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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM AUG 16 1982

TO: Robert J. Taylor (25)
Registration Division (TS-767)

THRU: Orville E. Paynter, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: Alachlor Teratology Study in Rabbits, #IR-79-022 (IRDC#401-060, 11/24/80), Accession#244369. A Response to Monsanto's Comments Concerning the 6/23/81 Review of this Study. CASWELL#11

Background:

A teratology study in rabbits, IR-79-022 (IRDC#401-060, 11/24/1980, Accession#244369), was submitted by Monsanto Company on 1/15/81 and reviewed on 6/23/81. The study was found to be invalid due to several considerations (see attached review of 6/23/81), and the Agency position was communicated to Monsanto in a letter dated 7/7/81 by Mr. Robert Taylor of the Pesticides Registration Division.

In light of an IRDC letter, dated 7/29/81, which discussed the issues associated with the invalidation of the rabbit teratology study, Monsanto requested on 8/26/81 that the Agency reconsider the position taken in the 7/7/81 letter in regard to the validity of this study.

Conclusions:

The rabbit teratology study is still classified as invalid and a new replacement study is requested. The issues regarding the invalidity of this study, which were presented in Mr. Robert Taylor's letter of 7/7/81 and addressed in the IRDC letter of 7/29/81, are discussed in the section below.

Discussion:

Issue #1

The study does not have valid control data due to the combination of the following factors:

a. The control group lost weight (59g) during the dosing period (day 6-28 of gestation) and two animals in this group died due to gavage errors.

b. The incidence of heart anomalies in this control group is high (8/66 fetuses and 2/10 litters) as compared to the historical control (2/741 fetuses and 2/118 litters). Also the incidence of scoliosis in this study is significantly higher than the historical control.

c. The fetuses in this control group are smaller (27.7 g) than the treatment groups (35.7 g in low dose, 28.5 g in mid-dose and 29.5 g in high dose) and the historical control (33.2 g).

d. Conjested lungs with red foci at necropsy (indicating the possibility of gavage error in more than the two animals that were reported dead due to gavage error).

IRDC response to issue #1 indicated that the effects seen in the control animals in this study are not remarkably different from what one might expect considering the species, nor is the magnitude of weight loss really significant; that rabbits are inconsistent eaters and maternal weight values are very reflective of this; and that it is not uncommon for control rabbits to show weight loss during the gestation period and have 6 or 7 normal size fetuses. Concerning death of 2 animals due to gavage error, IRDC indicated that it is not uncommon to lose one or two animals in a study as a result of intubation error and that the major concern is whether the deaths contributed to an insufficient number of fetuses available for teratologic evaluations. This was not the case in this study.

Toxicology Branch Response:

o Supportive data that demonstrate the invalidity of the control group include the combined factors, a, b, c, and d, listed above under issue #1. Among these factors is the body weight loss noted in the control animals during days 6 to 28 of gestation. In this study, this period was also the dosing period. We note that body weight loss is associated with the toxicity of many compounds, and we also note that the mean body weight loss

observed in the vehicle control group in this study was 59 g as compared to an average gain of 96 g in the low dose group during days 6 to 28 of gestation. The non gravid females and the gravid females that died or aborted before gestation day 28 were excluded from these calculations of the group mean maternal body weight changes (see our review of 6/23/82). However when these data were included in these calculations, the mean body weight changes were much higher, see table #1 below as presented in IRDC study#401-060, page 14:

Days of Gestation	Group Mean Maternal Body Weight Changes (g)			
	Control	Low	Mid	High
0-6	74	24	44	105
6-12	-33	68	-96	-52
12-18	14	11	-5	-25
18-24	-61	22	-59	-46
24-28	-16	18	-23	-78
0-28	-22	143	-139	-96
6-28	-96	119	-183	-201

In the above table the maternal control group mean body weight loss is 96g during gestation days 6 to 28. This figure is much higher (almost 2x) than the 59g figure we calculated for this group within the same period (see our review of 6/23/82).

