11-28-79
CASWELL FILE
TOX R. V 004?4

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: November 28, 1979

SUBJECT: EPA Reg. No. 707-75; Stampede 3E on Wheat; PP#8F2106; Addendum (Accession No. 098949) CASWELL#325

FROM: Laurence D. Chitlik, Toxicologist
Toxicology Branch (TS-769)

TO: Robert Taylor
Product Manager#25 (TS-767)
&
Residue Chemistry Branch
(TS-769)

Action Requested: Review of teratology study in support of petition.

RECOMMENDATIONS:

The submitted rabbit teratology study does not support the requested tolerance.

Although no terata related to compound administration were observed at any test level, a NOEL for fetal toxicity was not determined in this study. At the low dose level of 4 mg/kg, fetal toxicity was demonstrated by reduced mean fetal weights, increased resorptions/litter and reduced fetal viability. Maternal toxicity was also demonstrated at the low dose level by reduced body weight gain during the gestation period.

Interpretation of these low dose findings was made more complex by less prominent effects at the intermediate dose level and as a consequence, a dose response relationship was lacking. Detailed evaluation of the data has now revealed that GLP type problems may be associated with the irregular study results. (See Study Conclusions, Point 3, A-E)

This teratology study must be repeated.

BACKGROUND:

A meeting was held with representatives of Rohm and Haas, 11/14/79, to discuss submitted data and explanations of the increased incidence of resorptions and decreases of fetal viability. Representatives of Toxicology Branch (W. Dykstra and S. Biscardi) were not convinced as to the Rohm and Haas explanations and L.D. Chitlik of Toxicology Branch was then requested to make a final determination as to the existence of a NOEL for fetal toxicity in this study.

REVIEW:

Rabbit, Oral Teratology, Stam Technical, 85.4% A.I., (Lot No. 9287), Hazelton Laboratories, Project No. 417-393, March 16, 1979

The test material was administered orally by gavage from day 6 through day 18 of gestation to 3 groups of twenty pregnant female rabbits at levels of 4, 20, and 100 mg/kg/day. A vehicle control group received 2 ml/kg/day of corn oil on days 6-18 of gestation. Initial body weights ranged from 3180 to 4595 grams. Animals were individually housed.

MLG

Sperm from male New Zealand White rabbits maintained as breeding stock were used for artifical insemination of the does. Ovulation was induced in each female by intravenous injection of 250 IU of human chorionic gonadotropin. One half the does/group were inseminated on October 31, 1978 while the remaining half/group were inseminated on November 2, 1978.

Dosages were calculated from individual body weights recorded on Days 6, 9, 12, 15 and 18 of gestation. Test group dosages were prepared as a suspension in corn oil and 2 ml of the test suspension per kg of body weight was maintained.

Observations for survival and toxic signs were made daily. Body weights were recorded on days 0, 6, 9, 12, 15, 18 and 29 of gestation. The report indicated that "the scheduled day 23 weighing interval was inadevertently missed for all animals."

All animals found dead were necropsied and on day 29 all surviving animals were sacrificed by carbon dioxide asphyxiation and an injection of 95% absolute alcohol to the base of the brain. All fetuses were removed by cesarean section and the number of corpora lutea, implantation sites, resorptions, live fetuses and dead fetuses in each uterine horn were recorded. Fetuses were individually identified, weighed, externally examined, and the crown-rump distance measured and recorded.

The viscera of approximately 1/3 of the fetuses was examined by a modified Staples' technique. Heads of the fetuses were removed, fixed and sectioned by Wilson's free hand razor technique.

The report states that "one-half to two-thirds" of the fetuses of each litter were "examined internally, eviscerated, cleared and stained, and then examined for skeletal anomalies and the degree of ossification."

Changes in mean maternal body weights and mean fetal weights and lengths were compared to controls by Student's t-test when the variances did not differ significantly. When variances were statistically different, Cochran's approximation of t was utilized. Reproduction indices of control and treated groups were analyzed by chi-square.

RESULTS:

Mortality & Observations

One control female was sacrificed early because of premature delivery. Three control, one low-dose, four mid-dose and eight high-dose females were found dead. Clinical signs included anorexia, nasal discharge, wheezing, labored respiration, eye discharge, depression, rough coat, thinness and cyanosis.

Maternal Body Weight

Interpretation of maternal body weight data was complicated because the initial body weight range was so great. This would also indicate that does were not of approximately the same age. This initial body weight range is from 3180 to 4595 grams. Initial and final mean group body weights are as follows:

	Control	4 mg/kg	20 mg/kg	100 mg/kg
Mean Weight (Day 0)	4132.7	3935.8	3814.4	4056.6
(Day 29)	4506.4	4142.4	4130.6	4125.8

The above mean values are based on those Does that were pregnant and did not die on study. It should be noted that the 20 mg/kg group mean weight is 318.3 grams less than controls. Furthermore, in rabbits reduced litter size and an increase in the number of stillbirths have been seen with increasing maternal age.

