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

002343

12-16-82

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE:

SUBJECTS: I. Review of Five (5) Toxicological Studies of Technical Ordram, Including Reproduction-Fertility and Acute Inhalation Toxicities, Dose-Time Relationships on Coagulation Parameters, and Hematology and Blood Chemistry in Animals Fed in the Diet. EPA Registration No. 476-2107; EPA Accession No. 245675; Tox. Chem. No. 444.

II. Review of the Effect of Ordram on Nonhuman Primate Sperm Production - Stauffer Toxicology Report T-10714. EPA Registration No. 476-2107; EPA Accession No. 246520; Tox. Chem. No. 444.

FROM: Thomas S. S. Mao Thomas S. S. Mao 12/15/82
Review Section III
Toxicology Branch/HED (TS-769C) William M. Butler 12/15/82
12/15/82

TO: Richard Mountfort
Product Manager (23)
Registration Division (TS-767)

THRU: Orville E. Paynter
Branch Chief
Toxicology Branch/HED (TS-769C)

Applicant: Stauffer Chemical Company
1200 South 47th Street
Richmond, California 94804

Actions Requested: The following studies were requested to be reviewed by the Registration Division.

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I. EPA Registration No. 476-2107; EPA Accession No. 245675; Tox. Chem. No. 444.

Review 1. Ordram antifertility study in mice - Stauffer Toxicology Report T-10121 (December 1, 1980).

Review 2. Ordram fertility study in male rats: Mechanism/site of action - Stauffer Toxicology Report T-10421 (May 1, 1981).

Review 3. Acute inhalation toxicity of Ordram® Technical in albino rats - Stauffer Toxicology Report T-6598A (August 15, 1980).

Review 4. Acute inhalation toxicity of Ordram Technical in male albino CD-1 mice - Stauffer Toxicology Report T-6598B (July 24, 1980).

Review 5. Dose and time relationships on coagulation parameters, hematology and blood chemistry in male and female rats fed Ordram in the diet.

II. FPA Registration No. 467-2107; EPA Accession No. 246520; Tox. Chem. No. 444.

Review 6. The effect of Ordram on non-human primate sperm production - Stauffer Toxicology Report T-10714 (December 16, 1981).

Comment

The submitted studies can be considered toxicologically acceptable but with one point (in Review #5) recommended for supporting the future registration applications.

Recommendation

Sufficient experimental data of the hemorrhage in the testes of male rats be submitted for review and evaluation (in Review #5) for the future registration application.

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Review of the submitted data - Review 1.

Ordram Antifertility Study in Mice - Stauffer Toxicology Report T-10121 (By J. M. Killinger, P.D. Royal, G.M. Zwicker and R.I. Freudenthal of Stauffer Chemical Company - Environmental Health Center, Farmington, Conn. of December 1 , 1980)

I. Experimental

(1) This special experiment was designed to study the antifertility potential for Ordram, a herbicide, in male mice with its objectives of determining the dose-response relationship, the onset of action, and the reversibility of the observed antifertility effects.

(2) CD-1 mice were obtained from Charles River Breeding Labs., Wilmington, Mass. Upon receiving 140 male and 280 female, 8-week-old CD-1 mice, the animals were quarantined for 6 days before they were placed on study. Two females were assigned to each male using a random number generating program. The females were mated with the males for 5 days and they were observed for vaginal plugs each morning. At the end of 5 days the females were separated. The females were sacrificed 12-13 days later and the number of corpora lutea, viable fetuses and implant sites determined. Only the males which impregnated at least one female (as indicated by the presence of implant sites) were used in the study.

(3) The technical Ordram used in this study (Code #EHC-0009-46) was 98.2% purity. The corn oil was used as vehicle in dose preparation (Food Grade Mazola Corn Oil; Lot #48001-05840; expiration date August 23, 1980) from Best Foods in Englewood Cliffs, New Jersey. The Ordram was mixed with corn oil to obtain a 40 mg/ml stock solution. This stock solution was diluted with corn oil to make the less concentrated dose solutions: corn oil (vehicle control), 0.4 mg/ml, 4.0 mg/ml, 20.0 mg/ml, and 40.0 mg/ml. The male mice were weighed weekly and 0.5 ml of dose solution/100 g. of body weight was administered by oral gavage to obtain the following dose levels: 0, 2, 20, 100, and 200 mg/kg/day. The dose solutions were prepared three times during the study. Samples were taken from each dose solution for concentration analysis and retention.

(4) Twenty males were assigned to each dose group according to weight. They were treated daily at the dose levels of 0, 2, 20, 100 and 200 mg/kg/day for seven weeks by gavage.

Two groups of female mice were received at later dates for fertility testing. They were also quarantined and later randomly assigned to the males for scheduled mating periods when the females were 9 weeks old. After dosing the males for 2, 4, and 6 weeks, the males' fertility was tested by mating two females to each male as described previously. At the completion of the dosing period, five males per dose group were necropsied. The remaining males were allowed to recover for four weeks. Their fertility was further determined. After the completion of the final fertility test, the remaining males were necropsied. However, during the interim and final male necropsies, specified organs were weighed, collected, and processed for histopathology studies.

All mice were observed for mortality and overt signs of toxicity once daily prior to initiation of dosing, and twice daily after treatment began. The males were weighed weekly. The females were observed for vaginal plugs each morning during mating periods.

(5) After the female mice were sacrificed, the reproductive tract was opened and observed for the number of implant sites, resorbing fetuses, and viable fetuses. The corpora lutea in the ovaries were counted under a dissecting microscope. All the data were recorded and the reproductive tracts and ovaries were collected in 10% normal buffered formalin. Any abnormalities in the thoracic or abdominal areas were recorded.

In the case of male mice, the testes plus epididymides were weighed. The testes, epididymides, thyroids, and pituitaries were collected in Bouin's solution and processed for histopathology. Any tissue alterations were recorded and the altered tissues were collected and examined microscopically.

(6) The statistical analysis of the incidence data, i.e., mating and fertility indices, were processed by the Chi-square test. The fertility indices were also analyzed using a test for a trend in proportions. Analysis of continuous quantitative variables such as body weights were intercompared between the dose groups and the control by one-way analysis of Variance and Dunnett's t-test. Results of the female necropsies were comprised of enumeration data which were analyzed on a litter basis using the Mann-Whitney U, two sample rank test. The fiducial limit of 0.05 was employed to obtain the critical level of significance in all of the statistical tests used.

II. Results

(1) Technical Ordram in corn oil was found to be stable at 4, 25 and 60°C for at least four weeks. The dose solutions were stored at room temperature and used for no more than four weeks. However, the actual concentrations of the dose solutions at the time administered were somewhat different from the specific values at the time prepared as indicated in the following table:

Concentration Expected (mg/ml)	Concentration Measured (mg/ml)		
	Date Made: 12/31/79	1/16/80	2/8/80
	Number of Study Days Used 15	22	12
0	---	0.35 (0.00)	<0.09 (0.00)
2	2.75 (0.03)	1.75 (0.04)	1.95 (0.05)

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Table Cont'd.

