirm that these findings were "genetically related", the registrant proposed to conduct this study (WIL 82005). This reviewer (memo of Q. Bui to A. Heyward, 2/5/85) had indicated that, in case that these malformations would be determined as "genetically related", study WIL 82001 would be invalidated due to insufficient number of does per group.

To re-assess the scientific validity of study WIL 82001, this reviewer has compiled a table of the findings in Buckshire rabbits from studies WIL 82001, 82003 and historical control data.

The malformations were combined into three sub-groups: craniofacial, mid-line fusion, and spinal cord/tail. All three types of malformations were noted in both studies with the incidences in WIL 82001 slightly higher than those of WIL 82005. Cyclopia and diaphragmatic hernia were two findings of concern observed in the Larvadex study (WIL 82001). Cyclopia was not observed in study WIL 82005 but diaphragmatic hernia was noted in 1(1) fetus (litter) of the untreated control (group II) of study WIL 82005. Although cyclopia per se was not observed in study WIL 82005 (out of 368 fetuses/70 litters examined), it should be noted that defects of the midline structures of the head may range from cyclopia through arhinencephaly to near normal states. Cyclopia is characterized by a combination of lack of cleavage of the prosencephalon and separation of the eyes. Cyclopia can occur as isolated anomalies or be associated with other defects of the face or other organ systems. Cranio-facial defects such as astomia, microstomia, otocephaly, synotia, anencephaly, hydrocephaly, cleft palate, hare lip, etc., have been described as associated malformations with cyclopia (Sedano and Gorlin, 1963; Warkany, 1971). This is not unexpected since cranio-facial structures originate embryologically from the same anterior neural plate and neural crest cells. An insult on the anterior neural plate and the neural crest cells may, hence, express morphologically different depending on the stage of embryonic development and the severity of the insult. Therefore, all cranio-facial defects may be related etiologically.

As shown in the table on next page, the incidence of cranio-facial defects in litters sired by buck #2749 and treated with Larvadex is 20.8% (study WIL 82001; group A in table) compared to a litter incidence of 5.7% in control does and sired by buck #2749 (study WIL 82005; group B in table) and a litter incidence of 6.0% in the historical control data (group C in table). These findings suggested that (1) the cranio-facial defects were not associated with the presumably "genetic defects" in buck #2749 since the incidences in group A were not comparable to those in group B, (2) there was no clear evidence of genetic defects since the incidences in group B were neither statistically nor biologically different from the group C historical control data, and (3) the higher incidence of cranio-facial defects noted in group A as compared to group B likely results from the administration of Larvadex itself since the two groups differed mainly by the presence or absence of Larvadex administration.

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BUCKSHIRE MALFORMATIONS : LITTERS (%)

	GROUP A WIL 82001 Larvadex and sired by 2749	GROUP B [a] WIL 82005 Control and sired by 2749	GROUP C [b] Buckshire Historical Control Data
No. of litters	24	70	67
* Acrania	0	1 (1.4)	0
* Micrognathia	1 (4.2)	0	1 (1.50)
* Micro/anophthalimia	0	0	1 (1.50)
* Cyclopia	2 (8.3)	0	0
* Skull anomaly	1 (4.2)	1 (1.4)	1 (1.50)
* Hydrocephaly	3 (12.5)	3 (4.3)	1 (1.50)
* Cleft palate	0	1 (1.4)	0
† Spina bifida	1 (4.2)	3 (4.3)	0
† Tail anomalies	1 (4.2)	1 (1.4)	0
° Umbilical hernia	2 (8.3)	0	0
° Gastroschisis	0	1 (1.4)	0
° Thoraco-gastroschisis	0	1 (1.4)	0
Ventri. septal defect	0	1 (1.4)	0
Diaphragmatic hernia	2 (8.3)	0	0
* Cranio-facial defects	5 (20.8)	4 (5.7)	4 (6.0)
† Spinal cord/tail	2 (8.3)	3 (4.3)	0
° Mid-line fusion defects	2 (8.3)	2 (2.8)	0

(a) Groups I and III of study WIL 82005 - calculated by this reviewer
(b) Historical control provided by the testing facility from 6/28/83 to 5/8/85
(*) Cranio-facial defects: acrania, micrognathia, micro/anophthalmia, cyclopia, skull anomaly, hydrocephaly, cleft palate
(†) spinal cord and tail anomalies
(°) mid-line fusion defects: diaphragmatic hernia, gastroschisis, and thoraco-gastroschisis.

It is this reviewer's opinion that the registrant has failed to demonstrate that the findings in study WIL 82001 were "genetically related" based upon the following:

1. Insemination of does with semen from buck 2749 (groups I and III, WIL 82005) did not result in significant alterations in reproductive performance as characterized by similar pregnancy rate, fecundity, litter size, postimplantation loss, fetal weight, and fetal sex ratio as compared to concurrent and historical control values.

2. No significant differences relative to semen volume, sperm motility, sperm count, and sperm morphology were noted between buck #2749 and control bucks # 2272 and 1195 (WIL 82005).

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3. The malformation rates in untreated does and sired by buck #2749 were much lower than those in does treated with Larvadex and sired by buck #2749 (see page 13 of this memo).

4. The malformation rates in untreated does and sired by buck #2749 were not higher than both the concurrent control and historical control data (see pages 9 and 13 of this memo).

5. No increases in cranio-facial defects (including cyclopia) were noted in untreated does and sired by buck #2749 as compared with both the concurrent and historical control data.

6. Although several malformations, such as acrania, gastroschisis, cleft palate, malpositioned kidneys, and thoracogastroschisis) were noted only in litters sired by buck #2749 (groups I and III, WIL 82005), they occurred at a relatively low incidence. These isolated incidences could not be considered as "genetic-related" by this reviewer since they were within the historical control range and could well be of spontaneous origin.

