11-5-84

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

NOV 5 1984

MEMORANDUM

SUBJECT: A response to Rhone-Poulenc Inc.'s comments concerning the review of two teratology studies with Iprodione.
Accession#253443 CASWELL#470A

TO: Henry Jacoby, PM#21
Registration Division (TS-767C)

FROM: Quang Q. Bui, Ph.D.
Section V, Toxicology Branch
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THRU: Laurence D. Chitlik, DABT
Section Head, Sections V & VI
Toxicology Branch/HED (TS-769C)
and
William L. Burnam, Chief
Toxicology Branch/HED (TS-769C)

10/26/84

11/02/84

Registrant: Rhone-Poulenc Inc.
P.O. Box 125
Monmouth Junction, NJ 08852

Action Requested:

Evaluation of additional data submitted for two teratology studies with Iprodione in rabbits (IC. DREB#730925) and rats (IC. DREB#731016).

BACKGROUND:

The teratogenic potential of Iprodione has been studied in rabbits (IC. DREB#730925) and rats (IC. DREB#731016). Both studies had been submitted to the Agency (Accession #232712) and reviewed (6/22/81).

The rabbit study was classified as Supplementary Data by this reviewer (6/22/81) since a NOEL for maternal and fetal toxicity could not be demonstrated from the submitted data. The registrant, in this action, has submitted additional data and discussed the issues raised in the previous evaluation.

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The rat study was tentatively classified as Minimum Data (memo of 6/22/81). However, the historical control data and raw data were requested by the Agency to confirm the reported findings. The raw data were made available to the Agency in this action.

RECOMMENDATIONS:

A. The teratology study in rabbits (IC.DREB#730925) is still classified as Core Supplementary Data since a fetotoxic NOEL can not be demonstrated from the dosage levels selected (100, 200, and 400 mg/kg). Dose response relationships and statistically significant increases in skeletal anomalies are found at all dosage levels. Furthermore, the registrant is requested to provide explanations for the discrepancies noted between the final report and raw data (see Section I.6.B). The historical control data provided is of restricted value. To be acceptable, the historical control data should be collected within a 2 year period of the main study and should include study identification and date, findings on a per litter basis as well as individual fetal incidences. The teratogenic potential of Iprodione cannot be assessed with certainty due to the high incidence of embryolethality observed in the two highest doses (see discussion on p. 9).

B. The teratology study in rats (IC. DREB#731016) was previously tentatively classified as Core Minimum Data pending the submission of raw data and historical control data. This study is now classified as Core Supplementary Data after evaluation of the raw data (written in French) for the following reasons:

1. The results, as presented in the final report, are data combined from two studies (preliminary and primary study). This approach is of questionable scientific merit since the two studies were conducted 2-3 months apart. Recalculation of all parameters investigated (excluding preliminary study data) is therefore requested (see discussion on p. 10).

2. Sternum anomalies are the only skeletal findings in this study. In order to confirm that sternum anomalies are probably the only variations observed, the Agency has requested (memo of 6/22/81) that historical control data for this species and strain be submitted. These data are still not available.

3. Incidences of sternum anomalies other than chequered sternum are unreported (see discussion on p. 12).

4. Discrepancies between the final report and raw data are noted (see discussion on p. 12).

5. There are no indications in the raw data to verify that soft tissue examinations were performed in one third of the fetuses.

6. The registrant is requested to provide explanations for all issues listed under Section IIB of this memorandum.

DISCUSSION:

I. Teratogenic study in New Zealand rabbits with technical Iprodione (IC. DREB#730925, 2/5/76).

1. Issue #1

"There is no indication to reveal whether the rabbits were inseminated or received as pregnant by a commercial supplier".

The registrant's response to this issue indicates that mating of rabbits for teratology studies is a common procedure in its testing facility. Raw data to confirm the mating process are also submitted.

Toxicology Branch response:

Supportive raw data are presently available to confirm that the mating process was organized at the testing facility.

2. Issue #2

"Administration of technical Iprodione, as performed in this study from gestation days 6-16, did not encompass the entire period of major organogenesis in rabbits which is from gestation days 6-18".

The registrant emphasizes that this study was conducted in 1973 prior to published guidelines and standard protocols and only the FDA regulatory view point (1966) was available at that time. Based upon several references cited (Palmer, 1980; Delahunt; Tuchmann-Duplessis), the registrant concludes that the two days of missing treatment (days 17 and 18 of gestation) would not influence the results since malformations would unlikely be induced during those last two days of major organogenesis.