Concentration Expected (mg/ml)	Concentration Measured (mg/ml)		
	Date Made: 12/31/79	1/16/80	2/8/80
	Number of Study Days Used 15	22	12
20	16.0 (0.3)	19.0 (0.2)	19.5 (0.5)
100	90.0 (2.9)	100.0 (2.0)	105.0 (3.0)
200	175.0 (8.0)	190.0 (7.0)	210.0 (8.0)

---, Indicates not measured.
Concentrations were of technical Ordram in corn oil.

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(2) Several clinical observations were made during that study, but none was treatment-related, namely diarrhea, dehydration, eye irritation and minor injuries. Several animals also appeared to be somewhat more irritable during dosing.

(3) The male mice were weighed in the range of 30 - 36g prior to the test. There were no significant differences observed in the weekly body weights between the treated and control animals.

(4) No dose-related mortalities were observed. However, eight males died during the dosing period. One animal in the 200 mg/kg/day dose group died of bacterial infection and seven other deaths were due to dosing accidents which occurred in the treatment groups.

(5) The mating index was defined as the percentage of males whose females had one or more vaginal plugs during the mating period. The mating index was 89% (118/133) for the predose fertility test. The mating indices for the fertility tests after 2, 4, and 6 weeks of dosing and after the 4-week recovery period were recorded. None of these mating indices of the treated groups were found to be significantly different from those of the controls. Therefore, mating behavior of males was not affected by Ordram treatment.

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 getting pregnant. The results indicated that the predose male fertility index was 97% (129/133) and that the female

fertility index was 84% (223/264). The fertility indices for the fertility tests after 2, 4, and 6 weeks of dosing and after a 4-week recovery period were recorded. The data showed that the pregnancies from matings with the treated males were significantly fewer than the matings with the control males in the 100 mg/kg dose group after 2 weeks of dosing, the 200 mg/kg dose group after 4 weeks of dosing, and the 100 and 200 mg/kg dose groups after 6 weeks of dosing. There was a significant trend downward with increasing dose for the fertility indices after 2, 4 and 6 weeks of dosing. However, there was no significant trend of this kind for the fertility indices after the 4-week recovery period.

(6) The results of the female necropsies, which included only pregnant females, were recorded as the mean and the standard deviation. There were significantly fewer implants and viable fetuses in pregnancies from treated males as compared to pregnancies from control males in the 100 mg/kg dose group after 2 weeks of dosing and in the 100 and 200 mg/kg dose groups after 4 and 6 weeks of dosing. However, there were no significant differences between data from the treated and control mice after the 4-week recovery period.

Furthermore, the data also indicated that after dosing for 4 and 6 weeks, the mean number of viable fetuses and implants decreased, while the mean number of resorptions remained the same in the 100 and 200 mg/kg dose groups.

(7) The data from the male necropsies at both the interim sacrifice and the final sacrifice showed no dose-related changes in the mean testes plus epididymides weights. There were no treatment-related tissue alterations noted during the interim or final sacrifices.

The micropathology examination of tissues showed that the single tissue alteration observed with any frequency among the control and test mice from the interim sacrifice was generally mild testicular germinal cell degeneration affecting single or small clusters of seminiferous tubules. In general, a single testis was involved, but occasionally both were affected. None of the males in the 2 mg/kg dose group were affected, and there was no apparent incidence or severity pattern to suggest dose relationship with Ordram treatment.

In the case of the final sacrifice, very mild to mild testicular germinal cell degeneration affecting single or small clusters of seminiferous tubules was also the most frequently observed tissue alteration. The alteration affected the males in 57% of the control group, 100% of the 2 mg/kg dose group, 80% of the 20 mg/kg dose group, 86% of 100 mg/kg group, and 70% of the 200 mg/kg dose group. This tissue alteration was usually unilateral, but occasionally both testes were affected. Again, there was no clear-cut incidence or severity pattern which could be considered a treatment-related effect.

III. Discussions:

(1) The antifertility effects, namely a decrease in the number of pregnancies or fertility rate and a decrease in the number of implants per pregnancy,

were noted after the male mice were orally treated with Ordram. Both effects were observed in the 100 and 200 mg/kg dose groups and a dose-response relationship showed up only after 4 and 6 weeks of treatment. While there was a decrease in fertility rate and an increase in preimplantation losses after 2 weeks of dosing, these effects did not show a clear dose-response relationship. However, a no-effect level of 20 mg/kg/day in male mice showed up as far as these two effects were concerned.

The antifertility effects were reversible and were not observed after 4-week recovery period.

(2) No dose-related lesions or changes in the mean testes plus epididymides weights were observed during both interim and final male sacrifices. Furthermore, there were no histological changes in the testes, epididymides, thyroids, or pituitaries indicative of compound-related effects after treating animals with technical Ordram up to 200 mg/kg/day (a highest dose-level tested in this experimental design) for 7 weeks.

The NOEL for this study is 20 mg/kg/day.

Review of the Submitted Results - Review 2.

Ordran Fertility Study in Male Rats: Mechanism/Site of Action - Stauffer Toxicology Report T-10421 (by J. M. Killinger, C. R. Downs, J. L. Minor, G. M. Zwicker and R. I. Freudenthal of Stauffer Chemical Company - Environmental Health Center, Farmington, Conn. of May 1, 1981).

Experimental

(1) The technical Ordram used for this study and the corn oil used in dose solution preparation were exactly the same materials which were used in the previous experiment (Ordram antifertility study in mice - Stauffer Toxicology Report T-10121; EPA Accession No. 245675; EPA Registration No. 476-2107).

(2) Technical Ordram was mixed with Mazola Corn Oil to make the dose solutions at the concentration levels of 0, 0.04, 0.8, 2.4, 6.0 and 12 mg/ml. Five mls of the dose solution per kilogram of body weight was administered by oral gavage to obtain the following dose levels: 0, 0.2, 4, 12, 30 and 60 mg/kg, respectively. Samples were taken from each dose solution for concentration analysis and for archival retention.

(3) Sprague-Dawley rats were purchased from Charles River Breeding Labs. in Wilmington, Mass., for parts I, II, and III and from Charles River Canadian Breeding Farms and Laboratories, Ltd., La Prairie, Quebec, Canada, for part IV. During treatment, males were housed individually in cages. During mating, 1 male was housed with 1 or 2 females in one cage. Females were housed 2-4 per large cage before mating and singly in a small cage after mating.

All animals were quarantined at least 7 days before being placed on study. Test animals were assigned to the study groups on the basis of body weight. Permanent I.D. numbers were assigned and each animal was identified using Monel(R) ear tags.

(4) There were 4 parts in the experimental designs:

A. Part I: Twelve males were assigned to each of three dose groups. Each male was cohabited with a female for 7 days prior to the treatment in order to assure an adequate fertility rate. At the start of mating, the males were 9-10 weeks old and the females were 10-12 weeks old. At the end of the predose mating, the males received 5 consecutive daily dosages of 0, 12, or 60 mg/kg/day of Ordram in corn oil by oral gavage. Each male was then cohabited with a new female each week for 10 consecutive weeks. Nine to ten days after the completion of a cohabitation period, the females were sacrificed. The reproductive tract was examined to determine the number of corpora lutea, implants, viable fetuses, and resorptions. At the end of the last cohabitation period, the males were sacrificed.

