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

Caswell No 75A

Memorandum

OFFICE OF
PESTICIDES AND TOXIC SUBSTAN

TO: Henry Jacoby
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003042

THRU: Chad B. Sandusky, Ph.D.
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Hazard Evaluation Division (TS-769C)

6/27/83

SUBJECT: Benomyl, Additional Teratogenicity Data Reg. No. 352-354. Accession No. 249749 and 248563.

Action Requested:

Review of an additional teratogenicity study.

Recommendations and Conclusions:

1. The study indicates that a no-observed effect level (NOEL) for teratogenic effects (microphthalmia) is 30 mg/kg/day in rats.

2. The results, when considered with those from previous studies (Staples, 1980; and Kavlock, et al, 1980) at similar doses, suggest that the 2 fetuses with microphthalmia from a group of rats given 10 mg/kg/day are likely to be coincidental.

Background:

The Agency issued a Notice of its Rebuttable Presumptions Against Registration (RPAR) of products containing Benomyl on December 6, 1977. One of the presumptions was based on the fungicide's teratogenic potential. In a draft Position Document 4 (PD-4) dated October 1, 1982, the Agency concluded that a provisional NOEL of 30 mg/kg/day was established. In one study (Staples, 1980), the lowest effect level (LEL) was reported to be 30 mg/kg/day. However, animals given the 10 mg/kg/day dose in that study had 2 fetuses with unilateral microphthalmia. The Agency described that effect as biologically significant, and results from other studies, also described in PD 4, showed that similar effects occurred in fetuses of rats given doses of 62.5 mg/kg/day or more during gestation. The Agency noted in PD-4 that DuPont was conducting a follow-up study to investigate the occurrence of microphthalmia. The follow-up study is reviewed below.

DATA REVIEW

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Citation:

Staples, R.E. 1982. Benomyl Gavage: Teratogenicity Study in the Rat. Unpublished report prepared by Haskell Laboratory for E.I. DuPont De Nemours and Co. Report No. 587-82. EPA Acc. No. 248563 and 249749.

Materials and Methods:

Test substance: Benomyl (99.1% purity, contaminants were not identified) was used. The sample was numbered INT-1991-474, N.B. 5103-109, Lot F00117E

Test Species: Pregnant Crl: CD® (SD) BR rats were used. Day 1 of gestation was the day sperm were detected in vaginal smears.

Experimental Procedure:

Benomyl was suspended in stripped corn oil and administered by gavage at dosages of 0, 3, 6.25, 10, 20, 30, or 62.5 mg/kg. The dosages were administered in 1 ml of vehicle daily from day 7 through day 16 of gestation. There were 46, 47, 47, 48, 47, 47, or 19 animals in the 0, 3, 6.25, 10, 20, 30, or 62.5 mg/kg/day dose groups, respectively.

On day 21 of gestation dams were sacrificed and examined for gross pathological signs. Ammonium sulfide solution was used to determine the incidence of pregnancy in uteri of apparently non-pregnant dams.

Maternal body weights were obtained on day 5 of gestation for the purpose of dosage preparation.

At sacrifice the numbers of implantation sites, resorptions, live fetuses and dead fetuses were determined. Fetuses were individually weighted and mean live fetus weights per litter were calculated.

Fetal examinations were limited to the determination of the incidence of external hydrocephaly and microphthalmia. Eye diameters were measured in cases of suspected asymmetrical or small eyes. One measurement was made from the pinna through the center of the eye, and the other was made through the center and perplendicular to the first. The criteria for identification of microphthalmia considered the smaller of the two measurements. If both measurements were at least 0.4 mm less than those in the alternate eye, the smaller eye was classified as microphthalmic. Both eyes of a fetus were classified microphthalmic if the measurement was less than 1.8mm (the smallest diameter found in

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the control group). A transverse section through the center of both eyes was made freehand, and the eyes were examined for microphthalmia. All measurements and examination were made under magnification (10X).

A transverse section was made throught the widest portion of the head which was then examined for signs of internal hydrocephaly.

The author noted that the litter was considered the experimental unit for statistical analyses. The analyses included the Fisher's exact test for incidence of maternal and fetal mortality and occurrence of fetal effects, the Mann-Whitney U test for significant differences in maternal body weights, one-way analysis of variance and Dunnett's tests for maternal body weights after censoring those animals without live fetuses, dying before scheduled sacrifice, or those bred on the wrong date, and Jonckheere's test for significance of dose-response relationships.

Reported Results:

The author noted that no statistically significant differences between group mean meternal body weights were found.

One dam died on day 11 of gestation because of dosing error (30 mg/kg/day group). Other dams were excluded from the study because of errors in breeding date estimation (detected on the basis of unusually light or heavy litter weights). There were 4, 2, 3, 2, 3, 3, or 1 eliminated from the 0, 3, 6.25, 10, 20, 30 or 125 mg/kg/day groups, respectively.

Pregnancy rate varied from 84.2% (16/19) in the highest dose group to 95.7% (44/46) in the control group. No statistically significant differences were noted by the authors. Only one fetus was found dead (10 mg/kg/day). The highest dose group was reported to contain 1 fetus with microphthalmia and 1 fetus with hydrocephaly in separate litters). The number of litters containing fetuses with hematomas was comparable in the control and treated groups with the exception of the highest dose. In that dose group 11 of 16 litters contained an average of 11 ($\pm$ 6.0) % fetuses with hematomas (1 to 2 per litter) while 15 of 43 control group litters contained fetuses with hematomas (1 to 2 per litter). Mean fetal weight in each litter was statistically significantly less than that reported in controls (3.9 $\pm$ 0.08 g in the highest dose group compared with 4.1 + 0.04 g in the control group; p less than 0.05, Mann Whitney U test, two tailed). The author stated that no statistically significant dose-related effects were detected with respect to these observations as well as the other parameters measured.

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Discussion and Conclusions:

This study is intended to evaluate a specific effect on the development of the eyes in fetal rats. The data presented by the author supports the stated conclusion that the lowest teratogenic effect level (LEL) is 62.5 mg/kg/day and that under the conditions of the study described herein a no-observed effect level (NOEL) is 30 mg/kg/day..

Core Classification. Supplementary.* The study was intended to evaluate a specific effect noted in previous studies.

References

Kavlock R.J., N. Chernoff, L.E. Gray, Jr., J. Gray and D. Whitehouse. 1980.

Report on the teratogenic potential of benomyl administered via the oral and dietary routes in the Wistar rat. Health Effects Research Laboratory. Experimental Biology Division, Develpment Biology Branch, U.S. EPA, Research Triangle Park, North Carolina.

Staples, R.E. 1980. Benomyl: Teratogenicity in the rat after administration by gavage. Medical Research Project No. 3501-001. Haskell Laboratory Report No. 649-80.

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h/r CWS 6/30/83

8/18/83

* when these are combined with prior data from same study, the overall CORE grade is minimum

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8-18-83

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