Toxicology Branch response:

The Agency did notice that this study was conducted prior to the issuance of the 1978 EPA Proposed Guidelines. However, treatment of rabbits from gestation days 6-18 had been recommended by the FDA since 1966. The FDA approach has been evaluated and recognized by many investigators (Cook et al., 1969; Robson, 1970) prior to this study initiation (1973). Treatment from days 6-18 (inclusive) of gestation is also recommended "as a general rule" in rabbits in the Tuchmann-Duplessis book referenced by the registrant above.

It may be true that the two days of missing treatment (days 17 and 18 of gestation) would probably not significantly change the outcome of the results and the treatment period, as performed in this study, may coincide for the most part with the period of major organogenesis in rabbits. However, these statements are only correct in the strict sense of teratogenesis since susceptibility to teratogens decreases as organ formation advances.

It is generally accepted that developmental inhibition or, occasionally, pathologic degeneration may still be evident at a later stage. Treatment of dams throughtout the period of major organogenesis (days 6 to 18) is especially crucial in this study in which significant increases in skeletal variations were observed at all dosage levels tested. Those skeletal anomalies were indicative of developmental inhibition, hence, an extended treatment may significantly increase their frequency and/or severity.

Consequently, the registrant's approach, although basically sound, may not truly reflect all the effects induced by Iprodione in rabbits.

3. Issue #3

"The lowest dose tested (100 mg/kg) was associated with a significant decrease in maternal body weight gain during the period of treatment. Therefore, this reviewer concludes that a NOEL for maternal toxicity could not be demonstrated."

The registrant's response to this issue indicates that the decrease in body weight gain observed in the 100 mg/kg group is minimal and should be of no biological significance. This difference is attributed to the differences in mean body weight between the control and 100 mg/kg group at study initiation (3.33% difference in favor of the control). A maternal NOEL of 100 mg/kg is requested by the registrant since the litter size, mean fetal weight, and mean resorptions per litter between the 2 groups are comparable.

Toxicology Branch response:

In assessing body weight data, special considerations should be given to body weight gain data if differences in mean body weight exist among groups.

The following table illustrates the body weight gain data.

| Body weight changes (gram) | Control | 100 mg/kg | 200 mg/kg | 400 mg/kg |
|---|---|---|---|---|
| Days 0-6 | 90 | 60(66.7) | 90(100) | 60(66.7) |
| Days 6-16 | 120 | 20(16.7) | -20(-16.7) | -50(-41.7) |
| Days 16-28 | 170 | 200(118) | 200(118) | -230(-135) |
| Days 0-28 | 380 | 280(73.7) | 270(71.1) | -220(-57.9) |

Data extracted from Table 5, p.13.
Values in parentheses indicate percentage of the control for the same time interval.

During the dosing period (days 6-16), significant depressions in body weight gain were observed in all treated groups. The low, mid, and high dose groups averaged respectively 16.7, -16.7, and -41.7% of the control body weight gain. The body weight depressions observed were dose-related and compound related. Consequently, in terms of body weight gain, a maternal NOEL could not be demonstrated from the dosage levels selected. Other dose- and compound-related effects were also evident in the treated groups (see issues #4 and 5) throughout the entire study (days 0-28).

4. Issue #4

"Administration of technical Iprodione appears to produce a dose dependent increase in the percentage of resorptions, decrease in fetal weight and mean live fetuses per litter".

The registrant comments that statistically significant differences were not found between the control and 100 mg/kg groups in terms of percentage of resorptions, fetal weight, and litter size. The registrant also considers the dose-effect relationship observed as "purely forfuitous" and suggests a maternal and fetotoxic NOEL at 100 mg/kg.

Toxicology Branch response:

The percentage of resorptions, litter size, and fetal weight data are recaptured in the following table:

| | Control | 100 mg/kg | 200 mg/kg | 400 mg/kg |
|---|---|---|---|---|
| Mean Litter Size (All pregnant animals) | 7.38 | 6.83 | 5.23 | 1.50 |
| Mean Litter Size (excluding dams with total resorptions) | 7.38 | 6.83 | 6.80 | 6.00 |
| Resorptions (% of implantations) | 7.6 | 11.8 | 33.0* | 81.2* |
| Mean fetal weight (g) | 33.9 | 33.0 | 31.2 | 29.4 |

*Significantly different from control at $P < 0.05$.

From the above table, apparent dose-effect relationships are suggested by all the parameters investigated. A dose-effect relationsip may be considered as "purely forfuitous" if only one parameter is affected. It should not be regarded as occurring by chance by the Agency when a similar trend was recognized in all parameters. In addition, the Agency never indicates that statistical differences were found between the control and 100 mg/kg groups.

5. "Issue #5

"The data also demonstrate that administration of Iprodione produces a dose-dependent increase in ossification retardation with significant differences observed even at the lowest dose (100 mg/kg)".