B. Part II: Twenty males (10-11 weeks old) were assigned to each of two dose groups. The males received either 0 or 12 mg/kg/day of

Ordram in corn oil for 10 weeks by oral gavage. At the end of treatment, each male was cohabited with two females (10-12 weeks old) per week for two consecutive weeks.

Nine to ten days after cohabitation, the females were sacrificed. The reproductive tract was examined to determine the number of copora lutea, implants, viable fetuses, and resorptions present. At the end of the second cohabitation period, the males were sacrificed. Blood was collected for serum hormone measurements, adrenals, and testes plus epididymides were weighed, sperm samples were analyzed, and the testes and epididymides were collected for light microscopic examination.

C. Part III: Twelve males were assigned to each of three dose groups. The males were 9-11 weeks old when treatment began. The males received either 0, 12, or 30 mg/kg/day of Ordram in corn oil for 5 weeks by oral gavage. During the last week of dosing, each male was cohabited with two females (10-12 weeks old). After 15 days' cohabitation period, the females were sacrificed. The reproductive tract was examined as it was done in Part II above. The males were also sacrificed following cohabitation. The blood and sperm samples were collected and analyzed as it was done in Part II. The testes plus epididymides were weighed and collected for light microscopic examination.

D. Part IV: The same study design as in Part III was used, except that the dosages administered were 0, 0.2 and 4.0 mg/kg/day of Ordram in corn oil.

(5) All males were observed daily for overt signs of toxicity or ill-health. Furthermore, all males were weighed and examined thoroughly once each week.

(6) All females in this study and the males in the experimental design I were sacrificed by CO_2 inhalation, while the male rats in Parts II, III and IV were sacrificed by sodium pentobarbital injection and exsanguinated.

The sperm samples collected from several male rats during necropsy were submitted to the University of Connecticut Health Center in Farmington, Conn., for electron microscopic examination. Both scanning and transmission electronmicrographs were taken. The sperm samples were taken from the canda epididymis, suspended in 2 ml of media, and sperm viability, motility, abnormal shapes, and concentration were determined.

(7) The hormone concentrations in the sera are relatively very low and must be measured by the highly sensitive method of radioimmunoassays, which are the techniques derived from the sciences of Immunochemistry and radioisotopes (radiotracers). The serum was separated from the blood samples collected from males in the experimental design II, III, and IV

at termination. The sera were analyzed for hormones, triiodothyronine (T_3), thyroxine (T_4), thyroxine stimulating hormone (TSH), testosterone, luteininizing hormone (LH), and follicle stimulating hormone (FSH) concentrations. The components for the T_3 and T_4 assays were purchased from Antibodies Inc., Davis, Calif., and New England Nuclear, Boston, Mass. The components for the testosterone assay were obtained from Micromedics Systems, Horsham, Pa. and New England Nuclear. The components for the LH, FSH, and TSH assays were obtained from the National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Md., the National Pituitary Agency, Baltimore, Md. and New England Nuclear.

(8) Methods for Statistic Analyses: Incidence data, including fertility indices and frequencies of clinical, necropsy, and histopathology observations were analyzed by Fisher's Exact Probability test. Enumeration data from each litter, including implantion and implant viability indices and the number of corpora lutea, implants, viable fetuses, and resorptions were analyzed by the Mann Whitney-U Nonparametric Rank test. Quantitative data, i.e., body and organ weights, serum hormone concentrations, and sperm analysis parameters, were analyzed using the Bartlett's test for homogeneity, one-way analysis of variance, and Dunnett's t-test. The fiducial limit of 0.05 was employed to obtain the critical level of significance in all the statistical tests undertaken.

Results

(1) The prepared dose solutions were analyzed for actual dose levels used in the four experimental designs:

DOSE SOLUTION CONCENTRATIONS

Experiment Design	Desired Dose Level (mg/kg)	Dose Solution Concentration Desired (mg/ml)	Dose Solution Concentration Measured (mg/ml)	Actual Dose Level (mg/kg)
Part I	0	0	<.017	<0.085
5 day	12	2.4	2.3	11.5
treatment	60	12	10	50
Part II	0	0	<0.017	<0.085
10 week	Mean 12	2.4	2.2	11.0
treatment				
Part III	0	0	<0.017	<0.085
5 week	Mean 12	2.4	2.2	11.0
treatment	Mean 30	6.0	5.4	27
Part IV	0	0	<0.01	<0.05
5 week	0.2	0.04	0.053	0.26
treatment	4.0	0.80	0.95	4.75

Ordram was mixed with Mazola corn oil to produce these dose solutions and 5 ml/kg was administered to each animal. The solutions were prepared once (Part I and Part IV) and weekly (Part II and Part III).

(2) Both individual and weekly mean body weights of the male rats were recorded. No significant differences in body weights between the treated and control animals were observed. Clinical observations for males were also made, but there were no treatment-related clinical signs.

(3) Five animals died during treatment. The individual male necropsy records were made accordingly. In part II, 1 control male died as a result of a dosing accident, 1 male in the 12 mg/kg dose group died with a hematopoietic system neoplasia, and 1 male in the same dose group died due to esophageal impaction. Furthermore, 1 male in the 12 mg/kg dose group of part III and 1 male in the 4 mg/kg dose group of part IV died as a result of a dosing accident. None of the mortalities were treatment-related.

(4) Reproduction Results

A. Part I: This study phase was designed to determine which phases of spermatogenesis were affected by Ordram. A predose mating was conducted to ensure an adequate fertility rate after the treatment period. The males were treated with 0.12 or 60 mg/kg/day of Ordram for 5 days and then mated with a new female per week for 10 consecutive weeks. A reduction in fertility was the basis for determining the phase of spermatogenesis affected by Ordram treatment. A statistically significant reduction in the number of pregnancies (67%) was observed in the females mated to males of the 60 mg/kg dose group during the third week.

The results of necropsy for the pregnant females indicated that the female rats, which mated to the males receiving Ordram treatment at 60 mg/kg, had a statistically significant reduction in the number of implants and viable fetuses per litter after the third mating. There was also a statistically significant reduction in the number of implants per litter in this group during the fourth mating. The implantation indices (number of implants/number of corpora lutea) and the implant viability indices (number of viable fetuses/number of implants) were calculated accordingly. There was a statistically significant reduction in the implantation index in the 60 mg/kg dose group during the third mating. The data indicated that there was a significant increase in preimplantation loss but no significant increase in postimplantation loss in the 60 mg/kg dose group during the third mating. There was a significant reduction in male fertility in the 60 mg/kg dose group but no such reduction was observed in the 12 mg/kg dose group.

Further data correlated the timing of the rat spermatogenic cycle, which would have been affected by the Ordram treatment, with the reduction in the number of implants in the 60 mg/kg dose group. In the third mating week, significant reductions were observed in the number of pregnancies and in the number of implants and viable

fetuses per litter. This third week event corresponded to the late spermatid stage of the rat spermatogenic cycle. Although a significant reduction was also observed in the number of implants per litter during the fourth mating week, the magnitude of the effect was considerably smaller than in the third mating. Therefore, the available data indicated that Ordram treatment adversely affected the mid to late spermatid stages of the rat spermatogenic cycle, with the major effect occurring at the late spermatid stage.

