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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review Submission of "Weight-Of-Evidence" Evaluation of Cyromazine Potential to Cause Developmental Effects, from Ciba-Geigy. Comment on the worker safety in using the subject products according to the provided labels. EPA ID No. 100-631, 100-AAT, 100-ALA and 100-AAE; EPA Record No. 236828, 251360, 251361 and 251373; MRID # 409376-01; Caswell No. 167B; HED Project No. 9-0588 and 9-2104.

TO: Phil Hutton/Mike Mendelsohn (PM 17)
Insecticide-Rodenticide Branch
Registration Division (H7505C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 10/27/89
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Support/HED (H7509C)

THRU: Yiannakis M. Ioannou, Ph.D., D.A.B.T. Y M Ioannou 10/27/89
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and
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Registrant: Agricultural Division
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Action Requested: Review Cyromazine Weight of Evidence Evaluation to determine the potential for developmental effect and comment on worker safety in using the subject products according to provided labels.

Recommendations: The Agency has considered the "Weight-Of-Evidence" document submitted by Ciba-Geigy in support of the registrant's contention that cyromazine is not a developmental toxicant. Based on the document and additional data obtained by the TB-HFAS, the Wil teratology study number WIL 82001 has been reevaluated.

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The following are the reevaluated endpoints of the study:

MATERNAL NOEL = 10 MG/KG/DAY
MATERNAL LOEL = 30 MG/KG/DAY
DEVELOPMENTAL TOXICITY NOEL = 10 MG/KG/DAY
DEVELOPMENTAL TOXICITY LOEL = 30 MG/KG/DAY

Based on the above endpoints, cyromazine is no longer considered to be a developmental toxicant. The Agency agrees with Ciba-Geigy on the following "key observations" (partly extracted from the registrant's document):

1. Cyromazine is not teratogenic in the rat. This is born out in a study submitted in support of cyromazine registration.

2. There is a high spontaneous background malformation rate in Buckshire rabbits. This is evident from submitted historical control data.

3. The findings of cyclopia and craniofacial head defects apparently occur in "clusters" and these findings are not unusual. This is evident both from the submitted historical control data and from the reviewer's evaluation of the open literature data base both for cyromazine and for craniofacial birth defects.

4. The developmental effects observed in the submitted studies are not consistently reproducible across studies, are clearly not cyromazine dose-related, and appear to have occurred by chance. This is evident from reevaluation of the submitted studies and additional data and in the consensus of the Agency scientists involved in the evaluation of the cyromazine developmental toxicity data base.

It is the considered opinion of the Agency scientists that cyromazine does not represent a developmental hazard when used properly and the restriction on the labeling pertaining to developmental toxicity can be revised. Further, the labels submitted for the three (3) products containing cyromazine are adequate to address worker safety. Additional evaluation can be performed by the Non-Dietary Exposure Branch if neccessary.

The Agency was being conservative during the initial evaluation of the WIL 82001 study of cyromazine in rabbits due to the unusual findings of cyclopia in two dose groups (1 fetus in 1 litter in each of the 2 mid dose groups). Additional data were clearly required and have been met by the submitted weight-of-evidence document. The Agency does not agree with Ciba-Geigy's assertion that the single Buckshire male rabbit used in the WIL 82001 study was the cause of the observed malformation as the submitted data do not substantiate this claim.

DISCUSSION:

Ciba-Geigy has submitted 6 rabbit teratology studies to fulfill the Developmental Toxicity Guideline 85-2 (Teratology) for Cyromazine (table from the registrant's submission).

Study	Supplier/ Rabbit Strain	Lab/No.	Lab Conducted/Year
1,2	Langshaw, Dutch-Belted	IRDC 382-072, 382-072A	International Research & Development Corp. Mattawan, MI 1981
3	Buckshire New Zealand	WIL 82001	Wil Research Lab. Ashland, OH 1985
4	Langshaw, New Zealand	IRDC 382-104	International Research & Development Corp. Mattawan, MI 1985
5	Dutchland New Zealand	WIL 82008	Wil Research Lab. Ashland, OH 1985
6*	Buckshire, New Zealand	WIL 82005	Wil Research Lab. Ashland, OH 1985

* = (Cyromazine not tested; breeding study for background malformation rate)

Study 1 could not be evaluated alone (considered with Study 2) and is considered to be Supplementary Data by the Agency.