Based upon the above discussion, it is concluded that:

a) The possibility that the findings associated with buck #2749 in study WIL 82001 were genetically related has not been demonstrated by data from study WIL 82005.

b) Study WIL 82001 remains classified as Core Minimum Data

c) The maternal and developmental toxicity NOELs were established at, respectively, 10 and 5 mg/kg/day.

d) Developmental toxicity risk assessment for Larvadex should be based upon the developmental toxicity NOEL of 5 mg/kg/day.

DATA EVALUATION RECORD

Chemical: Cyromazine - Larvadex
Test Material: Cyromazine Technical, 95.2%
Lot FL 841538, pale yellow cristalline powder

STUDY IDENTIFICATION

"A Teratology and Postnatal Study in Albino Rabbits with Cyromazine Technical"

Testing Facility: WIL Research Lab., Inc.,
Ashland, Ohio
Final Report No.: WIL-82008
Report Date: 2/9/86
Study Authors: M. D. Nemec et al.,

EPA Accession No.: 262639

Study Reviewed by: Quang Q. Bui, Ph.D.
Toxicologist
Toxicology Branch/HED (TS-769C)

Review Approved by: Laurence D. Chitlik, D.A.B.T.
Section Head
Toxicology Branch/HED (TS-769C)

RECOMMENDATION AND CONCLUSIONS

It is recommended that this study be classified as Core Supplementary Data pending the submission of additional data to clarify the comments listed on pages 30 and 31 of this memorandum.

Under the conditions of this study, a maternal NOEL may be established at 10 mg/kg with decreased maternal weight gains and food consumption observed at 30 mg/kg during the period of treatment (days 6-19 of gestation). Several malformations were noted only in the treated groups (umbilical hernia, gastroschisis, omphalocele, agnathia, macroglossia, spina bifida, and diaphragmatic hernia) but evidence of a dose-response relationship was not demonstrated. There was an apparent increase in the incidences of "left carotid arises from brachiocephalic trunk", sternebrae 5/6 unossified, hyoid body and/or arch unossified, and 27th presacral vertebrae in the treated fetuses. However, no statistical differences were found.

Pups born from mothers treated with Larvadex during the period of organogenesis did not exhibit any adverse effects relative to survivability and growth development up to postnatal day 28. Scattered incidences of cyclopia (1 pup 30 mg/kg) and cataracts (2 pups 5 mg/kg and 1 pup 30 mg/kg) were found.

A developmental toxicity NOEL (including postnatal development) cannot be ascertained pending the registrant's clarification of the comments listed on pages 30 and 31 of this memorandum.

PROCEDURES

The investigators indicated that this study was conducted following the Good Laboratory Practice, 1982 FIFRA Guidelines, and the 1981 OECD Guidelines. A quality assurance statement was signed by R. Anderson and was appended with the final report.

A copy of the study procedures is appended and the following comments and highlights are noted:

1. The test material was received at the testing facility on 10/15/84 and the study was initiated on 8/6/85 and terminated on 11/9/85. Cyromazine technical was mixed with 0.5% carboxymethylcellulose and adjusted for purity. Daily test suspensions were prepared and analytical determinations were performed prior to study initiation and on the 1st, 6th, and last day of dosing. Methodology and results of the analytical determinations were appended with the final report.

2. Seventy-four inseminated New Zealand rabbits (Hazleton-Dutchland) were assigned to each of 4 groups administered with 0, 5, 10, or 30 mg/kg Technical Cyromazine. All animals were individually housed with food (Purina Certified Rabbit Chow) and tap water ad libitum under environmentally-controlled conditions.

3. Artificial insemination was performed with semen from 7 bucks of the same strain and commercial supplier. Semen evaluation was performed prior to insemination and a final concentration of greater than 3 million sperm/ml was used. Following insemination, all does received an injection of 100 USP units of HCG via the marginal ear vein to ensure ovulation.

4. Treatment of pregnant animals was conducted on days 7-19 of gestation. A minimum of 25 females with viable fetuses from each group were sacrificed on day 29 of gestation. The remaining females were allowed to deliver and raise their pups for the postnatal phase up to lactation day 28.

5. Fetuses were examined viscerally by a modified Staples' method to include the heart and major blood vessels. The authors reported that the fetal brain was examined by a mid-coronal slice. It would be better to examine fetal brain by serial section.

ANALYTICAL DETERMINATIONS

Analytical determinations of the test solutions were performed on 4 instances for all test groups. The submitted data indicated that all analytical concentrations were within ± 5% of the theoretical concentrations and, hence, are acceptable.

ARTIFICIAL INSEMINATION

Artificial insemination was performed with diluted semen from 7 bucks of the strain and commercial supplier (Hazleton-Dutchland). Semen from buck 1835, 2871, 2876, 2877, 2879, 3482, and 3486 were used to inseminate 26, 6, 6, 18, 12, 2, and 4 females from each group, respectively. The artificial insemination procedure, as performed, is acceptable.

Pregnancy rates of 83.8, 94.6, 78.4, and 81.1% were obtained for the 0, 5, 10 and 30 mg/kg groups, respectively, and were within the acceptable norm.

CLINICAL OBSERVATIONS

Clinical observations were performed daily for signs of morbidity, appearance, toxicity, and behavioral changes. A necropsy was conducted on all animals which died during this investigation.

Results

a. Mortality: Ten animals died during this investigation: 4 control, 2 in the 5 mg/kg, 3 in the 10 mg/kg, and 1 in the 30 mg/kg group. The authors stated that these deaths were not compound-related. One female of the 5 mg/kg group was sacrificed on day 9 of gestation due to "fracture of the vertebral column".

b. Abortion: Sixteen animals aborted: 4 control, 5 in the 5 mg/kg, 2 in the 10 mg/kg, and 5 in the 30 mg/kg group. Two control animals aborted and died.

c. Clinical: There were no definite clinical signs associated with the administration of Cyromazine except for a decreased incidence of defecation and urination observed in the 30 mg/kg group.

