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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Registration No. 4581-116.
Additional information concerning three previously submitted mutagenicity studies, and final rat teratology Evaluation of Cryolite.

TO: Ms. Marilyn Mautz, Product Manager Team #16
Registration Division (TS-767C)

FROM: William Woodrow, Ph.D. WSW 11/28/84
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Albin Kocialski, Section Head, Section VII, ABK 11/28/84
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Pennwalt Corp.
Three Parkway
Philadelphia, PA 19102

Tox Chem. # 264
Acc. No. 250968

Action Requested:

Following a Toxicology Branch team effort to rapidly review cryolite toxicity data for a cryolite registration standard, the Pennwalt Corporation has submitted additional information regarding three unacceptable mutagenicity studies reviewed by Dr. Mauer, and a final rat teratology report; a preliminary report was originally reviewed by Gary Burin (See Woodrow memo of 3/22/83 to James Yowell, Cryolite P.M).

Pennwalt submitted additional information for the three mutagenicity studies and a final report for the rat teratology studies mentioned above on August 3, 1983.

Dr. Mauer reassessed the mutagenicity studies in light of the new information received, and Woodrow reviewed the final rat teratology report.

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Recommendations:

1. Salmonella/Microsomal Mutagenicity Assay (Ames) of Kryocide Technical.

   Classified Supplementary Data (Dr. Mauer, Woodrow memo of 3/14/83). This study is now classified as Acceptable (Dr. Mauer 9/13/84)

2. DNA Repair Evaluation of Kryocide Technical.

   Classified Inclusive (Dr. Mauer 3/15/83)
   This study is now classified as Acceptable (Dr. Mauer (9/13/84)

3. In Vivo Cytogenetic Evaluation of Kryocide Technical.

   Classified Unacceptable (Dr. Mauer 3/15/83)
   This study is now classified as Acceptable (Dr. Mauer 9/13/84)

4. Rat Teratology Study.

   Classified Supplementary Data; preliminary report (Gary Burin, Woodrow memo fo, 3/14/83).

   Final Teratology report; Kryocide Insecticide, Rats

   Kryocide did not demonstrate a teratogenic potential, and was not fetotoxic when tested in rats.

   The NOEL for maternal and fetotoxicity is 3000 mg/kg body wt. (HDT)

   Classification: Core Minimium Data

Data Review

A. Reassessment by Dr. Mauer of the mutagenicity studies mentioned above. Following are summaries quoted directly from Dr. Mauer, dated Sept. 13, 1984:

   1. Salmonella/Microsomal Mutagenicity Assay (Ames) of Kryocide Technical.

      "Additional data and final report emendations satisfy the deficiencies listed in original review (3/14/83) and study is now acceptable."

   2. DNA Repair Evaluation of Kryocide Technical.

      "Additional data and revisions in amended final report satisfy deficiencies listed in original review (3/15/83) and the study is now acceptable."

3. In Vivo Cytogenetic Evaluation of Kryocide Technical.

"Additional data and re-evaluation of original review (3/15/83) satisfy requirements of an adequate test, and thus this study is considered acceptable."

B. Review of a final Kryocide teratology study by Woodrow. The preliminary report was reviewed (as mentioned above) by Gary Burin, 3/22/83.

Final Report for a Teratology Study of Kryocide Insecticide in Albino Rats

Sponsor: Pennwalt Corp. Tester: Science Applications, Inc., Division of Toxicology. Study No. 1182008, August 1, 1983.

Test Material

Kryocide Insecticide, Lot #NB No. 86-11-9; sodium fluoaluminate.

Control material - carboxymethyl-cellulose (CMC) - Lot No. 8M04, dissolved in distilled water to form a 0.2% solution. The CMC was refrigerated between use periods.

Dose levels of Kryocide were suspended in 0.2% CMC on a weight/weight basis, and were prepared for three and four day periods before new batches were made ready. Prepared suspensions were stirred at least 5 minutes prior to dosing, and were stirred continuously throughout dosing using a magnetic stirrer. The formulated doses were refrigerated between use periods.

Samples of the dose levels were taken on the first and last days of formulation batch use for determination of homogeniety and test material concentration.

Thirty female rats, 73 days of age per each of 0, 750, 1500, and 3000 mg/kg dose levels were bred to sexually mature male rats following a 7 day acclimation period, and were dosed by oral gavage, following vaginal smear for sperm and/or vaginal plug evidence of breeding. The day that breeding evidence was established was considered day 0 of gestation.