Study 2 was also considered to be Supplementary Data by the Agency (considered along with Study 1) as there appeared to be fetotoxicity at the lowest dose tested (10 mg/kg/day).

Study 3 was the study called into question by the Agency. The maternal NOEL is 10 mg/kg/day with a maternal LOEL of 30 mg/kg/day based on body weight gain depression and reduction of food consumption. Initially, based on a conservative approach by the Agency the Developmental Toxicity NOEL was 5 mg/kg/day with a Developmental Toxicity LOEL of 10 mg/kg/day based on findings of cyclopia and diaphragmatic hernia. This study is considered by the Agency as Minimum Data.

Study 4 was a repeat of the earlier studies (IRDC 382-072 and 382-072a) done at IRDC as a "backup" by the registrant in case of problems with the study conducted at Wil Laboratories. The animals in this study, however, had evidence of disease and was subsequently not reviewed by the Agency.

Study 5 was a repeat of the earlier Wil study (WIL 82001) and employed large numbers of pregnant animals per dose to allow for a post-natal evaluation of the effects of the chemical. This study identified a maternal NOEL of 10 mg/kg/day with a maternal LOEL of 30 mg/kg/day based on decreased body weight gain and decreased food consumption. Additional data were requested by the Agency to allow determination of the developmental toxicity endpoints. This study is considered as Supplementary Data by the Agency.

Study 6 was a specific study designed to demonstrate that the malformations noted in WIL 82001 were genetically related to buck #2749. Based on the information obtained in this study, no conclusions relative to the association between malformations and genetic defects could be made. Cyromazine was not tested in this study.

These studies will be discussed further in the following pages.

Other supporting studies include a rat teratology study (IRDC 382-070), where no specific developmental toxicity occurred. A 2-generation reproduction study in the rat with a systemic NOEL of 30 ppm and a systemic LOEL of 1000 ppm.

BACKGROUND

I. Specific Request from Ciba-Geigy.

CIBA-GEIGY has requested that the Agency reconsider the developmental toxicity classification for cyromazine. They provided the following rationale:

> 1. In the rabbit teratology study (Wil 82001) used to classify cyromazine, rabbits were obtained from a small, inbred population (Buckshire) which appears to have a higher background malformation rate than other strains. (The rate for this strain will be compared to some other strains in this document.) The incidence of rare malformations is also higher in the Buckshire strain control animals as observed in Wil Study 82005.
>
> Since the low incidence of the rare malformations, observed in Wil 82001 study was not dose-related, it is proposed that these malformations occurred by chance.
>
> 2. A single Buckshire male was used extensively in a non-random manner, more frequently in the treated groups. This single male was the sire for most of the malformed fetuses observed in this study.
>
> 3. Litter proportional analysis of the total malformations occurring across all dose groups indicate no significant increase in malformations.
>
> 4. A weight-of-evidence approach (similar to an oncogenic evaluation) is suggested using data from all of the teratology studies. Five studies have been conducted, with one study having 2X the normal number of litters. It should be noted that the malformations observed in the Wil 82001 study were not reproducible in any of the other cyromazine rabbit studies.
>
> 5. There is no evidence of developmental toxicity below maternally toxic levels in the rat teratology study with cyromazine.

II. Discussion of teratology studies conducted to fulfill Guideline 83-3, for cyromazine.

A. Study 1 - Conducted by International Research and Development Corporation (IRDC), Mattawan, MI (IRDC 382-072, 1981)

This study used Dutch Belted Rabbits from Langshaw Farms (Augusta, Michigan).

The registrant presented the following discussion:

> Although this study had insufficient numbers of pregnant animals to meet minimum guidelines, it provides useful data for a weight-of-evidence evaluation. Cyromazine did not cause any unusual malformations. When originally presented to EPA with study 2 included, CIBA-GEIGY concluded that both studies, together, provide sufficient data to judge the teratogenic potential of cyromazine in rabbits.