B. Part II: This study was designed to evaluate the effect of Ordram treatment on male rat fertility after 10 weeks of oral administration at 0 or 12 mg/kg/day, after which each male was mated with 2 females per week for 2 weeks.

The female fertility index is the number of females pregnant/the number of females mated. The male fertility index is the number of males impregnating at least one female/the number of males mated. There was a significant reduction in the female fertility index in the 12 mg/kg dose group during the second mating, observed as a reduction in the number of pregnancies.

The data of necropsy for the pregnant females indicated that in females mated to males in the 12 mg/kg dose group there were significant reductions in the number of corpora lutea in the second mating, the number of implants and viable fetuses per litter in both the first and second matings, and the number of total resorptions in the second mating.

As far as the implantation indices and implant viability indices for pregnant females were concerned, there were significant reductions in the implantation indices for both matings. There was also a significant increase in the implant viability index in the second mating. These results indicated that there was a significant increase in preimplantation loss but no increase in postimplantation loss in the females mated to males which received Ordram for a period of 10 weeks. In summary, there was a statistically significant reduction in male fertility in the 12 mg/kg dose group.

C. Part III: This work was designed to study the effect of Ordram treatment on male fertility after 5 weeks of oral administration. Male rats were treated with 0, 12 or 30 mg/kg of Ordram for 5 weeks and then each male was mated with 2 females for one week.

There was a significant reduction in both male and female fertility indices in the 30 mg/kg dose group. The necropsy data for pregnant females indicated that there were significant reductions in the number of implants and viable fetuses per litter, as a result of matings with males from either the 12 or 30 mg/kg dose group. There was also

a significant reduction in the number of resorptions per litter of 30 mg/kg dose group.

There were significant reductions in the implantation indices at both dose levels. There was also a significant increase in the implant viability index in the 30 mg/kg dose group. The results indicated an increase in preimplantation loss but not postimplantation loss in the 12 and 30 mg/kg dose groups. Therefore, Ordram treatment at either 12 or 30 mg/kg for 5 weeks had a statistically significant reduction in the male fertility.

D. Part IV: This experiment was designed to evaluate the effect of low-dose Ordram treatment on male fertility after 5 weeks of oral administration and a no-effect level of the test compound might be determined. The males were treated with 0, 0.2 or 4 mg/kg/day of Ordram for 5 weeks, and then each male was mated with 2 females for 1 week.

There were no significant reductions in the male or female fertility indices at 0.2 mg/kg dose level, but there were considerable reductions in both male (73%) and female (62%) fertility indices at 4.0 mg/kg dose level. The necropsy data for pregnant females showed that there was a significant reduction in the number of viable fetuses per litter in females mated to males in the 4.0 mg/kg dose group. There was a significantly unusual increase in the number of resorp- tions per litter as a result of matings with males in both 0.2 and 4.0 mg/kg dose groups.

There was a significant decrease in the implant viability index in the 0.2 mg/kg dose group. The control value for the number of resorptions per litter in this segment of study was very low compared to the other control groups and the implant viability index was very high. The number of resorptions per litter for control groups in parts I-IV of this study (T-10421) ranged from 0.4-1.4 and the implant viability indices ranged from 88.9-97.3. Therefore, the number of resorptions and the implant viability index for the 0.2 mg/kg dose group fall well within the control ranges for the study.

The data of fertility indices indicated that there was a reduction (73%) in male fertility in the 4.0 mg/kg dose group, but no reduction (100%) in male fertility was observed in the 0.2 mg/kg dose group. Therefore, 0.2 mg/kg was considered as the no-effect level of Ordram treatment for 5 weeks.

(5) Serum hormone levels were analyzed to see if any changes in hormone concentrations could be correlated with the reduced male fertility. Blood samples were obtained from the final male sacrifice in parts II, III and IV. Both individual hormone concentrations and a summary of the mean hormone concentrations of the male rats for each

dose group in three experimental designs (parts II, III and IV) were recorded.

There were no significant differences from the control values in the animals treated with 12 mg/kg for 10 weeks in the experimental design part II. However, the results from the part III indicated a significant increase in FSH concentration in the 30 mg/kg dose group, in testosterone at 12 and 30 mg/kg levels, and T_4 at 30 mg/kg level. While in part IV, there was an increase in FSH at 0.2 mg/kg level and a decrease in FSH at 4.0 mg/kg level compared with the control. The results from parts III and IV were then combined to determine if there were dose-related trends in hormone concentrations. There appeared to be slight increasing trends in testosterone, FSH, and T_4 concentrations with increasing doses. But the ranges of the individual values overlapped to a large extent and increases in the hormone concentrations did not correlate well with the reduction in male fertility. Therefore, the levels of the serum hormones may have no direct correlation with the Ordram antifertility under the aforementioned test conditions.

(6) Sperm samples from the Cauda epididymis were taken from 10 males per dose group in Part II and all animals from Parts III and IV during the final male scarifice. As it was previously stated, the sperm samples were suspended in 2 ml of media and then concentration, percent viable, percent motile and percent abnormal were measured and statistically analyzed. An overall examination of the data from Parts II, III, and IV showed a correlation between impaired fertility in the male rats and statistically significant alterations in sperm viability, morphology, and motility. A good dose-response relationship appeared at 4, 12, and 30 mg/kg dose levels, with 0.2 mg/kg clearly a no-effect treatment level.

Comparisons of the sperm analyses with the number of implants per female were performed to determine if the changes in sperm morphology, concentration, motility, and viability correlate with the reduction in implant number and the reduced male fertility. These comparisons were worked out by using rectilinear graph paper, so that the slopes of the straight lines which best fit the data and the correlation coefficient can be drawn. Good individual correlations were obtained between decreasing implant number and increasing abnormal sperm, decreasing motile sperm, and decreasing viable sperm. However, the correlation coefficient for the comparison of implant number with decreasing sperm concentration was lower than the others, and the magnitude of the slope was also lower. Thus, the sperm morphology, viabilty, and mortility appeared more useful than sperm concentration in detecting an ordram-related reduction in male rat fertility.

The mean number of implants per female for each dose group was also compared to sperm concentration, percent abnormal sperm, percent viable

sperm, and percent motile sperm for each dose group on rectilinear graph paper. There were good correlations between decreasing implant number and increasing abnormal sperm, decreasing motile sperm, decreasing viable sperm, and decreasing sperm concentration. The slopes of the lines which best fit the data had similar absolute values, except for the comparison with the sperm concentration which was higher. These results indicated that there may be two phenomena occuring which correlated with the reduction in male rat fertility: one that involves changes in sperm morphology, motility, and viability, and another involving reduction in sperm concentration.

The mean number of implants per female and the mean number of each of the sperm parameters measured in each dose group were also compared to the log of the actual dose administered for 5 weeks. Again, there were good correlations between increasing log dose and increasing abnormal sperm, decreasing motile sperm, decreasing viable sperm, decreasing sperm concentration, and decreasing implant number. The absolute values of the slopes of the lines which best fit the data had a four-fold range, with those from the abnormal sperm, motile sperm, and viable sperm being similar.

The impairment found in evaluation of sperm parameters correlated well with the reduced male fertility and the log of the administered dose of the test material. Thus, a dose-response correlation was demonstrated, with 0.2 mg/kg being the no-effect level.