(Reviewer's Note: For clarity sake, evaluation of the data was conducted separately for classical teratology and postnatal segments).

I. TERATOLOGY PHASE

MATERNAL BODY WEIGHTS

Individual body weights were recorded on days 0, 7, 10, 14, 20, 24, and 20 of gestation. Mean body weight changes, mean gravid uterine weights, and net body weight gains were calculated. Non pregnant animals were excluded from data analysis.

Results: The following table represents the body weight data during gestation.

MATERNAL BODY WEIGHT DATA (in grams)

	Control	5 mg/kg	10 mg/kg	30 mg/kg
Day 0 of gestation	3435(62)[a]	3478(70)	3483(58)	3487(60)
Wt gain, days 0-7	149(62)	159(70)	146(58)	138(60)
Wt gain, days 7-20	78(60)	141(69)	87(57)	-115(60)*
Wt gain, days 20-29	70(58)	41(64)	60(54)	206(55)*
Wt gain, days 0-29	301(58)	351(64)	306(54)	244(55)
$\bar{X}$ gravid uterine wt.	362(33)[b]	393(35)	374(26)	318(27)
Net weight gain †	-122(33)[b]	- 65(35)	- 88(26)	-115(27)

(a) = weight in grams (No. of does)
(b) = data collected from does sacrificed on day 29 of gestation
(†) = weight gain minus gravid uterine weight
(*) = differs significantly from control, $p < 0.05$

All groups had comparable body weights at study initiation (day 0) suggesting that randomization of the does had been properly conducted. Prior to treatment (days 0-7), no differences in weight gains were noted among the groups. However, during the treatment period (days 7-20) a significant decrease in weight gain was observed in the 30 mg/kg group. Animals in the 30 mg/kg group gained significantly more weight from days 20-29 suggesting a compensatory effect. Throughout the entire gestation period (days 0-29), the maternal body weight gain of the 30 mg/kg groups was slightly less than that of the control. No differences in uterine weights and net body weight gains were found among the groups.

FOOD CONSUMPTION

Individual food consumption was recorded daily throughout gestation. The food intake was presented as g/animal/day and g/kg body weight/day.

Results

No differences in food intake were found between the control, 5 and 10 mg/kg groups during gestation. A statistically significant decrease in food intake was noted in the 30 mg/kg group during the treatment period (days 7-20) with a rebound increase after cessation of treatment (days 20-29). During the period of treatment (days 7-20), the food intake of the 0, 5, 10, and 30 mg/kg groups was, respectively, 44, 45, 42, and 30 ($p < 0.05$) g/kg/day. Throughout the entire gestation (days 0-29), respective food intake of 41, 42, 41, and 38 g/kg/day was found.

MATERNAL STATUS AT LAPAROTOMY

A minimum of 25 does with viable fetuses per group were sacrificed on day 29 of gestation. Routine teratology procedures were used to collect data and uteri with no visible implantations were placed in ammonium sulfide for detection of early resorptions.

Results:

The number of females presumed gravid selected for C-section was, respectively, 36, 35, 36, and 33 for the 0, 5, 10, and 30 mg/kg groups. Respective 30, 33, 25, and 26 litters with viable fetuses were obtained and satisfied the 1982 FIFRA requirements. No significant differences in the number of does with complete resorptions were noted. Respective 3 (9.1%), 2 (5.7%), 1 (3.8), and 2 (7.1%) does (% pregnant) with complete resorptions were detected.

No significant differences in the means of corpora lutea, implantation sites, preimplantation loss, resorption, dead fetuses, fetal weight, and fetal sex ratio were found among the groups. A statistical decrease in postimplantation loss was noted in the 10 mg/kg group but should be considered as a biological variation. The means of viable fetuses for the 0, 5, 10, and 30 mg/kg groups were, respectively, 6.4, 6.8, 6.7, and 5.5. Respective fetal weights of 41.3, 41.0, 40.6, and 42.5 g were found. The slight increase in the 30 mg/kg fetal weight (42.5 vs 41.3 g/control) corresponded to a slight reduction in litter size associated with this group (5.5 vs. 6.4/control)

REPRODUCTIVE STATUS AS LAPAROTOMY

	Control	5 mg/kg	10 mg/kg	30 mg/kg
No. does sacrificed	36	35	36	33
No. does non gravid	3	0	10	5
No. does gravid	33	35	26	28
No. does with complete resorptions	3	2	1	2
No. does with viable fetuses	30	33	25	26
$\bar{X}$ corpora lutea	11.5	11.3	10.8	11.0
$\bar{X}$ implantation sites	7.5	7.7	7.0	6.5
Implantation Efficiency(%)[a]	64.7%	68.8%	64.8%	59.6%
$\bar{X}$ resorptions	1.1	0.9	0.3*	1.0
Postimplantation loss (%)[a]	14.6	12.1	4.9*	15.3
No. viable fetuses	210	238	173	155
Males/Females	114/96	130/108	85/88	72/83
$\bar{X}$ viable fetuses	6.4	6.8	6.7	5.5
$\bar{X}$ fetal weight (g)	41.3	41.0	40.6	42.5

(a) calculated by this reviewer
(*) differs significantly from control, $p < 0.05$

DEVELOPMENTAL TOXICITY

External, soft tissue, and skeletal examinations were conducted on all fetuses. The authors stated that a modified Staples technique was used for visceral examination to include the heart and blood vessels. Skeletal staining followed the Dawson technique with Alizarin Red-S. All findings were classified by the investigators as either malformations or variations. Non-viable fetuses were excluded from data tabulation. Fetal and litter incidences were presented by the authors and individual litter data were appended with the final report along with historical control data.