The EPA reviewer presented the following discussion (Memorandum, Q. Bui to H. Harrison, July 11, 1984) on this study:

> During the dosing period (days 6-28), maternal toxicity and/or stress is expressed by the decrease in maternal weight gain observed in all treated groups. Although the control animals did gain weight (8 grams during days 6-28), this gain is questionable for animals bearing each an average of 6.1 live fetuses. Furthermore, this finding could not be verified due to the lack of historical control data on maternal weight gain. The maternal weight gain depression is probably a consequence of either decreased food intake, which could not be verified, or unsatisfactory health status of the animals.
>
> The decrease in conception rate observed in all groups, in conjunction with a high incidence of preimplantation loss (48.1, 44.2, and 44.9% for the groups receiving 25, 50, and 75 mg/kg, respectively) suggest that either dosing was performed prior to completion of implantation or due to the unsuitability of the animals used. The latter is further illuminated by the presence of hydroceles on oviduct, pitted kidneys, and congested lungs found at necropsy.

In the performance of teratology studies, as in other biological tests, errors can arise if procedures are not followed accurately or from the selection of unsuitable and/or unhealthy animals. Due to the questionable dosing period and animal status, no scientific conclusion could be drawn from the submitted data.

The study was subsequently classified as Core-Supplementary Data, and was discussed in a further document (Memorandum, Q. Bui to A. Heyward, December 13, 1984) between the registrant and the Agency. When considered alone, the utility of the study is in question; however, the Agency agrees that this study did not show any unusual observations relating to developmental toxicity.

B. Study 2 - Conducted by IRDC (IRDC 382-072A, 1981).

This study used Dutch Belted Rabbits from Langshire Farms.

The registrant presented the following discussion:

> This study was classified by EPA as supplemental because of suspected diseased animals. This report also provides useful data as part of a weight-of-evidence evaluation of cyromazine's teratogenic potential.

The EPA reviewer presented the following discussion (Memorandum, Q. Bui to H. Harrison, July 11, 1984) on this study:

> 1. Suitability and/or health status of the animals used in this study are questionable due to the following reasons:
>
> a) Coccidiosis was found during the acclimation period and all animals were apparently treated with Purina Sulfa and only those that were free of infection were used. It is unclear why the testing facility did not replace those animals with a new and healthier population.
>
> b) The final report indicates that "congested lungs and/or foci on the lungs, pitted kidneys, and hydroceles on the oviducts" are "common findings in Dutch Belted rabbits of this age". These necropsy findings may be characteristic of pasteurellosis and/or nosematosis. Indeed, congested lungs and/or foci may be due to pasteurellosis and/or gavaging errors and nosematosis has been known to produce pitted kidneys. No statement is mentioned in the final report to affirm that these infections had been properly diagnosed and ruled out.
>
> c) Erratic findings in maternal body weight gain during the dosing period and throughout the entire gestation period were found between the two control groups of Experiment I and II. Variations in maternal body weight gain data between the two control groups render interpretation of the results difficult and also raise the question of health suitability of the animals tested. Dutch Belted rabbits are known to gain less weight during gestation than other strains

used in teratology testing. But, they are also known to perform considerably better than in the submitted studies. Due to the reduced weight gain of these rabbits, the need for food consumption data for proper assessment of maternal toxicity becomes even more important than when other strains are used. The food consumption data were not submitted for this study.

d) "Decreased amount of feces in several animals in the treated groups" was the only clinical observation summarized in the final report. Clinical observation data are not available to confirm the health status of the animals used.

e) The unexpected zero incidence of malformations observed in the control groups in both Experiment I and II makes it more difficult to properly evaluate the results.

2. Technical errors:

Technical errors, apparently, are characterized in this study by esophageal perforation and congested lungs possibly due to gavaging error and/or infection 5/6 animals that died and aborted.

Consequently, the two uncertainties mentioned above restrict the utility of this study.

3. Teratogenic potential:

The zero incidence of malformations in the control group makes it difficult to properly assess the significance of malformation findings appearing in the treated groups. Although anomalies were found in all treated groups, individual malformations were not present in a dose related manner. However, the increased litter incidence of malformations (33.3, 30.8, and 40.0% for the groups receiving 10, 30, and 60 mg/kg, respectively) cannot be overlooked in terms of teratogenic potential. Furthermore, two malformations - fetal anasarca observed in both Experiments I and II and cataract found in Experiment II - that were non-existent in the provided compiled historical control data were seen in the treated groups and also cannot be neglected.