The pregnant female rats were dosed as described above at 10 ml/kg body weight on days 6 through 19 of gestation.

Food and water were available ad libitum. The dose levels chosen were based upon the results of a prior dose range-finding study. All study dams were observed for mortality 2 x daily, and were observed for feces and urine output and condition or activity at least once daily. Females showing signs of premature birth were sacrificed on the day of observation.

Body weights were recorded on days 0, 6 through 19 of gestation and prior to laparohysterectomy on day 20 of gestation.

Dams were sacrificed on day 20 of gestation by CO_2 asphyxiation.

At necropsy, the thoracic and abdominal cavities were examined for gross lesions. The ovaries were examined and the number of corpora lutea were recorded. The uterus was opened and the number and distribution of live and dead fetuses in each uterine horn was recorded. Each fetus was examined for external anomalies; individual fetal body weights and sex were recorded. Approximately one-half of the fetuses from each litter were randomly selected for decapitation and later head examination for internal changes following fixing and cross-sectioning in 1 mm sections.

Fetuses selected for head examinations and the remaining fetuses from each litter were subject to fresh tissue visceral examinations. The umbilical blood vessels, stomach, spleen, pancreas, liver, and diaphragm were observed for size, shape and location. The fetal sex was verified and the kidneys, adrenal glands, uretus and urinary bladder were examined for size, shape and location. All abnormalities were recorded.

The fetal thoracic cavity contents examination included heart blood vessels, heart valves, lungs, trachea, esophagus. Abnormalities were recorded and atypical tissues were removed for further examination.

Each fetus was fixed in 70% ETOH and stained with alizarin prior to skeletal examinations. Bones of the neck region, the sternum, pectoral girdle, ribs, extremities, pelvic girdle, and the vertebral column were examined. Bones not fully ossified, absent, misshapen, fused, bifurcated, malaligned, or of unusual size were described and recorded.

Statistical tests were conducted on a DEC-10 computer using standardized statistical programs from the Statistical Package for the Social Sciences[1] or a Fortran Applications Program for the Gladen[2], and Modified Jonckheere[3] Analyses.

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1 Nie, N. H., Jenkin, C. H., Steinbrenner, K. and Bert, D. H. Statistical Package for the Social Sciences, 2nd Ed., McGraw Hill Book Co., 1975.

2 Gladen B. The Use of the Jackknife to Estimate Proportions from Toxicological Data in the Presence of Litter Effects, J. Am. Stat. Assoc., 74, 278-283, 1979.

3 Lin, F. O. and J. K. Hasseman. A Modified Jonckheere Test Against Ordered Alternatives when Ties are Present at a Single Extreme Value, Biomed. Z Bd, 18 , 623-631, 1976.

The dam weight gain was analyzed using a two-way analysis of variance. The number of implantations, number of corpora lutea and litter size were analyzed using one-way ANOVA. Live fetal body weights were analyzed using an Analysis of Covariance with litter size as the Co-variant. Embryo-lethality (no. dead + no. resorbed no. implantations) was analyzed using the modified Jonckheere test and Kruskal-Wallis[4] test; malformations and variations were analyzed using the Kruskal-Wallis and Gladen tests.

Results

A. Maternal Effects

Clinical observations - The only clinical observations were confined to three dams: No. 48 dosed at 750 mg Kryocide/kg delivered easily on day 20 of gestation. No. 74 dosed at 1500 mg/kg exhibited respiratory congestion on day 16-20 of gestation. No. 102 dosed at 3000 mg/kg displayed a porphorin discharge from both eyes, unthrifty fur, was hypoactive and moribund; this animal was removed from the study on day 11 of gestation and was subjected to necropsy.

The only clinical sign attributable to Kryocide treatment was whitening of dam tooth enamel for all of the treated animals (750, 1500, or 3000 mg/kg) beginning on day 16 or 17 of gestation. None of the described clinical signs (excepting teeth whitening) appeared to be treatment-related. Control dam teeth were unaffected.

A variety of lesions were observed in control and treated dams during laparohysterectomy, following CO_2 sacrifice:

Two control animals showed right or left hydronephrosis, and one control animal displayed purulent material in the right lung lobe.