Results

1. External Observations

In the control group, only one fetus with short tail was found. However, several external abnormalities were noted in the treated groups. Umbilical hernia and omphalocele were noted in one fetus each in the 5 mg/kg group. Macroglossia and agnathia were found in one fetus each in the 10 mg/kg group whereas gastroschisis, spina bifida, and abnormality of the pinna were detected in one fetus each in the 30 mg/kg group.

The percentages of litters with external malformations were, respectively, 3.3, 9.1, 8.0, and 7.7 for the 0, 5, 10, and 30 mg/kg groups.

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STRUCTURAL MALFORMATIONS

	Control	5 mg/kg	10 mg/kg	30 mg/kg
# fetuses (litters)	210(30)	238(33)	173(25)	155(26)
Umbilical hernia	0	1(1)	0	0
Gastroschisis	0	0	0	1(1)[c]
Omphalocele	0	1(1)	0	0
Pinna abnormality	0	0	0	1(1)[a]
Macroglossia	0	0	1(1)	0
Agnathia	0	0	1(1)	0
Micro/Anophthalmia	0	1(1)	0	0
Spina bifida	0	0	0	1(1)
Short tail	1(1)	0	0	1(1)[a]
Diaphragmatic hernia	0	1(1)	3(2)[b]	1(1)[a]
Kidney/ureter absent	0	0	0	1(1)[a]
Retroesophageal aortic arch	0	0	1(1)[b]	0
Rib anomaly	0	3(2)	1(1)	0
Vertebrae anomaly	3(2)	1(1)	4(4)	3(3)[a]
Costal cartilage anomaly	0	1(1)	1(1)	0
Sternebrae, extra site	1(1(	1(1(	0	0
Fused sternebrae	2(2)	1(1)	1(1)	0
Sternebrae malaligned	0	1(1)	0	1(1)[c]
Skull anomaly	2(2)	0	2(2)	1(1)
Cranio-facial defects[d]	2(2)	1(1)	3(2)	1(1)
Mid-line fusion defects[e]	0	2(2)	0	1(1)
Axial skeleton[f]	3(2)	5(3)	6(4)	3(3)
Sternebral defects[g]	3(2)	3(3)	1(1)	1(1)
# with external malform.	1(1)	3(3)	2(2)	2(2)
# with soft tissus malform.	0	1(1)	4(3)	2(2)
# with skeletal malform.	7(6)	8(5)	8(5)	5(4)
Total # with malformations	8(7)	11(8)	12(8)	6(5)

(a) findings in fetus 3, doe 1267 - (b) findings in fetus 1, doe 2013
(c) findings in fetus 4, doe 1450
(d) sum of pinna defect, macroglossia, agnathia, micro/anophthalmia, skull anomaly; calculated by this reviewer
(e) sum of umbilical hernia, gastroschisis, and omphalocele; calculated by this reviewer
(f) sum of short tail, spina bifida, rib anomaly, vertebral anomaly, costal cartilage anomaly; calculated by this reviewer
(g) sum of sternebrae extra site, fused, and malaligned; calculated by this reviewer

2. Soft tissue Examinations

Diaphragmatic hernia was found only in the Larvadex-treated groups affecting 1(1), 3(2), and 1(1) fetuses (litters) in the 5, 10, and 30 mg/kg groups, respectively. The 30 mg/kg fetus with diaphragmatic hernia (fetus 3, doe 1267) also had short tail, pinna defect, kidney and/or ureter absent, and vertebral anomaly. The percentages of litters with soft tissue malformation were, respectively, 0.0, 3.0, 12.0, and 7.7, for the 0, 5, 10, and 30 mg/kg groups.

3. Skeletal Observations

The incidences of skeletal anomalies (e.g. rib anomaly, fused sternebrae, skull anomaly, vertebral anomaly, and sternebrae malaligned) occurred in all groups, including the control, at a low incidence and did not follow any dose-response pattern. The incidences of litters with skeletal malformations were 20.0, 15.2, 20.0, and 15.4% for the 0, 5, 10, and 30 mg/kg, respectively.

When the incidences of cranio-facial defects (pinna defect, macroglossia, agnathia, microphthalmia and/or anophthalmia, and skull defect), mid-line fusion defects (gastroschisis, umbilical hernia, and omphalocele), axial skeleton defects (short tail, spina bifida, rib anomaly, vertebral anomaly, and costal cartilage anomaly), and sternebral defects (fused, extra site, and malaligned) were combined, no clear dose-response relationship was found (see table on previous page). No dose-related increases in the total number of litters with malformations (external, visceral, and skeletal) were found. Respective litter incidences of malformations were 23.3, 24.2, 32.0, and 19.2%.

STRUCTURAL VARIATIONS (Litter %)°

	Control	5 mg/kg	10 mg/kg	30 mg/kg
# litters examined	30	33	25	26
Blood vessel[a]	26.7	42.4	48.0	38.5
Gall bladder absent/small	6.7	15.2	32.0	15.4
13th rudimentary ribs	50.0	57.6	52.0	57.7
Sternebrae 5/6 unossified	40.0	48.5	52.0	57.7
27 presacral vertebrae	50.0	66.7	64.0	53.8
Hyoid body unossified	0.0	6.1	4.0	11.5

(°) Only findings of interest are tabulated
(a) Left carotid arises from brachiocephalic trunk

Although no statistical significances were found, there was an apparent increase in the incidences of "left carotid arises from brachiocephalic trunk", absent/small gall bladder, sternebrae 5/6 unossified, and 27 presacral vertebrae in the treated groups as compared to controls. Unossified hyoid body and/or arch was the only finding that was absent in the concurrent control. A historical control litter incidence of 6.6% for hyoid body and/or arch was provided by the testing facility. All findings in the treated groups did not follow a clear dose-response relationship except for the finding of sternebrae 5/6 unossified, which was higher than both the concurrent control (40.0%) and historical control data (41.9%).