The registrant in several instances indicates that malformations observed in the treated groups were also mentioned in the compiled historical control data and hence did not reflect a teratogenic response. In this reviewer's opinion, anomalies found in a study have to be compared with the concurrent control findings and cannot be dismissed because the treated data "fell within the range of the historical control". To be useful, the historical control data should be provided in a format containing information concerning collection time, date, and study number.

"Rib anomalies" was described by the testing facility as a malformation. If a full description of "rib anomalies" is given, this incidence may possibly be classified as variations instead of malformations.

4. Fetal toxicity:

Fetal toxicity is characterized by dose-related increase in "27 presacral vertebrae" and "13th full ribs" with statistical differences in "13th full ribs" and "rib variations" found at all dosage levels including the lowest dose used (10 mg/kg) and by dosage levels including the lowest dose used (10 mg/kg) and by the presence of "talus unossified" observed only in the treated groups.

5. A NOEL for maternal toxic effects could not be determined due to the questionable health status of the animals. However, in terms of body weight gain, the 10 mg/kg dose level was comparable to the concurrent control values.

No NOEL for fetotoxicity was determined (NOEL < 10 mg/kg). The teratogenic potential of CGA 72662 could not be properly assessed by this study due to the above listed considerations, although some increased incidences in malformations were noted in all treated groups.

In conclusion, to adequately assess the teratogenic potential of CGA 72662, a new study in rabbits is requested (with adequate historical control data presented). Maternal clinical observations food consumption data, and appropriate individual litter and fetal data should be submitted. In consideration of the fetotoxicity noted at the lowest dose level, the registrant should consider retesting with at least one dose level below 10 mg/kg.

This study was also subsequently classified as Core-Supplementary Data, and was discussed in a further document (Memorandum, Q. Bui to A. Heyward, December 13, 1984) between the registrant and Agency. The health status of the animals in this study was in question; however, it was determined that these animals were healthier than the first stuay (IRDC 382-072) and the maternal NOEL was determined to be 10 mg/kg/day. The NOEL for developmental toxicity could not be determined in this study due to the zero incidence of malformations in the concurrent control and the significant increase in rib variations noted in the low dose group. The Agency does agree that no unusual observations were noted in this study.

C. Study 3 - Wil Research Laboratories (WIL), Ashland, Ohio (WIL 82001, 1985).

This study used New Zealand Rabbits from Buckshire Farms, and was the study called into question.

The registrant presented the following discussion:

> This study is classified by EPA as core guideline. The fetotoxic NOEL was set at 5 mg/kg and the maternally toxic NOEL was 10 mg/kg. EPA considers this study as an indication that cyromazine may be positive for developmental toxicity based primarily upon the occurrence of cyclopia in one fetus in each of the low mid and high mid dose groups of the 10 and 30 mg/kg, respectively. A single Buckshire male was used extensively in a non-random manner, more frequently in the treated groups. This single male was the sire for most of the malformed fetuses observed in this study.

Further,

> although a very limited number of unusual malformations occurred, a weight-of-evidence evaluation shows that their appearance is by chance and are not compound-related. This position is further supported by evidence which shows cyromazine does not cause an increase in the malformation rate (litter proportion analysis) in rabbits except at maternally toxic levels of 30 mg/kg or higher. The occurrence of the type of malformations observed in this study is at a very low incidence and not dose responsive, a strong indication that they are not compound-related.
>
> Additional historical data from several reference sources on the specific occurrence of cyclopia...Mid-Atlantic Regional Teratology Association (MARTA) has published an incidence of cyclopia as 1.4 per 10,000 Although the occurrence of cyclopia is considered unusual, craniofacial defects which include cyclopia have been cited as one of the more common findings in rabbits and cyclopia has been cited to occur as a cluster effect in rabbits.

The EPA reviewer presented the following discussion (Memorandum, Q. Bui to A. Heyward, February 5, 1985) on this study:

> 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 (sic) 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).
>
> 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 the 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 cannot be attributed to random occurrence since both malformations were observed at the next highest dose (30 mg/kg).

The investigators mentioned that 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.

This study was classified as Core-Minimum Data and the reviewer made these following recommendations:

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 impregnation for each study were not mentioned.