(7) The major types of sperm abnormalities produced after ordram treatment for 5 or 10 weeks were detached sperm heads and tails, heads and tails bent at abnormal angles, and rupture of sperm membranes at the head-midpiece junction and the midpiece-tail junction. Both junctions are areas of stress on the sperm membranes.

Both scanning electron micrographs and transmission electron micrographs illustrated the major types of abnormal sperm observed. From observing the electron micrographs and other light micrographs, it appeared that the following sequence of events occurred: the plasma membrane did loosen around the head-midpiece or midpiece-tail junction, allowing the section to be bent abnormally, followed by a rupture of the membrane, and, finally, separation of the head and midpiece or midpiece and tail.

(8) Organs from the final male necropsies were collected. The testes plus epididymides were weighed in Parts II, III, and IV. The adrenals were weighed in Part II. The individual organ, body, and relative organ weights were recorded. The mean organ weights and mean relative organ weights per dose group were also presented. It appeared that there were no significant differences in organ weights between treatment and control groups.

(9) The results of the individual male necropsy in Parts II, III, and IV were recorded. The summaries of the incidence of tissue alterations observed during the final male necropsies from Parts II, III, and IV were also presented. The relative high incidence of fatty livers observed throughout the study was considered a result of using corn oil as a vehicle. There were no tissue alterations with an incidence pattern that could be associated with ordram treatment.

(10) The testes and epididymis from the final male sacrifice in the study parts II, III, and IV were individually and histopathologically examined. While there were no apparent differences in the character of observed microscopic lesions in the testes and epididymis of the treated animals compared to those of the controls, there appeared to be a treatment-related increase in the number of seminiferous tubules containing degenerating spermatids/spermatocytes per testis. This histopathological alteration was characterized by the presence of generally round bodies in or near the lumen of seminiferous tubules. The bodies varied in size from that of spermatids to somewhat larger than primary spermatocytes. Most of these bodies were near the luminal surface and infrequently deeper than the inner third of the tubular cell layer. These cells were distinguishable from spermatids and were present often throughout the inner surface of the tubule. These abnormalities were considered to be degenerate spermatids and were present in all testes, including controls. However, all the available data indicated that the degree of degeneration in the testes (number of spermatids/spermatocytes per testis) was clearly much more severe in the treated males.

There was no increase in the number of tubules containing degenerating spermatids/spermatocytes in the testes of male rats in the 0.2 mg/kg dose group compared to the control animals. Therefore, there is a possibi- lity, which cannot be ruled out at this point, that Ordram treatment may enhance the formation of abnormal seminiferous tubules containing degenerating spermatids/spermatocytes per testis at dose levels higher than 0.2 mg/kg.

Conclusions

(1) There were no treatment-related clinical signs observed in this overall experiment design.

(2) Part I: A statistically significant reduction in the number of pregnancies was observed in the females mated to males of the 60 mg/kg dose group during the third week. On the other hand, the results of necropsy for the pregnant females indicated that the female rats, which mated to the males receiving Ordram treatment at the 60 mg/kg level, had

a statistically significant reduction in the number of implants and viable fetuses per litter after the third week.

The antifertility effect of Ordram in the male rats resulted in increased preimplanation loss, but no significant increase in postimplantation loss was observed. Ordram treatment adversely affected the mid to late spermatid stages of the rat spermatogenic cycle, with the major effects occurring at the late spermatid stage.

(3) Part II: There was a significant reduction in the female fertility index in the 12 mg/kg dose group during the second mating, observed as a reduction in the number of pregnancies. This finding correlated in that there was a statistically significant reduction in male fertility in the 12 mg/kg dose group.

(4) Part III: There was a significant reduction in both male and female fertility indices in the 30 mg/kg dose group. Further experimental data indicated that Ordram treatment at either 12 or 30 mg/kg level for 5 weeks had a statistically significant reduction in the male fertility.

(5) Part IV: The 0.2 mg/kg dose level was found as the no-effect level of Ordram Treatment for 5 weeks as far as antifertility was concerned.

(6) Ordram treatment resulted in an increase in abnormal sperm morphology, and a decrease in sperm viability, motility, and concentration, all of which correlated well with the reduction in male fertility.

(7) There was no increase in the number of tubules containing degenerating spermatids/spermatocytes in the testes of male rats in the 0.2 mg/kg dose group compared to the control animals. However, there is a possibility that Ordram treatment may have enhanced the formation of abnormal seminiferous tubules containing degenerating spermatids/spermatocytes per testis at the dose levels higher than 0.2 mg/kg.

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Review of the Submitted Results - Review 3.

Acute Inhalation Toxicity of Ordram(R) Technical in Albino Rats - Stauffer Toxicology Report T-6598A (by Jeffrey L. Miller, J. W. McCabe and G. L. Zwicker of Richmond Toxicology Laboratory, Inhalation Facility, Stauffer Chemical Company, Richmond, Calif. 94804); August 15, 1980.

Experimental

(1) The test material used for this study was Ordram Technical (Lot No. CGB-1802, 98.2% pure), a composite sample that was typical of production material. The sample was characterized for all identifiable impurities greater than 0.1%.

(2) The test animals were Sprague-Dawley derived rats (males 186-302g; females 166-243g), purchased from Charles River Labs. (Portage, MI). Rats were maintaind according to the acceptable laboratory standards. Feed (Purina Rat Chow(R), Ralston Purina, St. Louis, MO) and tap water were provided ad libitum except when rats were in the exposure chambers. Rats were acclimated to laboratory conditions for 10 days prior to testing. Ten animals (male or female) were used in each group at different dose levels.

(3) Aerosal Generation System: The aerosol was delivered through stainless steel tubing into an aerosol discharger that removed excess static charges on the droplets. The "conditioned" aerosol was introduced into the chamber inlet using stainless steel liners to prevent subsequent recharging of the aerosol. The air, which was purified, was delivered into a manifold containing three calibrated rotameters that controlled the distribution of air to the generator, and to the auxiliary air and dilution air devices.

(4) Aerosol Exposure Chamber: The inhalation chambers used in this study were obtained from a commercial supplier (Young and Bertke Co., Cincinnati, OH). The chambers were constructed of stainless steel and glass and e osed a volume of 447 liters. The chamber make-up air was supplied by awing conditioned room air through an absolute filter and into the inhalation chamber under negative pressure supplied by a centrifugal exhaust blower. The total air flow through the chamber was adjusted for each exposure and ranged from 110 to 130 l/min (15-17 air changes per hour). The relative humidity, air temperature and percent oxygen inside the chamber were monitored using a certified hydrometer and a gas phase oxygen meter. The chamber exhaust was passed through an absolute filter to remove particulate contaminants and through an activated charcoal element to remove vapor contaminants.

(5) Aerosol Concentration and Particle Size Measurements: Actual chamber aerosol concentrations were determined at 60±15 min. intervals during the 4-hour exposure. Samples of chamber atmosphere were collected at the breathing zone of the rats by drawing chamber atmosphere at 1.0 l/min. through a stainless steel filter housing containing a 47 mm fiberglass filter. In the lower exposure levels, a 2-stage charcoal column was placed downstream of the filter to collect Ordram vapor. The filter and charcoal columns were extracted with organic solvent and the amount of the test material was determined by gas-liquid chromatograph equipped with a flame ionization detector.