Also, the actual decrease in the maternal mean body weight of the control group during the whole gestation period (days 0 to 28) is much higher than the figure presented in table #1 above (-22g) if we compute these changes on the basis of the actual maternal mean body weight at day 28 of gestation (i.e. less the mean weight of the pups):

1) Maternal body weight of control animals at day 0 of gestation = 2955g.

2) Maternal body weight of control animals at day 28 of gestation = 2933g.

3) Maternal fetal weight of control pups = 27.7g.

4) Average number of fetuses per control female = 6.6 fetuses/dam.

5) Mean weight of all pups per control female :

6.6 x 27.7 = 182.82g

6) Actual maternal control body weight on day 28:

Step#1 - Step#5

2933g - 182.82g = 2750.18g

7) Actual maternal control body weight loss during gestation (days 0 to 28):

Step#1 - Step#6

2955g - 2750.18g = 204.82g

Similarly, the actual maternal body weight changes for the treatment groups are 190.93, -284.84, and -275.95g for the low, mid and high-dose groups respectively.

Evaluation of data from other IRDC studies and studies from other testing facilities indicates that control groups do not generally lose weight during gestation. Furthermore in the absence of any feed consumption data, it is impossible to determine if the noted decrease in the body weight of the control group during the indicated gestation periods is due to the rabbits feeding pattern or to other stresses (i.e. gavage, animal husbandry).

o In this study, the mean body weight of the control fetuses was lower than that of the treatment fetuses (27.7, 35.7, 28.6 and 29.5 g for the control, low-, mid-, and high dose groups respectively). The mean weight of this control group was also lower than the mean weight of the vehicle control fetuses (31.1 g) of the positive control study (IRDC#999-014, submitted with this study) and of the historical data (33.2 [30.7-36.2]) collected for 5 months, between 12/7/79 and 4/22/80.

A new set of historical data covering the period of 19 months (12/7/79-7/13/81) was submitted on 8/26/81. These data indicate that the mean weight of fetuses is 32.8 [27.7-36.2]. One can only assume that the addition of the control data under discussion in this study to the previously submitted IRDC historical data (11/24/80) contributed to the decrease of the minimum mean fetal weight range (27.7) reported in the new IRDC historical data.

This reviewer concludes that the the mean weight of the control fetuses in this study is smaller than what is usually noted in other IRDC studies and studies from other laboratories. Thus data from this control group are not useful as a basis for comparison of the mean fetal weight of the treatment groups.

o IRDC also stated that it is not uncommon to lose one or two animals in a study as a result of intubation error and that the major concern is whether the deaths contributed to an insufficient number of fetuses available for teratologic evaluation. Toxicology Branch emphasizes that the issue here is not the death by gavage of 2/16 control animals, but the general health status of the surviving rabbits (i.e. lung red foci; weight loss) which may also indicate poor animal husbandry conditions. We may also add that a poor gavage technique does not necessarily mean perforation of the lungs or deposition of the test substance in these tissues but it may mean irritation of these tissues.

It is true that a major concern in a teratology study is whether the death of the mothers contributed to an insufficient number of fetuses available for teratologic evaluations. However the issue in this study is not whether we have an adequate number of control fetuses, but the condition of these fetuses allowing use for valid comparison to the test groups. One problem in this study is that the maternal health appeared to have been affected by an outside uncontrolled factor, potentially to the extent that the control fetuses demonstrated reduced weight. Thus, as previously noted, data from these control fetuses are not useful as a basis for comparison to the treatment groups.

o Concerning the noted increase in the incidence of major heart anomalies in the control litters and the reported increase in the incidence of scoliosis in this study (see Issue#1, item b), IRDC responded by submitting the new set of historical data and indicated that these historical data should provide a more complete assessment of the incidence of heart anomalies and scoliosis seen in the control group of study 401-060 than the historical data originally submitted with this study. IRDC also indicated that the incidence of heart anomalies in eight (8) fetuses from two (2) litters in the controls is admittedly rather high, but that the malformation in this case is one of the more commonly observed in this strain and species as evidenced by the updated historical control, and that their frequency of occurrence in this study represents biological variation and has no bearing on the validity of the concurrent control data.