B. POSTNATAL PHASE

Animals that were not sacrificed on day 29 of gestation were allowed to deliver and raise their offspring up to postnatal day 28.

MATERNAL BODY WEIGHT DURING LACTATION

Maternal body weights were recorded on lactation days 1, 7, 14, 21, and 28.

Result:

During lactation, animals in the 0, 5, 10, and 30 mg/kg groups gained 72, -28, 78, and 178 g, respectively. No evidence of a treatment-related effect was noted.

MATERNAL REPRODUCTIVE STATUS

Females were allowed to deliver and raise their offspring. Animals, which failed to deliver, were sacrificed on postmating days 35-36 to assess the reproductive status.

Results

	MATERNAL REPRODUCTIVE STATUS			
	Control	5 mg/kg	10 mg/kg	30 mg/kg
# allowed to deliver	32	31	35	36
# non-gravid	9	4	6	9
# gravid	23	27	29	27
No. delivered	19	25	26	26
No. gravid not delivered†	4	2	3	1
Total # pups delivered	150	182	160	167
Litter size°	7.9	7.3	6.2	6.4
# dead pups	29	20	25	10
$\bar{X}$ dead pups	1.5	0.8	1.0	0.4*
# live pups	121	162	135	157
$\bar{X}$ live pups at birth°°	6.3	6.5	5.2	6.0
# litters with live pups	17	25	24	25
# litters with dead pups only	2	0	2	1
Live birth index°°°	7.1	6.5	5.6	6.3

(†) pregnancy status determined at necropsy on postmating days 35-36
(°) total # pups delivered / # litters delivered; calculated by this reviewer
(°°) # live pups / # litters delivered; calculated by this reviewer
(°°°) # live pups / # litters with live pups.
(*) Differs significantly from controls, $p < 0.05$

The percentages of females, which delivered in the 0, 5, 10, and 30 mg/kg groups, were 82.6, 80.6, 89.7, and 96.3, respectively. No evidence of a treatment-related effect was found relative to fertility.

Although the litter size in all treated groups was slightly reduced as compared to control, a statistical difference was not attained. A significant decrease in the number of stillborns was found in the 30 mg/kg group and was biologically irrelevant. No significant differences in the mean numbers of live pups at birth were found. When the live birth index was calculated, a slight decrease in this index was noted in all treated groups as compared to controls, but evidence of a dose-response relationship was not demonstrated.

PUP SURVIVABILITY

The authors stated that pup survivability was monitored twice daily. On day 4 of lactation, each litter was randomly reduced to 6 pups. The viability index was calculated at different intervals during the lactation period.

Results

The following table summarizes the offspring survivability

OFFSPRING SURVIVABILITY

	Control	5 mg/kg	10 mg/kg	30 mg/kg
Total # pups born	150	182	160	167
# pups alive day 0	121	162	135	157
# stillborn	29	20	25	10
Survivability at birth (%)	80.7	89.0	84.4	94.0
# pups alive day 1	115	158	119	153
# pups dead day 0-1°	6(5.0)	4(2.5)	16(11.9)	4(2.5)
# pups alive day 4	82	122	97	118
# pups dead days 1-4 °	33(28.7%)	36(22.8%)	22(18.5%)	35(22.9%)
Total pups dead days 0-4	39(32.2%)	40(24.7%)	38(28.1%)	49(24.8%)
Viability Index[a]	67.8	75.3	71.8	75.2
# pups after culling	62	91	76	99
# pups sacrificed[b°]	20	31	21	19
# pups alive day 21	47	79	66	78
Day 21 weaning index[c]	75.8	86.8	86.8	78.8
# pups alive day 28	47	76	63	71
Day 28 weaning index[d]	75.8	83.5	82.9	71.7

(°) calculated by this reviewer
(a) Pups viable on day 4 before culling / Pups viable on day 0
(b) Pups viable on day 4 before culling minus pups viable after culling
(c) Pups viable on day 21 / Pups viable on day 4 after culling
(d) Pups viable on day 28 / Pups viable on day 4 after culling

No statistically or biologically significant differences in offspring survivability were noted among the groups at birth as well as throughout lactation. The day 4 viability index was similar between the control and treated groups. The day 21 and 28 weaning indices did not demonstrate any evidence of a treatment-related effect.

OFFSPRING BODY WEIGHT

Pups were weighed individually on lactation days 1, 4, 7, 14, 21, and 28.

Results:

On day 1 of lactation, pups from the 0, 5, 10, and 30 mg/kg groups weighed an average of 57.7, 61.9, 68.5, and 60.5 grams, respectively. By day 28 of lactation, respective body weights of 495.3, 470.4, 584.5, and 540.7 were found. There was no evidence of an adverse effect on pup body weight during the lactation period.

NECROPSY DATA

The authors stated that the Staples technique was used to examine stillborn as well as all pups which died from days 0 to 4. A detailed necropsy was performed on all pups dying after lactation, pups that were culled, as well as on all pups at terminal sacrifice (day 28 of lactation). Pups were eviscerated, preserved, and stained for possible future evaluation.

Results:

Findings of interest are tabulated on next page

a) Stillborn and Pups dead from days 0-4 of lactation:

The authors indicated that 55(15), 59(15), 59(14), and 54(17) pups (litters) were examined (Table 21 of final report).