Considering the unacceptable initial weight range of the rabbits, Dr. Jerry Smith of Rohm and Haas was telephoned 11/16/79, and the age of the rabbits was requested. Rohm and Haas checked with Hazelton Laboratories and found that no records substantiating the age of the rabbits were kept.

Mean weight change during the conduct of this study is reflected below:

Days	Group 1 0 mg/kg	Group 2 4 mg/kg	Group 3 20 mg/kg	Group 4 100 mg/kg
0-29 (total)	373.7	206.6	316.2	71.2
6-18 (dosing period)	22.3	-58.9	9.4	-150.4

Considering that mean maternal body weight gain in the 4 mg/kg group was 167.1 grams less than controls during gestation and does actually lost 58.9 grams during dosing (days 6-18), maternal toxicity was evident at the low dose level. The dose response relationship is complicated by less pronounced effects at the 20 mg/kg level, but some of this may be explained by the age and weight variation as noted previously.

Gross Pathology - maternal

Liver alteration, described as discolored, friable or soft, thickened, or pale livers were noted at a higher incidence only in high dose does that died or were sacrificed early. No other significant findings were noted.

Cesarean Data

If "Implantation Efficiency" for group 2 and group 3 is recalculated using the total number of implantations rather than excluding some values (which the report attempted to do) then Group 2 implantations totaled 114 (instead of 78) per 164 corpora lutea and Group 3 totaled 101 implantations (instead of 94) per 161 corpora lutea. Therefore, Group 2 implantation efficiency is 69.51 while Group 3 would be 62.73.

In comparison to these revised values, this reviewer notes a low implantation efficiency in controls (43.60) and also in group 4 (56.45). Compound intake should not have an effect on the number of corpora lutea nor the number of implantations, and such values are therefore very suggestive of questionable experimental procedure. This point is further demonstrated by the irregularity in number of corpora lutea and implantations on a per litter basis:

	Group 1	Group 2	Group 3	Group 4
Mean corpora lutea	13.54	8.73	10.12	5.66
Mean implantations	5.90	6.00	6.31	5.16

Also of significance in Table 3 is the fact that mean litter size is below expected numbers in all groups including controls (5.35 fetuses/litter). High dose litters only averages 2.49 fetuses/litter. Resorptions were increased in all test groups. This was evident on a per litter basis and by group totals (Incidence of Resorption.)

	Group 1	Group 2	Group 3	Group 4
Resorptions/litter	.54	2.26	1.00	2.66
Incidence of Resorption (%)	9.23	37.71*	15.84	51.61*

*Group 2 and 4 values were indicated as significant increases at $p < .05$.

From the above parameters, it is evident that fetal toxicity as manifested by increased resorptions was present at the low dose level of 4 mg/kg (2.26 resorptions/litter or 37.71%). Effects are not as pronounced in group 3 (20 mg/kg) but this may likely be due to procedural error. The incidence of fetal viability (live fetuses/implantations) was also significantly reduced at 4 mg/kg and 100 mg/kg.

There was a dose related decrease in fetal weight and length. Low dose mean fetal weights reflect a slight compound effect (42.37 grams) when compared to the control mean of 45.71 grams.

A

	Group 1 (control)	Group 2	Group 3	Group 4
Fetal Weight	45.71 g	42.37 g	42.84 g	37.9 g
Fetal Length	9.55 cm	9.64 cm	9.15 cm	8.97 cm

Fetal Examinations

Skeletal

Fused sternebrae were reported for only one low dose fetus. Two fetuses with fused or forked ribs, one fetus with unossified areas in the parital bones, and one fetus with an extra ossification center in the sternum were reported in the mid-dose group. Incomplete ossification of the hyoid body was observed in one high-dose fetus.

The above list of variations are common, but more variations should be expected in any study and it appears that reporting of skeletal variations is not complete (ie - fetuses with more than 12 pairs of ribs are not included in the table 7, although the text on page 13 indicates that these variations occurred.)

No anomalies were reported.

Visceral

Only 3 visceral findings were reported in the entire study. Dilated ureters in one control group female fetus, a single olfactory lobe and dilated lateral ventricles which communicated anteriorly in a second control group fetus, and an umbilical hernia in one low dose female fetus.

There was no evidence of compound related effects.

Conclusions:

1. A NOEL for fetal toxicity was not determined. The NOEL < 4 mg/kg.

   Fetal toxic effects at the low dose level included reduced mean fetal weights, increased resorptions per litter, and reduced fetal viability while maternal toxicity at this level was demonstrated by reduced body weight gain during gestation.

2. No terata were observed at any dose level.

3. An audit of this study should be considered because of:

   A. Irregular implantation efficiency values.

   B. Variation between test and control groups in the mean number of corpora lutea and implantations per litter.

C. The mean litter size is unexpectedly low.

D. An unacceptable initial body weight range of nearly 1.4 kg.

E. Reporting of skeletal variations is not complete.

4. This study is classified as core supplementary because of points 1 and 3 above.