Aerosal particle size analyses were conducted twice during each exposure period using a low-volume cascade impactor operated at a flow rate of 0.30 l/min. The amount of test material on each impactor stage was quantitated by gas-liquid chromatography and the particle size distribution was determined using a graphical method. Aerosol particle size is reported in mass mean aerodynamic resistance diameters ($MMAD_{ar}$).

A recovery study established the efficiency of recovery of the test material from the aerosol filter and/or vapor column samples. Using the extraction procedure, the quantitative recovery can be achieved.

(6) Exposure Procedures: During the exposure, rats were caged individually in two compartmented stainless steel wire cages. The generation of aerosol was started and attained 99% of the final concentration within 16-19 minutes at the chamber flows used in the previous studies (P.G. Hinners et al., Animal inhalation exposure chamber. Arch. Env. Health 16:194-199; 1968). This atmosphere was presented to the test animals for a 4-hour period.

After the exposure period, the rats were kept in the chambers for an additional one hour at an increased flow rate (~40 air changes/hr.) to facilitate evaporation of the test material from their pelts. Finally, the rats were transferred to holding cages and they remained there for the duration of the test (total 14 days).

(7) All rats were observed twice daily for toxic signs except on weekends and holidays when they were observed once daily and were weighed on days 0 (the day of exposure), 3, 7 and 14. At necropsy, rats were killed exsanguination under ether anesthesia.

The tissues, such as trachea, larynx, nasal passages, lung, liver, kidney and heart, were removed and placed in 10% neutral buffered formulin. Testes from all treated and control males were removed and placed in Bouin's solution. These preserved tissues were then microscopically examined.

Results

A. Aerosol Particle Size Distribution and Chamber Concentration Determination

For high exposure levels of Ordram (4.9-0.9 mg/l), aerosol was collected on glass-fiber filters. Filters were extracted with ethyl acetate and extracts were analyzed for Ordram using a GLC equipped with a flame ionization detector. Recovery from Ordram filters was 98±4%. However, Ordram vapor was not measured at the afore-mentioned high exposure levels.

In the lower exposure levels (0.89-0.059 mg/l), both Ordram vapor and aerosol concentrations were measured. Filters were used to collect aerosol and 2-stage charcoal columns were used to collect vapor. Filters and both stages of the charcoal columns were extracted with carbon disulfide and extracts were analyzed for Ordram using a GLC equipped with

a nitrogen-phosphorus flame ionization detector. Recovery of Ordram from filters and charcoal columns using this procedure was 91±1% and 80±1%, respectively.

All chamber concentrations of Ordram aerosol and vapor were corrected using the recovery values indicated above. The data on the variability in chamber concentrations of Ordram measured in this study indicated a constant amount of Ordram aerosol and vapor (0.06, 0.12, 0.28, 0.83, 0.9, 1.6, 2.2, 2.4, 2.8, 4.0, and 4.9 mg/l of chamber air) was presented to the animals at all times during the test.

This method gave even greater consistency in the aerosol particle size distribution. Values for the mass mean aerodynamic diameter ($MMAD_{ar}$) and the estimated geometric standard deviation (g) of the aerosol ranged from 3.1-4.5 μm and 1.6-2.1δg, respectively.

B. Observations and Histopathology of Male Exposure Groups

(1) All 10 rats died of inhalation of 4.9 mg/l of Ordram aerosol and vapor for 4 hours. Deaths occurred between days 2 and 7. Other toxic signs showed up during and/or following exposure including severe depression, prostration, ataxia, shallow and audible breathing, salivation, brown or red stains about the face, and hindleg weakness. Individual terminal body weights were markedly decreased from day 0 values (19±9%). Necropsy data indicated dark red lungs and reddened intestinal mucosa in 10 rats and red and/or black foci in the stomach mucosa of 9 rats and on the thymus of 7 rats.

The testes of 9 rats had generalized purple appearance, with occasional white and/or red mottling. The testes from one male rat, that died two days postexposure, were examined histologically. Moderately severe lesions included vasculitis, edema, congestion, interestitial hemorrhage, necrosis of germinal cells, and necrosis of interestitial cells. There was also moderate reduction of spermatozoa in seminiferous tubules. Autolysis precluded histopathological evaluation of the testes from other males of this group.

(2) Eight of ten rats were killed as a result of inhalation of 4.0 mg/l of Ordram aerosol and vapor for 4 hours. All deaths occurred between days 2 and 6. Other toxic signs present during and/or following exposure were similar to those observed at 4.9 mg/l exposure level but were generally less severe. Two surviving rats appeared normal by day 14. Terminal body weights for the dead rats were markedly decreased from day 0 values (18±7%). Body weights of surviving rats measured on days 3, 7 and 14 were decreased compared with controls (33, 41 and 35%, respectively). Necropsy findings included black aeras and/or red patches on the lungs (8 rats).

The testes of 8 rats had a generalized purple appearance and the testes of 2 rats that survived to day 14 appeared small and white. Histopathological examination of the testes was conducted on 6/10 rats of this group. Four rats that died between postexposure days 2 and 6 had discolored testes that were sufficiently autolyzed to preclude microscopic examination. The testes of 4 rats that died 2 days after exposure had lesions similar in character and severity to those found in one male of the 4.9 mg/l dose level. The 2 rats, which were killed 14 days after exposure, had somewhat different changes that included marked necrosis of germinal cells involving all of the tubules and interestitial fibrosis. There was also slight focal infiltration of inflammatory cells and atrophy.

(3) Seven of 10 rats died of inhalation of 2.8 mg/l of Ordram aerosol and vapor for 4 hours. All deaths occurred between days 2 and 7. The observed toxic signs included depression, ataxia, red or brown stains about the face, aggression and/or hyperexcitability, shallow and rapid breathing, and hindleg weakness. Ocular changes (cloudy and/or protruding cornea) were observed beginning on day 5 and were generally unimproved on day 14, while all other toxic signs had disappeared by day 12. Individual body weights of the rats that died and and those that survived were markedly decreased.

Both testes of 10 rats had a generalized purple appearance, some also had white mottling. Testes were microscopically examined from one rat that died 7 days after exposure and 3 rats killed 14 days postexposure. The testes of these rats were atrophic and had whitish discoloration. The testes from other rats of this group were discolored and autolytic, precluding microscopic evaluation. Furthermore, the testes examined from rats killed on day 14 showed moderate thickening of the capsule, minimal necrotizing vasculitis, residual evidence of interstitial hemorrhage and moderate dystrophic calcification.

(4) Two of the 10 rats died of inhalation of 2.4 mg/l of Ordram aerosol and vapor for 4 hours. Toxic signs included depression, brown or red stains about the face, aggression and/or hyperexcitability, and hindleg weakness. All surviving rats appeared normal by day 9. Body weights measured on days 3, 7 and 14 were substantially decreased compared with controls (23, 29 and 21%, respectively). Necropsy findings included red and/or gray areas on one or more lung lobes (3 rats) and apparent atrophy and glossy white appearance of one or both testes (9 rats).