Pup 1 of doe 1394 in the 30 mg/kg group had multiple defects (cyclopia, cleft palate, and omphalocele). Cranio-facial defects including hydrocephaly were noted only in the 30 mg/kg group affecting 3(2) pups (litters). There were no major malformations in the 5 and 10 mg/kg groups but one pup with omphalocele was found in the control.

b) Pups culled on day 4 of lactation:

Table 19 of the final report revealed that 13(5), 12(7), 17(8), and 11(5) pups (litters) culled on day 4 were examined. A single case of cataract was noted in the 5 mg/kg group.

c) Pups dead from days 5-28 of lactation:

There were no findings of major malformations.

d) Pups sacrificed on days 28 of lactation:

One control pup was found with carpal flexure. Cataract was observed in one pup each in the 5 and 30 mg/kg groups.

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	CONTROL	5 MG/KG	10 MG/KG	30 MG/KG
DEAD PUPS FROM DAYS 0-4				
# examined (litters)	55(15)	59(15)	59(14)	54(17)
Omphalocele	1(1)	0	0	1(1)°
Cyclopia	0	0	0	1(1)°
Cleft palate	0	0	0	1(1)°
Tarsal flexure	0	0	0	1(1)
Hydrocephaly	0	0	0	2(1)
Absent kidney	0	0	0	1(1)
Absent ureter	0	0	0	1(1)
PUPS CULLED ON DAY 4				
# examined (litters)	13(5)	12(7)	17(8)	11(5)
Eye cataract	0	1(1)	0	0
DEAD PUPS FROM DAYS 5-28				
# examined (litters)	10(6)	13(7)	13(6)	26(10)
Findings	0	0	0	0
PUPS SACRIFICED DAY 28				
# examined	47(10)	76(18)	63(16)	71(17)
Carpal flexure	1(1)	0	0	0
Cataract	0	1(1)	0	1(1)
TOTAL PUPS EXAMINED †	125	160	152	162
TOTAL PUPS BORN °°	150	182	160	167
TOTAL PUPS NOT EXAMINED †	25	22	12	5

(°) Findings in same pup (#1, doe 1394)
(°°) Dead and alive pups (see table on page 23)
(†) Calculated by this reviewer

DISCUSSION

I. C-SECTION PHASE FINDINGS

A. Maternal Toxicity

Administration of 5, 10, or 30 mg/kg of Technical Cyromazine to pregnant rabbits from days 6-19 of gestation did not result in overt treatment-related signs of toxicity except for the presence of decreased urination and defecation observed in 30 mg/kg does. Significant decreases in body weight gains were noted at the 30 mg/kg dosage level during the treatment period (days 6-19) followed by a compensatory increase in weight gain after cessation of treatment (days 20-29). Throughout the entire gestation period, only does treated with 30 mg/kg gained less than controls. These findings indicated that the effects observed at the 30 mg/kg dosage level were treatment-related.

At laparotomy (day 29 of gestation), no statistical differences in reproductive parameters were found. The numbers of corpora lutea, implantations, resorptions, dead fetuses, fetal weights, and fetal sex ratio were biologically similar between the control and treated groups. A slight decrease in the live litter size was noted in the 30 mg/kg group, but a statistical difference was not attained (5.5 vs. 6.4 of control).

Based upon the above findings, a maternal toxicity NOEL is suggested at the 10 mg/kg dosage level.

B. Developmental Toxicity

1. Embryonic death

No differences relative to resorptions, dead fetuses, and postimplantation loss were found between the control and treated groups including the highest dose tested (30 mg/kg).

2. Growth development

The fetal weights of all groups ranged from 40.6 (5 mg/kg), 41.0 (10 mg/kg), 41.3 (control), to 42.5 (30 mg/kg) grams and were comparable to the historical control data (40.6 g). The slight increase in fetal weight observed in the 30 mg/kg may result from a relatively smaller litter size.

3. Structural malformations

Cranio-facial defects characterized by abnormal pinna, macroglossia, agnathia, skull anomaly, and microphthalmia and/or anophthalmia were observed in 2(2), 1(1), 3(2), and 1(1) fetuses (litters) of the 0, 5, 10, and 30 mg/kg groups, respectively. Mid-line fusion defects (umbilical hernia, gastroschisis, and omphalocele) were noted in 0(0), 2(2), 0(0), and 1(1) fetuses (litters) in the 0, 5, 10, and 30 mg/kg groups, respectively. Respective defects of the axial skeleton (short tail, spina bifida, rib anomaly, vertebral anomaly, and costal cartilage anomaly) were observed in 3(2), 5(3), 6(4), and 3(3) fetuses (litters). Sternebral defects (fused, malaligned, and extra site) were noted in 3(2), 3(3), 1(1), and 1(1) fetuses (litters) in the 0, 5, 10, and 30 mg/kg groups. Diaphragmatic hernia

was found only in the treated groups affecting 1(1), 3(2), and 1(1) fetuses (litters) in the 5, 10, and 30 mg/kg groups, respectively. It should be noted, however, that the 30 mg/kg fetus with diaphragmatic hernia (fetus 3, doe 1267) also had short tail, malformed pinna, absent ureter, and vertebral anomaly.

The following table represents these findings as percent of fetuses and litters:

PERCENT OF FETUSES (LITTERS) WITH MALFORMATIONS †

	Control	5 mg/kg	10 mg/kg	30 mg/kg
Fetuses (litters) exam.	210(30)	238(33)	173(25)	155(26)
Cranio-facial defects	0.9(6.7)	0.4(3.0)	1.7(8.0)	0.6(3.8)
Mid-line fusion defects	0.0(0.0)	0.8(6.0)	0.0(0.0)	0.6(3.8)
Axial skeleton defects	1.4(6.7)	2.1(9.1)	3.5(16.0)	1.9(11.5)
Sternebral defects	1.4(6.7)	1.3(9.1)	0.6(4.0)	0.6(3.8)
Diaphragmatic hernia	0.0(0.0)	0.4(3.0)	1.7(8.0)	0.6(3.8)
Total with malformations	3.8(23.3)	4.6(24.2)	6.9(32.0)	3.9(19.2)

(†) Calculated by this reviewer

No evidence of a dose-response relationship was found with respect to the findings of cranio-facial, mid-line fusion, axial skeleton, and sternebral defects. Diaphargmatic hernia was observed only in the treated groups and the historical control data incidences for this finding are 0.2 (1.1)% fetuses (litters).