This study was also discussed further between the registrant and the Agency (Memorandum, Q. Bui to A. Heyward, April 24, 1985) and a peer review of this study was conducted by the Reproductive Effects Assessment Group (REAG) of the Office of Research and Development (ORD). The REAG peer review document presented the following conclusions (Memorandum, C. Kimmel to Q. Bui, April 5, 1985):

> The data from this study clearly indicate a NOEL for maternal effects at 10 mg/kg/day. The data on fetal effects are not as clear because of the small number of litters at the 10 mg/kg dose level. I think that both the 30 and 60 mg/kg dose groups show an increased incidence of malformations. Since there are several malformations at 10 mg/kg which also occur at higher doses, this dose is somewhat suspect. Therefore, under these circumstances it would seem appropriate to set 5 mg/kg as the fetal NOEL.

Specific details of the defect observed in this study (WIL 82001) will be discussed later.

D. Study 4 - IRDC (IRDC 382-104, 1985)

This study used New Zealand White Rabbits from Langshaw Farms.

The registrant presented the following discussion:

> This study was conducted as a contingency plan, if the Wil 82001 study failed, and planned at the same time as the Wil study, but not initiated because of animal supply; it was subsequently reported later.
>
> The study report has been submitted to EPA but CIBA-GEIGY believes the report has not been reviewed and/or classified by EPA at this time. Some of the animals in this study were patently ill, having hemorrhagic GI tracts, abortions, and in some cases, death. Upon consultation with EPA, it was agreed that this would most likely not satisfy minimum EPA guidelines. Although this study does have some ill animals, there were sufficient numbers of healthy animals, having litters to allow an evaluation. Thus, this study has merit when performing a weight-of-evidence evaluation. Animals in the 30 mg/kg group were below the acceptable number but it has already been established that this dose is clearly a maternally toxic level. Evidence shows that cyromazine did not cause any increase in developmental toxicity in rabbits in this study at any dose level.

This study has not been reviewed by the Agency due to indications of disease in the animals.

E. Study 5 - Wil (Wil 82008, 1985)

This study used New Zealand White Rabbit from Dutchland Farms.

The registrant presented the following discussion:

> The purpose of this study was to provide a large number of animals on test, with increased numbers of litters, to thoroughly evaluate the developmental toxicity potential of cyromazine in rabbits.
>
> This study (Wil 82008) was designed with protocol input from EPA scientists and had at least 25 litters per group for a routine prenatal developmental evaluation of the fetuses (at C-Section) and at least 25 females allowed to deliver, nurse and wean their kits. The objective of the prenatal evaluations were met and it is CIBA-GEIGY scientists' position that adequate data exists from this portion of the study to completely evaluate the developmental toxicity of cyromazine. The postnatal phase used female rabbits for a first time pregnancy and these animals were allowed to deliver. Because of apparent poor motherhood for these animals across all groups, a high fetal death rate occurred and in many cases total litter loss occurred. The reliable data that can be evaluated from this postnatal phase are the kit survival indices through day 28 and the kit growth (as evaluated by pup body weights) through day 28.

The EPA reviewer presented the following discussion (Memorandum, Q. Bui to T. Gardner, November 21, 1986) on this study:

> 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. 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 investigators stated that a necropsy using the Staples' technique was conducted on all stillborn and pups dying from days 0-4 of lactation and that 55, 59, 59, and 34 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, 58, 60, 63, and 40. 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.

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, 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 10mg/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.

The above mentioned deficiencies have not been resolved (additional data have been submitted, but not reviewed and the results of the lab audit, if conducted, have not been submitted to HED) and therefore, the developmental toxicity NOEL cannot be determined for this study. The last paragraph as stated above still stands; however, the developmental toxicity NOEL has been reevaluated and will be discussed later.

The following comments on this study were received from Dr. Carol Kimmel (Memorandum, C. Kimmel to Q. Bui, April 6, 1987) of the Reproductive Effects Assessment Group of the Office of Health and Environmental Assessment (OHEA):

> I have reviewed the original data from this study and concur completely with the review done by Dr. Bui (memo dated November 21, 1986). I agree with a maternal NOEL of 10 mg/kg. Although there was not a clear dose-response relationship for malformations in this study, there was an increase in certain types of malformations, particularly external and visceral malformations in Larvadex-treated groups which was in all cases greater than that seen in the control group. Since many of these individual defects were seen at very low incidence, this may explain why there was not a clear dose-response relationship. Until the postnatal data are clarified according to the request for additional information in the review by Dr. Bui, a NOEL for developmental toxicity cannot be determined. When this information is made available, it may be appropriate to combine the defects observed prenatally with those detected postnatally to determine a developmental toxicity NOEL.
>
> The assertion in this study that several cases of umbilical hernia from litters sired by male #2871 are "genetically-related" again appears to be unfounded strictly on the basis that this same male also sired a number of completely normal litters. All of the comments made above about this issue under the review of study #82008 apply here as well, and I believe that the observed frequency of the observations could be explained by chance.