Testes from 8 rats of this group, which were killed 14 days postexposure, were microscopically examined. Autolysis precluded examination of tissue from the two rats that died. All of the testes examined were atrophic, lesions included slight to moderate capsular thickening, slight vascular congestion, slight to moderate necrotizing vasculitis, marked necrosis of germinal cells, moderate

dystrophic calcification and moderate hyperplastia of interstitial cells.

(5) All 10 rats survived inhalation of 2.2 mg/l of Ordram aerosol and vapor for 4 hours. Toxic signs observed during and/or following exposure were similar in character and duration to those noted in the 2.4 mg/l exposure group. All rats appeared normal by day 11. Body weights measured on days 3, 7 and 14 were substantially decreased compared with control (18, 26 and 22%, respectively). Necropsy findings included apparent atrophy and glossy white appearance of one or both testes in 10 rats.

The testes of all rats in this group were atrophied. The micropathologic changes were generally similar in severity and character. Most of these changes were similar to those noted in 2.4 mg/l treatment group.

(6) Again, all rats survived at a dose level of 1.6 mg/l of Ordram aerosol and vapor for 4 hours. Toxic signs observed during and/or following the exposure were similar in nature and duration to those noted in the 2.4 mg/l exposure group, but were generally less severe. Except for the ocular anomalies which did not improve during the study, all rats appeared normal by day 11. Body weights taken on days 3, 7 and 14 were significantly decreased compared with controls. Necropsy findings included a glossy white and atrophied appearance of one or both testes in 8 rats.

The testes of 8 of 10 rats in this group were atrophied. For all rats, micropathological changes were similar in nature and duration to those observed in the 2.2 mg/l group.

(7) All 10 rats survived the exposure to 0.9 mg/l of Ordram aerosol and vapor for 4 hours. Toxic signs observed during and/or immediately after the exposure included depression or aggression. All rats appeared normal by day 2. Body weights taken on days 3 and 7 were still substantially decreased compared with controls (16 and 15%, respectively). Necropsy findings included apparent atrophy and glossy white appearance of one or both testes in 2 rats, apparent atrophy and glossy white with a purple tinge white discoloration of one or more testes in 2 rats.

The testes of 4 male rats, that were killed 14 days after exposure, were microscopically examined. The testes were atrophic and had slight to moderate capsular thickening, slight vascular congestion and trace focal necrotizing vasculitis (phlebitis) marked germinal cell necrosis.

(8) One of 10 rats died of inhalation of 0.83 mg/l of Ordram aerosol and vapor for 4 hours. Toxic signs observed during and/or after exposure included depression, shallow and audible breathing, brown or red stains about the face, hindleg weakness and dark pupils and/or opaque

corneas. All remaining rats appeared normal by day 9. Body weights measured on days 3,7, and 14 were substantially decreased compared with controls (15,21 and 15% respectively). At necropsy, lesions were noted on one or both testes in 8 rats and included slight to moderate purple discoloration (5 rats) and/or pink and white mottling (3 rats) in addition to other findings such as a distended and red fluid filled bladder in one rat.

One rat of this treatment group died 6 days post-exposure and had tissue autolysis, so the testes were not examined. Testes from the 9 rats killed 14 days post-exposure were examined microscopically. 8 rats had generally similar alterations both in character and severity. Lesions included trace focal to moderate capsular thickening, moderate to marked germinal cell necrosis, and trace focal to moderate interstitial cell hyperplasia.

(9) All 10 rats survived the exposure to 0.28 mg/l of Ordram aerosol and vapor for 4 hours. Toxic signs observed after exposure included slight to moderate depression and hindleg weakness. All rats appeared normal by day 4. Body weights measured on days 3, 7 and 14 were moderately decreased compared with controls (11,8 and 7% respectively). At necropsy, one or both testes of 4 rats had a slight purple discoloration. No other gross lesions were observed.

Micropathological examination of the testes from all rats of this group revealed no apparent exposure related alterations.

(10) Again, all 10 rats were alive after exposure to 0.12 mg/l of Ordram aerosol and vapor for 4 hours. Toxic signs after exposure included slight to moderate depression. All rats appeared normal by day 2. Body weights measured on days 3,7 and 14 were decreased (9,7 and 6% respectively) compared with controls. At necropsy, no gross lesions were observed. Micropathological examination of the testes from all male rats in this group showed no apparent exposure related alterations.

(11) All 10 rats survived the inhalation of 0.06 mg/l (the lowest dose level in the tests on male rats) of Ordram aerosol and vapor for 4 hours. Toxic signs after exposure included slight to moderate depression and brown stains around the anogenital area. All animals appeared normal by day 1 with the exception of 2 rats which had dark scabs over the left eye on days 5 and 7. Body weights measured on days 3,7 and 14 were decreased compared with controls (6,9 and 9% respectively). At necropsy, no gross lesions were noted. Furthermore, micropathological examination of the testes from all male rats of this treatment group revealed no apparent exposure related alterations.

B. Observations and Histopathology of Female Exposure Groups

(1) All 10 rats died as a result of inhalation of 4.9 mg/l of Ordram aerosol and vapor for 4 hours. All deaths occurred by day 2. Toxic

signs observed during and/or after exposure included severe depression (lethargy), prostration, shallow and audible breathing, ataxia, salivation, and brown or red stains about the face. Terminal body weights of rats that died were decreased from day 0 values (12 + 1%). Necropsy findings included red and/or darker areas on the lungs of 10 rats, and reddened intestinal mucosa in 2 rats. Other findings included black foci on the thymus (3 rats), green liquid and/or solids in the urinary bladder (8 rats).

(2) All 10 rats were killed as a result of exposure to 4.0 mg/l of Ordram aerosol and vapor for 4 hours. All deaths occurred by day 2. Toxic signs were similar to those showed up in the 4.9 mg/l group. Terminal body weights of rats that died were moderately decreased compared with day 0 valued (12 $\pm$ 4%). Necropsy findings were also similar to those occurred in the above treatment group.

(3) 9 of 10 rats died of inhalation of 2.8 mg/l of Ordram aerosol and vapor for 4 hours. All deaths also occurred by day 2. Toxic signs observed during and/or after exposure included depression, shallow and audible breathing, ataxia, aggression (fighting) and/or hyperexcitability, hindleg weakness and abnormal appearing eyes (opaque corneas or dark appearing pupils). Terminal body weights of rats that died were moderately decreased compared with day 0 values (14$\pm$2%). The mean body weight of the surviving rats measured on days 3,7 and 14 were markedly decreased from controls (23,26 and 16% respectively). Necropsy findings included red and/or darker patches on the lungs (9 rats), reddened intestinal mucosa and/or stomach mucosa (9 rats), etc.

(4) 4 of 10 rats died of inhalation of 2.4 mg/l of Ordram aerosol and vapor for 4 hours. All deaths occurred between days 2 and 4. Toxic signs observed during and/or after exposure included hyperexcitability and hindleg weakness in addition to those which occurred in the aforementioned treatment groups. All surviving rats appeared normal by day 4. Body weights measured on days 3 and 7 were moderately decreased compared with controls (16 and 10% respectively).

(5) 3 of 10 rats were killed as a result of inhalation of 2.2 mg/l of Ordram aerosol and vapor for 4 hours. All deaths occurred between days 2 and 3. Toxic signs observed during and/or after exposure were similar to those occurred in the 2.4 mg/l treatment group. All surviving rats appeared normal by day 8. Body weights measured on days 3 and 14 were significantly decreased compared with controls (15 and 12% respectively). Necropsy findings were also somewhat similar to the fore-mentioned 2.4 mg/l treatment group.