4. Structural variations

Although no dose-response relationship was demonstrated, the incidences of "left carotid arises from brachiocephalic trunk", gallbladder absent/small, 13th rudimentary ribs, 27 presacral vertebrae, and hyoid body/arch unossified were slightly increased in all treated groups as compared to concurrent control data. There was an apparent dose-related increase in sternebrae 5/6 unossified affecting 40.0, 48.5, 52.0, and 57.7% litters in the 0, 5, 10, and 30 mg/kg groups, respectively. The historical control litter incidence of sternebrae 5/6 unossified is 42.0%.

5. Developmental toxicity potential

Several types of malformations were noted in the Larvadex-treated groups but could be considered as spontaneous in light of their presence and similar incidence in the historical control data and in the absence of a dose-response relationship in this study. However, diaphragmatic hernia was of concern since:

i. Its absence in the concurrent control group
ii. Low historical control data incidence = 0.2 (1.1)% fetus (litter).
iii. It was found in a previous developmental toxicity study with Larvadex in rabbits (WIL 82001) affecting 1(1) and 3(2) fetuses (litters) in the 10 and 30 mg/kg groups

However, a thorough examination of the insemination procedure and individual litter data revealed that most of the fetuses with diaphragmatic hernia as well as other malformations were from does sired by buck 2871. From August 6-14, 1985, semen from buck 2871 was used to inseminate 6 does from each group for a total of 32 does. Two were non-pregnant (does 1421 and 1299) and one was found with 100% resorption (doe 1372).

The total number of litters with viable fetuses examined in this study (WIL 82008) was 114 (control=30; Larvadex groups=84) of which 29 were sired by buck 2871 representing 25.4%. If the Agency were to consider the position taken by the registrant that buck #2871 was "genetically defective" then excluding data from those inseminated does, the number of litters available for examination for the 0, 5, 10, and 30 mg/kg groups was 24, 27, 20, and 22, respectively and the malformation rate is as follows:

STRUCTURAL ABNORMALITIES (EXCLUDING DOES SIRED BY BUCK 2871)†

	Control	5 mg/kg	10 mg/kg	30 mg/kg
# litters examined	24	27	20	22
Cranio-facial defects	2(8.3%)	1(3.7%)	1(5.0%)	0
Mid-line fusion defects	0	2(7.4%)	0	0
Axial skeletal defects	0	2(7.4%)	4(20.0%)	2(9.1%)
Sternebral defects	2(8.3%)	2(7.4%)	1(5.0%)	1(4.5%)
Diaphragmatic hernia	0	0	1(5.0%)	0
Blood vessel°	7(29.2%)	10(37.0%)	10(50.0%)	7(31.8%)
Gall bladder absent/small	2(8.3%)	4(14.8%)	8(40.0%)	3(13.6%)
13th rudimentary ribs	14(58.3%)	16(59.3%)	11(55.0%)	13(59.1%)
Stern. 5/6 unossified	9(37.5%)	10(37.0%)	9(45.0%)	12(54.5%)
27 presacral vertebrae	13(54.2%)	22(81.5%)	12(60.0%)	11(50.0%)
Hyoid body/arch unossified	0	0	1(5.0%)	3(13.6%)
Total with malformations	5(20.8%)	5(18.5%)	7(35.0%)	3(13.6%)

(°) Left carotid arises from brachocephalic trunk
(†) Calculated by this reviewer

From the above data, no apparent dose-response relationship was observed except for a trend increase in the incidence of sternebrae 5/6 unossified. With respect to diaphragmatic hernia, only one fetus (#1, doe #2013) in the 10 mg/kg group had this defect. The total number of litters with malformations and not sired by buck 2871 was 5, 5, 7, and 3 for the 0, 5, 10, and 30 mg/kg groups.

The question as to whether the abnormalities found in 2 control and 6 Larvadex litters were related to "genetic defects associated with buck #2871" would require further investigation and the investigators' statement was not demonstrated by supporting data. Further, the postnatal segment of this investigation (WIL 82008) did not provide any insight into this question since all does in the postnatal phase were not inseminated with buck #2871.

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II. POSTNATAL PHASE FINDINGS

A. MATERNAL TOXICITY

During the lactation period, there is no evidence of a compound-related effect relative to maternal absolute weights, weight gains, and reproductive performance. The mean litter size was not significantly different among all groups. Although a small decrease in the live birth index was noted, a statistical difference was not obtained.

B. DEVELOPMENTAL TOXICITY

At birth and throughout lactation, pups from the treated groups gained similar weight to the controls. The survivability of the offspring was monitored at different intervals during lactation and no biological differences were found. The day 4 viability index as well as the day 21 and 28 weaning indices were comparable between the control and treated groups.