One dam dosed at 750 mg/kg displayed a whitish-green nodule in a diaphragmatic lung lobe.

Two dams dosed at 1500 mg/kg showed hydronephrosis of right or left kidneys, and a third animal showed mottling throughout the lungs, the left lung was absent, the right lobe was small and contained white purulent material.

Three dams dosed at 3000 mg/kg displayed right or left kidney hydronephrosis. One dam dosed at this rate was necropsied on day 11 of gestation (in a moribund state), and showed stomach-scattered

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4 Conover, W. J., Practical Nonparametric Statistics, John Wiley and Sons, Inc., p. 256, 1971.

pinpoint hemorrhages; the cause of this animal's illness could not be determined. None of the lesions observed at necropsy or terminal laparohysterectomy appeared to be treatment related.

Quoted from the tester's report:

Table 1
SUMMARY OF MATERNAL DATA IN RATS
ADMINISTERED KRYOCIDE® INSECTICIDE

Dose Level (mg/kg)	Control	750	1500	3000
Number of Dams Pregnant/ Number Sperm Positive (%)	29/30 (96.7)	28/30 (93.3)	26/30 (86.7)	30/30 (100)
Mortalities (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Actual Mean Body Weight Change (g)[a]	89.1 ±17.84*	92.1 ±22.69	90.8 ±21.18	90.3 ±15.71
Corrected Mean Body Weight Change (g)[b]	30.7 ±13.51	28.5 ±17.11	26.9 ±12.26	25.3 ±13.95
Mean Number of Corpora Lutea	10.7 ±1.87	11.2 ±1.39	11.3 ±2.36	11.0 ±1.52

* Data accompanied by plus or minus one standard deviation.
a Day 20 weight (grams) - Day 6 weight (grams).
b Day 20 weight (grams) - Day 6 weight (grams) - gravid uterus weight (grams).

End of quotation.

An analysis of variance applied to the dam weights at day 6 of gestation and at cesarean section (day 20 of gestation) showed no significant effects were observed for weight gain during pregnancy between the control and treated groups ($p = .894$). No significant differences in numbers of corpora lutea were determined when analyzed statistically ($p=.381$). Only one dam was removed from the study due to moribund condition; all other control and test animals survived to experimental term.

B. Prenatal Effects

A summary of prenatal effects prepared by the tester is shown below:

TABLE 2

SUMMARY OF PRENATAL DATA IN RATS ADMINISTERED KRYOCIDE INSECTICIDE

Dose Level (mg/kg)	Control	750	1500	3000
Number of Dams Pregnant	29	28	26	30
Number of Litters Examined (Fetuses)	28[a] (278)	27[b] (285)	26 (275)	29[c] (311)
Number of Implantations	300	300	286	325
Mean Number of Implantations/Litter	10.3 ± 2.42*	11.1 ±1.69	11.0 ± 2.95	11.2 ±1.47
Percent Live Fetuses[d]	92.7	95.0	96.2	95.7
Mean Number of Live Fetuses/Litter	9.6 ±3.24	10.6 ±2.12	10.6 ±2.89	10.7 ±1.49
Mean Fetal Weight (g) All:	3.8 ±0.58	3.8 ±0.30	3.8 ±0.28	3.8 ±0.31
Males:	3.9 ±0.58	3.9 ±0.31	3.9 ±0.29	3.9 ±0.32
Females:	3.8 ±0.59	3.6 ±0.31	3.7 ±0.26	3.7 ±0.28
Number of Resorbed Fetuses	22	15	11	14
Number of Dead Fetuses	0	0	0	0
Percent Dead and Resorbed Fetuses	7.3	5.0	3.8	4.3
Mean Sex Ratio (M:F)	1.1:1.0	1.1:1.0	1.0:1.0	1.1:1.0

* Data accompanied by plus or minus one standard deviation.
a One dam had entire litter resorbed (number 15).
b One dam delivered early (number 48).
c One dam was removed from study on day 11 of gestation due to moribund condition (number 102).
d Percent of live fetuses calculated as the number of live fetuses divided by the number of implantations.

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End of quotation.

No differences between control and test animals were found when various prenatal parameters were analyzed statistically:

a. The number of implantations in control and test groups were not significantly different; p=.521

b. Litter sizes were not significantly different between control and test groups; p=.691.

c. Live fetal weights for control and test animals were not significantly different, at a p value of .370.

d. A statistical trend analysis to determine the possibility of a trend in embryolethality difference between control and test animals showed no significant differences; p=.354.

e. A test for embryolethality between control and test groups of animals, also showed no significant differences; p=.496 (embryolethality was defined as the number of dead fetuses plus the number or resorptions divided by the number of implantations).