F. Study 6 - Wil (WIL 82005, 1985)

This study used New Zealand White Rabbits from Buckshire Farms.

The registrant presented the following discussion:

> The purpose of this study was 1) to evaluate the naturally occurring malformation rate in the Buckshire strain of rabbits and 2) to specifically determine if one male, used extensively as a semen donor in Wil 82001 study, would sire fetuses with an increase incidence of head malformations.
>
> Protocol input was also received from WPA scientists on this study. Cyromazine was not tested. Three large groups of Buckshire does (>25/group) were evaluated. The one male from Wil 82001 study was mated to two groups of does with one group stressed by sham-gavage and the second group remained unstressed during gestation. The sham-gavage group was done to evaluate the effects of normally caused stress during a standard teratology study. A third group was mated to another Buckshire male for comparison purposes.

The EPA reviewer presented the following discussion (Memorandum, Q. Bui to T. Gardner, November 21, 1986) on this study:

> It is recommended that the study of the incidence of fetal malformations in the control populations of Buckshire New Zealand rabbits in 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 #2794 (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."

The following comments on this study were received from Dr. Carol Kimmel (Memorandum, C. Kimmel to Q. Bui, April 6, 1987):

A breeding study submitted to the Office of Pesticide Programs (WIL #82005) was conducted with the objective of determining "the incidence of spontaneous fetal malformations in NUK:(CRL)NEWZBR rabbits using selective males." However, the study as conducted appeared to have more specific objective, that of determining the spontaneous malformation rate in litters sired by one particular male (#2749) that had sired two litters (each of which had one fetus with cyclopia) treated with Larvadex in a previous study (WIL #82001). In that study, the authors concluded that this malformation was "genetically-related" rather than being due to Larvadex. The objective for study #82005, as I have restated it, may have been valid but instead of comparing the influence of male #2749 with the general population in this rabbit strain, the comparison was made to essentially one other male (#2272; male

#1195 was used to inseminate only 6 out of 69 females in the comparison group). No instances of cyclopia were seen in any litter in this study, and the total incidence of malformations in litters sired by male #2749 was similar to that in the group sired by #2272 and #1195, as well as in other historical control data from this strain of rabbits. Even if one considers only craniofacial defects, the incidence in the groups sired by #2749 was similar to other groups.

As for the assertion made in the earlier study that cyclopia was "genetically-related" to male #2749, study #82005 did not address the issue of heritability. The frequency of craniofacial malformations in litters sired by this male in either study #82001 or #82005 does not support a heritable trait, and appropriate crosses and backcrosses would need to be done in order to show that this was a heritable trait. It is my opinion and that of Dr. Vicki Dellarco (geneticist in RRAG) that the observed frequency of the malformations in question could be explained by chance.

I would also like to comment on several observations listed in the individual fetal visceral records as major blood vessel variations. The first observation, retroesophageal right subclavian, was seen in 4 fetuses out of 3 litters in group 2 and 2 fetuses out of 2 litters in group 3. The second observation, stenosis of the left carotid, entire length, was seen in 1 fetus out of 1 litter in group 2. The third, accessory left subclavian, was seen in 2 fetuses out of 2 litters in group 2. I would argue that all of these observations should be listed as malformations since they constitute major abnormalities in the development of the great vessels. However, this would not change my overall conclusion about the study.

The registrant offered the following comments in summary:

> When considering 1) the weight-of-evidence approach with data from all of the cyromazine rabbit studies, 2) the unusually high spontaneous background malformation rate with Buckshire animals, 3) the overuse of the Buckshire male in the one study with the associated malformations, and 4) cyclopia, and related head defects, is found as one of the more common type of malformations in rabbits and seems to appear in clusters in some studies, it is proposed that EPA not classify cyromazine as a developmental toxicant.