The registrant's response to this issue states that no major abnormalities nor malformations were observed at this dosage level. Furthermore, the registrant indicates that the incidences of retarded ossification observed in the 100 mg/kg group are similar to those of the of the historical control data collected over a 3 year period (1979-81) by the same testing facility.

A fetotoxic NOEL of 100 mg/kg is requested by the registrant.

Toxicology Branch response:

Anatomic variations of the skeleton, especially in the number and morphology of ribs and vertebrae, are common in all mammalian species. Because of their frequency, skeletal variations may be attributed to normal occurrences, to genotype, to assorted environmental factors, to maternal-related effects, as well as to compound-induced effects. An increase in the incidence of a skeletal variation known to occur in control animals can be regarded as treatment-related if the increase is statistically significant and if the increase is dose dependent. In this study, the incidences of skeletal variations in all treated groups are significantly different from those of the control and follow a dose-dependent fashion. These incidences are summarized as follows:

| | Control | 100 mg/kg | 200 mg/kg | 400 mg/kg |
|---|---|---|---|---|
| Litters with variations/ litters examined (%) | 6/13(46) | 8/12(67) | 9/10(90) | 1/1(100) |
| % fetuses with variations | 8.3 | 21.9* | 36.7* | 66.7* |

*Significantly different from control at $P < 0.05$.

Consequently, due to the presence of statistical differences and dose-response relationship increases, the skeletal variations observed even at the lowest dosage level (100 mg/kg) could not be ignored since they can provide valuable supportive evidences of fetotoxicity. Note that the Agency did not consider the skeletal variations reported as malformations and, in fact, had clearly indicated that in its review of 6/22/81.

6. Other Issues

In this action, in addition to the comments addressing the deficiencies noted by the Agency, the registrant also includes the historical control data and raw data for this study.

A. Historical Control Data

The historical control data submitted is unacceptable by the Agency for the following comments:

a. Skeletal ossification data were collected from 1979-81 (six years after the completion of the main study).

b. Only a summary of cummulative findings is provided.

c. Lack of study identification.

d. Lack of individual study data.

e. Number of litters with skeletal variations as well as the number of litters examined are unknown.

To be appropriate, the historical control data provided should be collected within a 2-year period of the main study. These data should include study identification, date, findings on a per-litter basis as well as individual fetal incidences.

B. Raw Data

The raw data, written in French, were evaluated by this reviewer. The following comments are noted:

a. The incidences of delayed ossification (retard ossification), unossified (non-ossifié) sternebrae, and shortened ribs (côte plus petite que l 'autre) are not included in the final report.

b. Dam 22 - 100 mg/kg group: The raw data indicate:

"6 foetus pied arrière gauche tordu" (6 fetuses, twisted left hind paw).

"1 foetus patte droite tordue" (1 fetus, twisted right front paw).

These findings are not addressed in the final report.

c. Discrepancies between the final report and raw data.

i. The final report indicates 13 pups for Control #98 but only 12 are accounted for in the raw data.

ii. The final report indicates a zero (0) incidence of "missing rib" for the 100 and 200 mg/kg groups.

From the raw data, "missing rib" (abscence d'une côte du côté) is found in the following dams:

Low dose: #19: 1 fetus
Mid dose: #37: 1 fetus
#93: 1 fetus
#39: 2 fetuses

iii. Incidences of missing sternebrae.

| | Final Report | Raw Data |
|---|---|---|
| Control #7 | 0 | 3 fetuses |
| Low dose #23 | 2 | 3 |
| Mid dose #39 | 1 | 2 |

CONCLUSION:

This study is still classified as Supplementary Data due to the lack of a fetotoxic NOEL as evidenced by dose-response relationships and significant increases in skeletal variations observed at all dose levels selected (100, 200, and 400 mg/kg). The teratogenic potential of Iprodione in rabbits cannot be assessed with certainty due to the high incidence of resorptions found in the 200 and 400 mg/kg groups. Teratogenesis and embryolethality often have a similar threshold : they begin to appear at approximately the same dosage. Beyond this dosage level, embryolethality eventually overtakes malformation and precludes an acceptable number of survivors at term for teratogenic evaluation.

The registrant is requested to explain the discrepancies noted between the reported findings and the raw data as listed under Section I.6.B. The historical control data, as provided in this action, is of limited value.

II. Teratogenic study in Sprague-Dawley rats with technical Iprodione (IC. DREB #731016, 2/5/76.

A. Issue

This study has been previously reviewed by the Agency (6/22/81) and was tentatively classified as Core Minimum Data. In this study, anomalies of the sternum were the only reported findings. Consequently, historical control data and raw data for this study were requested by the Agency to confirm the findings.

