UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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SUBJECT: EPA Registration No. 476-2107. Review of Ordram Antifertility Study in Rabbits

Tox. Chem. No. 444
EPA Acc. No. 243816

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THRU: William L. Burnam, Acting Branch Chief
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Applicant: Stauffer Chemical Company
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Requested Action: Review of the Data of Ordram antifertility study in rabbits, which is unsolicited data to support registration, was requested by the Registration Division.

Comment:

1. This study was reasonably performed and reported. The results in this study could be used for the future registration support.

2. A note is hereby made as it was done in the previous ordram antifertility review (EPA Reg. No. 476-2107; EPA Acc. No. 241965): In the interim of review processes of ordram antifertility studies, Toxicology Branch recommends that Registration Division not register any further uses of either technical or formulation products of ordram in order to avoid any possibly additional field-worker exposure to this specific chemical (please see point (8) under Discussion of this review).

Review of the Submitted Materials

Ordram antifertility study in rabbits (By J.M. Killinger, G.M. Zwicker, P.D. Royal, R.I. Freudenthal, et al., Environmental Health Center, Stauffer Chemical Company; Project No. T-10176 of November, 1980). This study was to determine whether subchronic ingestion of technical Ordram has adverse effect on male fertility.

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A. Experimental

1. The test material was technical Ordram (Code #EHC-0009-46) which was 98.2% purity. The corn oil used for dose preparation was food grade Mazola corn oil (Lot #48001-0584C, expiration date 8/23/80) from Best Foods in Englewood Cliffs, N.J.

2. Dutch belted rabbits (6-9 month old upon receiving) from Dutchland Laboratories, Inc. in Denver, Pennsylvania, were used as test animals. The rabbits were quarantined 5-7 weeks before study started. Both males and females were used in the predose fertility test in which two females were assigned to each male. Each male was hand-mated with one female one day and with the second female three days later. In subsequent matings, the males were paired with the same two females. Immediately after mating, the females were returned to their cages and held until littering occurred. When littering occurred, the number, weight, and gross condition of the litter were recorded. The pups were separated from the females and sacrificed using sodium pentobarbital.

3. The dose solutions were prepared as: Corn oil (Vehicle control), Ordram in corn oil at 10 mg/ml and 100 mg/ml, and undiluted technical Ordram (1000 mg/ml). The male rabbits (the body weights prior to initial exposure were 1.6-2.8 kg) were weighed weekly and 0.2 ml of dose solution/kg of body weight was administered in gelatin capsule to obtain the following dose levels: 0, 2, 20 and 200 mg/kg, b.w. The dose solutions were stored at room temperature and used no longer than four weeks. Samples were taken from each dose solution for concentration analysis and retention. The capsules were filled weekly.

4. Only males of proven fertility were used for the final tests. The males were assigned to the 4 dose groups according to the weight. There were 9 males in each of the treatment groups and 10 males in the control group. The males received Ordram daily by capsule for 6 weeks at dose levels of 2, 20, and 200 mg/kg, b.w./day. During the 6th week of dosing, the male rabbit's fertility was tested by mating each male with the same females with which the male was paired previously, using the aforementioned method. At the end of the dosing period, 5 males from the control group and 4 males from each treatment group were necropsied. The remaining males were allowed to recover, and their fertility was tested again during the 5th week of their recovery period. After the postrecovery littering, the remaining

males were necropsied. The testes plus epididymides, thyroids plus parathyroids, adrenals, and pituitary were weighed. The testes, epididymides, vas deferens, prostate, thyroids, adrenals, and pituitary were collected and fixed in Bouin's solution for microscopic examination. All observed tissue alterations were recorded, and the affected tissues were collected.

5. The male rabbits were observed once daily for mortality and overt signs of toxicity prior to and after the dosing period. During the dosing period, the males were observed twice daily. The females were observed once daily after delivery to the laboratory. After eye irritation was observed in several males, an opthalmic examination was performed on the control and high-dose males near the end of dosing period.

6. The mating and pregnancy rates, clinical observations, and necropsy and histopathology lesions were statistically analyzed using the Fisher exact probability test. Analysis of quantitative variables, such as body weights, organ weights, pups weights, number pups per litter, number of viable pups per litter, and gestation length, were intercompared between the dose groups and the control by Bartlett's test for homogeneity of variance, one-way analysis of variance, and Dunnett's t-test.

B. Results

1. One male in the 2 mg/kg dose group died during the dosing period due to asphyxiation with a dosing capsule found in the larynx. Another male of the same dose group was sacrificed in moribund condition with severe spinal cord damage during the recovery period. One female was sacrificed because of developing an inner ear infection. Another female died from an intestinal blockage due to a gastric hair ball. However, there were no mortalities that might be attributed to the doses of Ordram administered in the test.