C. Fetal Malformations and Variations

Table 3 below quoted from the tester's report, presents data as mean proportions to facilitate comparison of statistical tests calculated as proportions, versus the raw data:

Quoted from the tester's report:

Table 3

SUMMARY OF MEAN LITTER PROPORTIONS[a] OF MALFORMATIONS AND VARIATIONS IN RATS ADMINISTERED KRYOCIDE INSECTICIDE

Dose Level (mg/kg)	Control	750	1500	3000
Total Number of Litters Examined	28	27	26	29
External[b] Malformations	0.036 ±0.189[c]	0.016 ±0.060	0.005 ±0.025	0.007 ±0.027
Visceral[d] Variations	0.000 ±0.000	0.000 ±0.000	0.003 ±0.018	0.000 ±0.000

Skeletal				
Malformations	0.006 ±0.023	0.027 ±0.091	0.000 ±0.000	0.009 ±0.046
Variations	0.177 ±0.210	0.110 ±0.192	0.152 ±0.166	0.130 ±0.185
Head Examinations				
Malformations	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000
Variations	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000
Other[e]	0.007 ±0.038	0.034 ±0.161	0.000 ±0.000	0.003 ±0.017
Abnormal Fetuses[f]	0.042 ±0.189	0.044 ±0.143	0.005 ±0.025	0.016 ±0.065

[a] Mean litter proportions were derived by obtaining the proportion of viable fetuses per litter which had at least one malformation or variation, and then calculating the grand mean of these proportions for the entire group. Individual fetuses may have more than one type of malformation and/or variation.
[b] No external variations were observed in any dose level.
[c] All data accompanied by plus or minus one standard deviation.
[d] No visceral malformations were observed in any dose level.
[e] Specimens which were inadvertently damaged during dissection or processing and, therefore, were considered unsuitable for evaluation.
[f] All fetuses with one or more malformations.

End of quotation.

Table 3 footnotes state that no external variations or visceral malformations were seen at any dose level.

Table 3 indicates that two types of external fetal malformations were found in control and all treated group fetuses; the litter from control dam no. 7 contained all runt animals, and one fetus from a dam treated with 750 mg/ml displayed an umbilical cord hernia.

Visceral variations were confined to one fetus from a dam treated with 1500 mg/kg (left ureter - slight hydroureter).

One dam treated with 750 mg/kg contained a number of fetuses that displayed a number of skeletal malformations, and a number of fetuses from one dam treated with 3000 mg/kg showed absent sacral vertebrae. The thoracic centrum was absent in each of two fetuses from two untreated dams, and was also absent from two fetuses from one dam and in two fetuses from two different dams treated with 750 mg/kg. A classification of all malformations combined showed no significant differences existed between control and treated groups ($p = .331$).

Various kinds of skeletal variation were observed in both control and fetuses from treated dams, as indicated in Table 3; the most common variations included absent or incompletely ossified sternbrae. An examination of Table 3 shows that the occurrence of skeletal variations was greater in the control fetuses; statistical analyses indicated that no significant differences in skeletal variations existed between fetuses from control or treated dams ($p = .261$).

Conclusions:

Kryocide insecticide tested at 750, 1500, or 3000 mg/kg in rats did not demonstrate a teratogenic potential, and was not shown to be fetotoxic. A whitening of the dam teeth in treated animals was the only change in either dams or fetuses that was attributable to Kryocide treatment.

No significant differences between control and treated dam body weight gains, or numbers of corpora lutea were found.

Parameters of prenatal comparisons between control and treated dams showed no statistically significant differences, including: number of implantations, mean number of implantations per litter, the percent of live fetuses, the mean number of live fetuses per litter, all mean fetal weights, the number of resorbed fetuses, the number of dead fetuses, the percent of dead and resorbed fetuses, and the mean male and female sex ratio.

No statistically significant differences were found to exist between litters from control or treated dams for external, visceral, skeletal or head examinations for malformations or variations.

The NOEL for maternal and fetotoxicity is 3000 mg/kg body weight (HDT).

Classification: Core Minimum Data