In this action, the raw data (written in French) are submitted and subjected to a thorough evaluation by this reviewer.

B. Evaluation of additional data submitted

1. Historical Control Data

Historical control data collected from teratology studies in Sprague-Dawley rats conducted by the same testing facility are not included in this action. Consequently, the background incidences of sternum anomalies as well as of other skeletal variations in this species and strains (OFA-Sprague-Dawley rats) could not be determined. To this reviewer, it is still unlikely that no skeletal variations (except sternum) were noted in a study of this kind.

2. Raw Data

From the presently available raw data, this reviewer is now able to recognize that this investigation was conducted in two studies: preliminary (3/28-4/17) and main study (6/13-7/4). The data collected from both studies were combined and presented in the final report. The investigators then evaluated and discussed the results based upon the combined data. Combination of the data, as presented in the final report, is unacceptable by this Agency's standards since the two studies were not conducted concurrently. The following sets of data from the preliminary study were included in the final report: 3 (dams #26, 27, and 28), 0, 5 (dams #60, 61, 62, 63, and 64), and 5 (dams #115, 116, 117, 118, and 119) for the groups receiving 0, 100, 200, and 400 mg/kg, respectively. Omission of the preliminary study data from the final report will probably alter the outcome of all results reported. Recalculation of all parameters investigated (excluding preliminary study data) for all groups is requested.

3. Maternal Mortality

The final report, on page 16, indicates that "no deaths occurred in any of the groups". The raw data reveal that dam #99 of the 400 mg/kg group was found dead on July 3 (trouvee morte le 3/7).

4. Fetal Gross Observations

Gross observation of the fetuses was not addressed in the final report.

Evaluation of the raw data indicates the following incidences:

a. Dam #32 - 100 mg/kg group

1 fetus with a hemorrhagic spot on the femur (1 foetus avec 1 tâche hémorragique au membre posterieur droit)

b. Dam #72 - 200 mg/kg group

1 fetus: hematoma (1 F avec hematome)

c. Dam #73 - 200 mg/kg group

1 fetus: hematoma on right hind leg (1 foetus avec hematome à la patte arrière droite)

d. Dam #79 - 200 mg/kg group

1 fetus: absence of digits on front legs; small fetus (manque les doigts des pattes avant; foetus petit)

e. Dam #95 - 400 mg/kg group

1 hemorrhagic spot on the snout between the eyes (1 tâche hemorragique sur le museau entre les deux yeux)

f. Dam #102 - 400 mg/kg group

1 fetus: hematoma on right hind leg

g. Dam #106 - 400 mg/kg group

1 fetus: hematoma left front leg
2 fetuses: hematoma on mandible

These findings should be indicated in the final report and discussed.

5. Soft Tissue Examination

The final report states that about one third of the fetuses were examined in situ for visceral anomalies.

There are no indications in the raw data that visceral examination was performed for fetuses of the main study. The Wilson technique apparently was conducted on half of the fetuses of each litter of the preliminary phase.

6. Skeletal Examination

Skeletal staining apparently was performed on all fetuses of the main study. The incidences of delayed ossification, unossified, and absent sternebrae were not reported. For each of these findings, the registant is requested to calculate the percentage of fetuses and litters affected.

Furthermore, discrepancies between the raw data and final report are noted by this reviewer.

| Chequered sternum (number of fetuses) | Final Report | Raw Data |
|---|---|---|
| Control #5 | 0 | 9 |
| Control #11 | 0 | 1 |

CONCLUSION:

Based upon the presently available raw data, this study (IC. DREB#731016) can only be classified as Supplementary Data due to the following:

1. The results as presented in the final report are combined data collected from two different studies conducted at different time periods.

2. The incidences of delayed ossification, unossified, and absent sternebrae are not reported.

3. Discrepancies are noted between the final report and raw data.

4. Lack of historical control data to confirm that "sternum anomalies" are the major findings for the species and strain selected.

5. There are no indications in the raw data to confirm with certainty that visceral examinations were performed in one third of the fetuses.

6. The registant is requested to provide explanations for all issues mentioned under Section II.B.

## REFERENCES

Cook, M.J., Fairwealter, F.A. and Hardwick, H.: Further thoughts on teratogenic testing. In Proceedings of a symposium organized by the Italian Society of Experimental Teratology, 1967. Excerpta Medica Foundation, Armsterdam and London, 1969.

Robson, J.M.: Testing drugs for teratogenicity and their effects on fertility. The present position. British Med. Bull. 26, 212-216, 1970.

Tuchmann-Duplessis, H.: In Drug effects on the fetus - Monograph on Drugs Vol. 2, ed. H. Tuchmann-Duplessis, Adis Press.