2. Several clinical observations, such as anorexia, diarrhea, and minor injuries were reported in several animals. Cataracts, a tongue polyp, and reduced iris pigmentation were also observed in one animal each, but these observations were not related to Ordram treatment.

   There were few observations being considered related to the dosing method. One male (2 mg/kg dose group) died of suffocation after a capsule lodged in his upper esophagus,

blocking the laryngeal opening. One male developed respiratory rales after dosing. Blood was observed in another animal's mouth (200 mg/kg dose group) twice after dosing and once in an animal's nasal passages.

Other adverse effects of the test material on the skin and eyes appeared to be dose-related. Two males (200 mg/kg dose group) had dry desquamating skin and mild alopecia at the corners of their mouths for 3-5 days. One male in the control group, 3 males in the 2 mg/kg dose group, 3 males in the 20 mg/kg dose group, and 7 males in the 200 mg/kg dose group had eye irritations. The severity and duration increased with increasing dose. The eye and skin irritation apparently resulted from the rabbits occasionally biting into the capsules, getting the dose solution on the fur around their mouths, and then rubbing it in their eyes. During the recovery period, two males from the 200 mg/kg dose group had residual eye irritation for 4 days.

There were no dose-related changes in the mean body weights of the treated male rabbits.

3. The mating behavior of the males was not affected by the treatment of test material. The mating index was defined as the percentage of males who mated with at least one female during the mating period. The mating index was 98% for the predose fertility test, while it was 100% for both postdose and postrecovery mating in all dose groups.

Results from the postdose and postrecovery fertility tests showed no treatment-related decrease in fertility. The male fertility index was defined as the percentage of males impregnating at least one female. The female fertility index was defined as the percentage of females pregnant. The predose male fertility index was 77% and predose female fertility index was 54%. The fertility indices for the postdose and postrecovery male and female fertility tests in all three dose groups are comparable to the controls.

The litter data collected and analyzed were the mean number of pups/litter, the mean weight/pup, and the mean gestation length, as well as the mean number of viable or nonviable pups/litter. These data from both postdose and postrecovery fertility tests indicated no dose- related changes in the aforementioned analysis.

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4. Necropsy data, including mean relative organ weights from both interim sacrifice and final sacrifice, lesion incidence from both interim sacrifice and final sacrifice, and analysis of the incidence of histological lesions from both interim sacrifice and final sacrifice, showed no apparent dose-related changes in lesion incidence or organ weights. There were numerous spontaneous tissue alterations in both control and test animals. However, none could be considered with a frequency or severity pattern being treatment-related.

5. A significant increase in eye irritation with increasing dose was noted. This effect was evidently due to the animal rubbing the dose solution in their eyes and was reversible after the dosing was completed.

C. Discussions

1. There were no mortalities that might be attributed to Ordram treatment.

2. There were no treatment-related changes in body weight.

3. There were no apparent treatment-related lesions observed during the postdose and postrecovery necropsies.

4. The observed increase in eye irritation and desquamate skin and mild alopecia around the mouth were apparently resulted from the animal biting the capsule, getting dose solution around the mouth, and rubbing it in the eyes.

5. There were no apparent treatment-related changes in the mean weights of the testes plus epididymides, thyroids plus parathyroids, pituitary or adrenals.

6. The mating behavior of the males and the fertility patterns of females in both postdose and postrecovery tests were not affected by the treatment of test compound. In other words, antifertility effects were not observed in the Dutch Belted male rabbits treated with and up to 200 mg (a highest concentration tested)/kg, b.w./day of the technical Ordram for 6 weeks. Only three dose levels, namely 2, 20 and 200 mg/kg, were used in this experimental design.

7. A true NOEL may be etablished for Ordram antifertility effects by using higher dose levels in a new experimental design.

8. Previous review of Technical Ordram antifertility effects in the rats (Sprague-Dawley CD(R) species for a 3-month inhalation study-Bio/dynamics Project No. 78-2346 of Dec. 13, 1979 that was submitted by Stauffer Chemical Compnay; EPA Registration No. 476-2107; EPA Accession No. 241965) indicated that this test material has the adverse effects on the testes and spermatoza in male rats at the relatively low dose levels (CMEC at 2.2 ~ 42 mg/m^3). It appeared that the antifertility effect of Technical Ordram is relatively weaker in the rabbits (non-rodent).

However, under review at present are still oral antifertility studies in mice and rats with Technical Ordram. When these reviews are completed, a detailed discussion will be made on this subject which is of potentially serious concern to the fieldworker exposure.