The authors reported that the Staples' technique was used to examine all stillborns and pups dying from days 0-4 of lactation whereas only a detailed necropsy was performed on culled pups, pups dying from days 5-28 of gestation, and pups at final sacrifice. It is the opinion of this reviewer that data from pups which died from days 0-4 are of utmost importance since existing developmental defects may be related to early death of these pups during this period. Mortality of the offspring after postnatal day 4 is important as well since many factors including maternal neglect, food deprivation, and hormonal alterations, may also affect offspring' survivability

a. "Days 0-4 dead" pups

Cyclopia, omphalocele, and cleft palate were noted in one "days 0-4 dead" pup (#1, doe 1394) of the 30 mg/kg dosage level. Hydrocephaly was found in two "days 0-4 dead" pups of the 30 mg/kg dosage level. No malformations were noted in "days 0-4 dead" pups in the 5 and 10 mg/kg groups but one control pup was reported with omphalocele. Although only one single case of cyclopia was found at the 30 mg/kg dosage level with no similar finding in the cesarean phase of this study, this occurrence cannot be ignored completely due to its zero incidence in the historical control data and due to its presence in one fetus each in the 10 and 30 mg/kg of study WIL 82001 (Larvadex in Buckshire rabbits). This finding cannot be attributed to "genetic defect" since none of the delivered mothers were sired by buck 2871. Using the classification of cranio-facial defects to include cyclopia, hydrocephaly, and cataract, then the fetal (litter) incidences of 0(0), 2(2), 0(0), and 3(2) were found at the 0, 5, 10, and 30 mg/kg dosage levels, respectively.

b. Pups culled on day 4

In pups culled on day 4, one pup in the 5 mg/kg group was found with cataract. Cataract was also noted in 1 pup each in the 5 and 30 mg/kg groups in the offspring at terminal sacrifice. The investigators did not consider the presence of cataract as a compound-related effect since "this finding had been observed in the historical control data". However, it should be noted that cataract was not observed in the Dutchland historical control data (Appendix D of the final report) and at a very low incidence in the historical control data for New Zealand rabbits from

all suppliers (Appendix C of the final report) affecting 2/2569 fetuses (0.08%) and 2/434 litters (0.5%). Since this study was conducted with Dutchland rabbits, use of the Dutchland rabbit historical control data is more appropriate. The fetal and litter incidences with cataract in this postnatal segment were 2/160 fetuses (1.3%) and 2/25 litters (8.0%) for the 5 mg/kg group, 1/162 fetuses (0.6%) and 1/25 litters (4%) for the 30 mg/kg group. The fetal and litter incidences of cataract in all Larvadex-treated groups were 3/374 (0.8%) and 3/74 (4.1%), respectively, as compared to 0% in both the concurrent control data and historical control data for Dutchland rabbits.

CONCLUSIONS FOR STUDY WIL 82008

Under the conditions of this investigation (WIL 82008), a maternal NOEL may be established at 10 mg/kg/day with decreased maternal weight gains and food consumption noted at the 30 mg/kg/day (highest dose tested).

Several malformations were noted only in the Larvadex-treated groups but evidence of a dose-response relationship was not demonstrated. Nevertheless, three findings were of concern to this reviewer: diaphragmatic hernia, cyclopia and cataracts.

a) Diaphragmatic hernia was found in all Larvadex-treated groups at C-section examination affecting 1(1), 3(2), and 1(1) fetuses (litters) in the 5, 10, and 30 mg/kg groups, respectively (see pages 20 and 21 of this memo). Although evidence for a dose-response relationship was not demonstrated, its presence should be of concern since (i) it was previously noted in study WIL 82001 at the Larvadex 10 and 30 mg/kg dosage levels, (ii) its zero incidence in the concurrent control group, and (iii) its low historical control data incidence [0.2% fetuses, 1.1% litters].

b) Although only one single case of cyclopia was found at the 30 mg/kg dosage level of the postnatal phase, cyclopia is of concern since (i) it is an extremely rare abnormality with a zero incidence in the historical control data and (ii) its previous occurrence in one fetus each in the 10 and 30 mg/kg Larvadex groups of study WIL 82001.

c) Cataract was noted in 3 Larvadex-treated pups as compared to none in the concurrent control group and a zero incidence in the Dutchland historical control data.

In this investigation (WIL 82008), assessment of the developmental toxicity of Larvadex in rabbits is further complicated by the apparent discrepancy relative to the number of pups examined. The number of pups and litters examined at necropsy are presented by the authors in tables 19, 20, and 21 of the final report and are summarized by this reviewer on page 25 of this memo. The investigators stated that a necropsy using the Staples' technique was conducted on all stillborn and pups dying from days 0-4 of lactation (page 14 of final report) and table 21 of the final report indicates that 55, 59, 59, and 54 pups of the 0, 5, 10, and 30 mg/kg groups were examined, respectively. A re-examination of individual data revealed that the numbers of stillborns and dead pups from days 0-4 for the 0, 5, 10, and 30 mg/kg groups were, respectively, 68, 60, 63, and 49. The number of pups not examined for the 0, 5, and 10 mg/kg groups were, respectively, 13, 1, and 4 pups.

This reviewer could not explain the over-reporting of 5 pups examined in the 30 mg/kg group.

Table 15 of the final report summarizes the pup survival indices and it was calculated that 20, 31, 21, and 19 pups were culled from the 0, 5, 10, and 30 mg/kg groups, respectively. However, table 19 of the final report indicates that necropsy was conducted for only 13, 12, 17, and 11 pups in the 0, 5, 10, and 30 mg/kg groups, respectively. Apparently, 7, 19, 4, and 8 culled pups were not examined, respectively. Likewise, 15, 15, 13, and 28 pups were found dead from days 5-28 in the 0, 5, 10, and 30 mg/kg groups, but only 10, 13, 13, and 26 pups were examined, respectively. No explanations were given in the final report.

It is recommended that this developmental toxicity study with a postnatal phase (WIL 82008) be classified as Core Supplementary Data pending the registrant's submission of clarification for the above discrepancies. A developmental toxicity NOEL cannot be established at the present time but a maternal toxicity NOEL is tentatively determined at 10 mg/kg/kg.

The registrant's statement relative to "genetic defects associated with buck #2871" was not supported by definitive data.

It should be noted that a new developmental toxicity study with Larvadex in rabbits was not required since the registrant has fulfilled the regulatory requirements for rabbit teratogenicity: study WIL 82001 in rabbits was classified as Core Minimum Data (memo of Q. Bui to A. Heyward, 2/5/85) with a developmental toxicity NOEL of 5 mg/kg/day.

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