III. Discussion of the Developmental Toxicity Potential of Cyromazine.

In reference to Study 3 - Wil Research Laboratories (WIL), Ashland, Ohio (WIL 82001, 1985). This study used New Zealand Rabbits from Buckshire Farms, and was the study called into question. The registrant provided a historical background check on the incidence of cyclopia in the rabbit. MARTA historical control data states that cyclopia occurs in an incidence of 1.4 per 10,000 examined fetuses. A further examination of this anomaly was discussed by A.K. Palmer in an article entitled "Drugs and Fetal Development" (Adv. Exp. Biol. Med. 27, 45-60, 1972) where he stated:

> In all species craniofacial malformations are the most common and among the craniofacial malformations of the rabbit there is a fascinating sequence of reduction progressing from monorhina through varying degrees of cebocephaly, in which the inter-ocular distance is reduced until a cyclopian (or rhinencephalic) condition is reached. Further reduction brings about arrhinencephaly (or anops), otocephaly, and finally complete acrania or acephaly.

The stated incidence of cyclopia in this article was 7.94 per 10,000 New Zealand White Rabbit fetuses. Historical data from Wil Laboratories did not separate out individual anomalies.

There appears to be a high spontaneous background malformation rate in Buckshire rabbits with the observations of cyclopia and craniofacial head defects occurring in "clusters". This is evident both from submitted historical control data and from this reviewer's evaluation of the open literature data base both for cyromazine and for craniofacial birth defects.

An examination of the open literature data base by this reviewer found some interesting observations. As far back as the early 1960's, it was found that the steroidal alkaloid, Veratrum Californicum would induce cyclopia and other craniofacial defects in sheep and rabbits with a tendency towards this defect in chick embryos and mice. One of the alkaloids extracted from the plant is called cyclopamine. Other steroidal alkaloid bearing plants such as the Veratrum Lupinus and Conium also produce this defect in livestock (cattle, sheep and swine). Other species are affected including fish and amphibians (the hydrazine compound, UDMH induced cyclopia in South African clawed toads). Further research has shown that steroid biosynthesis is affected by the ingestion of these plant alkaloids. There is also evidence that some antifungal drugs can induce cyclopia in cats and certain anticancer drugs have produced this anomaly in chick embryos.

The chlorinated pesticide heptachlor was found to cause cyclopia in chick embryos. There is evidence of the production of cyclopia in humans from exposure to the salicylates during pregnancy.

The above information indicates that the rabbit has a unique response to certain agents in the form of cyclopia; however, these agents have a steroidal structure which cyromazine does not possess. Further, a dose related increase in the numbers of observations would be expected from treatment with a compound that would induced such a malformation. There is an indication in the open literature that cyromazine was tested in pregnant sheep during the first trimester with "three treatments at twice the recommended concentration" and this did not affect "their progeny".

The developmental effects observed in the submitted studies are not consistently reproducible across studies, are clearly not cyromazine dose-related, and appear to have occurred by chance. This is evident from reevaluation of the submitted studies and additional data and in the consensus of the Agency scientists involved in the evaluation of the cyromazine developmental toxicity data base.

It is the considered opinion of the Agency scientists that cyromazine does not represent a developmental hazard when used properly and the restriction on the labeling pertaining to developmental toxicity can be revised. The Agency was being conservative during the initial evaluation of the WIL 82001 study of cyromazine in rabbits due to the unusual findings of cyclopia in two (2) dose groups (1 fetus in 1 litter in each of the 2 mid dose groups). Further, the observations of diaphragmatic hernia and hydrocephaly were not dose related and were within the historical control range for this strain of rabbits.

The Agency does not agree with Ciba-Geigy with the assertion that the single Buckshire male used in the WIL 82001 study was the cause of the observed malformation as the submitted data do not substantiate this claim.

The following are the endpoints of the study:

MATERNAL NOEL = 10 MG/KG/DAY
MATERNAL LOEL = 30 MG/KG/DAY*

DEVELOPMENTAL TOXICITY NOEL = 10 MG/KG/DAY
DEVELOPMENTAL TOXICITY LOEL = 30 MG/KG/DAY**

* Based on depression of body weight gain and reductions in food consumption.
** Based on increases in skeletal anomaly observations.

Based on the above endpoints, cyromazine is no longer considered to be a developmental toxicant.