This reviewer notes that the new set of historical data probably includes the data of the vehicle control group, the validity of which is the subject of our discussion. In fact, the upper range limit of heart anomalies in this new historical control reflects exactly the levels noted in this vehicle control group (i.e. incidence of heart anomalies 12% and 20% in fetuses and litters respectively). Consequently we do not agree with IRDC comments that this effect represents a biological variation and has no bearing on the validity of the control group in this study. This decision is further ascertained by the review of other IRDC teratology studies and studies from other laboratories where fewer major heart anomalies were reported in the control groups.

Concerning the noted incidence of scoliosis in this study in comparison to the new historical data and the IRDC statement that "scoliosis in rabbits per se is considered of genetic origin in this laboratory" we can only mention that animals with major genetic defects lead only to confusion in assessing the teratologic potential of a compound and should be substituted by animals with a better genetic background. We also suggest that if IRDC notes an increase in the incidence of scoliosis or/and an increase in the incidence of heart anomalies in their present strain of rabbit to consider whether this strain of rabbit is suitable for future use or if it would be more appropriate to substitute it with another strain.

In summary, if the assumption is made that Alachlor is not a teratogen, the incidence of major heart anomalies and the incidence of scoliosis in all the animals (control + low + mid + high dose rabbits) used in this study are yet remarkably higher (4x) than the incidences noted in the new set of historical data, see table below:

	Historical Control		Animals in this Study	
	Fetuses	Litters	Fetuses	Litters
Total No. of Fetuses (F) & Litters (L) Examined	1156	184	244	43
Major Heart Anomalies				
No. affected	10	4	10	4
Percent	0.87	2.17	4.1	9.3
Scoliosis				
No. affected	7	7	6	6
Percent	0.61	3.8	2.5	14.0

Issue #2

Low level of implantations/dam (5.4) is noted in the low dose group as compared to the control group (6.9) and other treatment groups (6.3 in the mid-dose and 6.5 in the high dose).

IRDC stated in response to issue #2 that Implantation in rabbits occurs between gestation days 6 and 7, and that conceivably, implantation could be affected in rabbits as initial administration occurs on gestation day 6. IRDC also indicated that these findings at the low dose level are considered due to random occurrence and of no biological significance.

Toxicology Branch Response:

It appears that the low-dose group was treated before implantation was complete. The noted decrease in the number of implantations/dam at the low-dose level (5.4 implantations/dam) as compared to the control group (6.9 implantations/dam) appears to reflect a biologically significant level of embryolethality. According to J.G. Wilson "The early embryo appears to be more susceptible to adverse influences than any other developmental stage. The effects on early embryos are usually not seen until near term when resorption sites or dead, malformed, or growth-retarded fetuses are found in routine examination. Some chemical agents, particularly if given before or during the time of implantation, however, are capable of interfering with the process or of killing the blastocyst while implanting. In either case the products of conception are lost or are implanted for so short a time as to leave no recognizable implantation site. The apparent result would be failure of maternal animals to produce any issue (s.c. tissue) or to produce litters of the expected size." (Ref. Environmental and Birth Defects, J.G. Wilson, Chapter 9, 1973).

Conclusions:

No conclusion can be drawn from this study due to the following two points:

1) Invalidity of the control group.

2) The incidence of heart anomalies and the incidence of scoliosis are high and not comparable to historical data presented by the test laboratory. If the assumption is made that Alachlor

is not a teratogen, by totaling the incidence of heart anomalies in the control, low, mid and high-dose groups or by totaling the incidence of scoliosis in these groups, a 4x increase in each of these 2 anomalies is noted in the animals used in this study as compared to the recently submitted historical control data.

JDC 8/10/82

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8/15/82

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