(6) All 10 rats survived the exposure to 1.6 mg/l of Ordram aerosol and vapor for 4 hours. Toxic signs observed during and/or after exposure included hindleg weakness, hyperexcitability and opaque corneas and/or dark appearing pupils. All rats appeared normal by day 10.

Body weights measured on day 3, 7 and 14 were substantially decreased compared with controls (16, 16 and 12% respectively).

(7) All 10 rats survived the inhalation of 0.9 mg/l of Ordram aerosol and vapor for 4 hours. All rats appeared normal by day 2. Body weights measured on days 3, 7 and 14 were slightly decreased compared with controls (7, 8 and 7%, respectively).

C. LC_{50} Records

The acute LC_{50} of Ordram Technical was determined to be 2.9 (2.5-3.3) mg/l in male rats and 2.4 (2.2-2.6) mg/l in female rats.

Conclusions

(1) The particle size distributions of Ordram Technical, which were analyzed by a low-volume cascade impactor (Aries, Inc., Davis, California) operated at a flow rate of 0.30 l/min, showed mass median aerodynamic diameters of 3.1-4.5 µm with standard geometric deviations of 1.6-2.1, resulting in greater than 95% of the particles having diameters <9 µm.

(2) The acute LC_{50} was determined to be 2.9 mg/l in male rats and 2.4 mg/l female rats (minimum data).

(3) Exposure-related clinical signs included depression, prostration, ataxia, shallow and audible breathing, agressive behavior, and hindleg weakness. These signs disappeared in rats that survived the 14-day observation period.

(4) Body weights measured on days 3, 7, and 14 were significantly decreased in all exposure groups compared to the controls.

(5) The detailed micropathic evaluation of testes from male rats, which were exposed for 4 hours to concentrations in the range of 0.83 mg/l to 4.9 mg/l showed acute testicular damage, apparently related to vasculitis (small veins); and apparent decreases in both absolute testes weights and relative testes weights. Further, male rats exposed to lower concentrations, such as 0.28, 0.12 and 0.06 mg/l, indicated no significant micropathological evidence of testicular lesions.

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Review of the Submitted Results - Review 4.

Acute Inhalation Toxicity of Ordram Technical in Male Albino CD-1 Mice - Stauffer Toxicology Report T-6598B (by J. W. McCabe and J. L. Miller and G.M. Zwicker of Richmond Toxicology Laboratory, Stauffer Chemical Company, Richmond, Calif., July 24, 1980.

Experimental

1. Ordram Technical (Lot No. CGB-1802, 98.2% pure) used for this study was a composite sample that was typical of production material.

2. Male albino CD-1 mice (21-33g) were obtained from Charles River Labs. (Portage, MI) and housed (10 per group) in clear plastic cages containing hardwood chip bedding. Feed (Purina Rat Chow) and tap water were provided ad libitum except when mice were in the exposure chamber. Mice were acclimated to laboratory conditions for 10 days prior to testing.

3. The aerosol generation system, aerosol exposure chamber and aerosol and vapor concentration and particle size measurements were very close to what were described in previous reviews (Review of Acute Inhalation Toxicity of Ordram Technical in Albino Rats - Stauffer Toxicology Report T-6598A) of this series of investigation.

4. Exposure procedures: During the exposure, mice were housed individually in a stainless steel wire cage containing ten compartments. The cage was placed in the middle shelf of the exposure chamber. The generation of aerosol and vapor was initiated and attained 99% of the final concentration within 12-24 minutes depending upon the chamber air flow. This Ordram Technical atomosphere was supplied to the test animals for a 4-hour period.

   After the exposure period, the test animals were immediately transferred to holding cages equipped with automatic watering and lighting systems. Mice remained under these conditions for the duration of the test.

5. Mice were observed twice daily for toxic signs except weekends and holidays when they were observed once daily. The test animals were weighed on days 0 (the day of exposure), 3, 7, and 14.

6. The tissues, such as trachea, larynx, nasal passages, lung, liver, kidney and heart, were removed at necropsy of male mice and placed in 10% neutral buffered formalin. Testes and epididymides were placed in Bouin's solution. Histopathological evaluation of testes and epididymides was performed accordingly.

Results

A. Aerosol particle size distribution and chamber concentration determination: These described sensitive and reproducible procedures used for analysis of Ordram in inhalation exposure atmospheres provided accurate determinations of the mean exposure concentration of Ordram (0.034, 0.09, 0.23, 1.1, 1.8, 2.0, 2.3 and 3.2). These data indicated a constant amount of aerosol was presented to the test animals at all times during the test.

The consistency obtained in the aerosol particle size was even greater. The values for mass mean aerodynamic diameter ($MMAD_{ar}$) and the estimated geometric standard deviation (6g) for the aerosol ranged 3.1~ 4.7 um and 1.5-2.0, respectively.

B. Observation of toxic signs, necropsy and histopathology

The toxic signs were recorded daily for the 14-day test period and individual body weights were measured on days 0, 3, 7 and 14. Testicular histopathology findings for individual mice were also presented.

1. All 10 mice were killed as a result of inhalation of 3.2 mg/l of Ordram aerosol for 4 hours. All deaths occurred on day 1. Other toxic signs included severe depression, prostration, ataxia, dyspnea and wet fur. Individual body weights were moderately decreased from day 0 values (9±1%). Necropsy findings included red lungs in 10 mice and prominent blood vessels of the surface of the right testicle of one mouse. Although there were few testicular lesions noted in these mice, it was probable that the treated mice did not survive sufficiently long enough for the testicular vascular or germinal cell lesions to develop.

2. All 10 mice were killed after inhalation of 2.3 mg/l of Ordram aerosol for 4 hours. All deaths occurred on day 1. Toxic signs observed at the 2.3 mg/l exposure were similar to those observed at the 3.2 mg/l exposure level but were generally less severe. Terminal body weights were moderately decreased from day 0 values (8±2%). Necropsy findings included red lungs in 10 mice. Only few testicular lesions were observed in these mice, again it was possible that the treated mice did not survive sufficiently long for the testicular vascular or germinal cell lesions to develop.

3. 3 of 10 mice died of inhalation of 2.0 mg/l of Ordram aerosol for 4 hours. All deaths took place between days 1 and 8. Other toxic signs included depression, prostration, ataxia and moderate to severe leg weakness. All survivors appeared normal by day 2. Body weights of surviving mice measured on days 3, 7 and 14 were significantly decreased compared with control (22, 27 and 21% respectively). There was slight to moderate germinal cell necrosis accompanied by formation of germinal giant cells in 5 of 7 remaining males killed on post exposure day 14.

4. 4 of 10 mice died of inhalation of 1.8 mg/l of Ordram aerosol for 4 hours. All deaths occurred between days 1 and 11. Toxic signs observed in this group of mice were similar to those noted at the 2.0 mg/l exposure level. All survivors appeared normal by day 5 except for one mouse that appeard emaciated and had ruffled fur on day 14. Body weights of surviving mice measured on days 3, 7 and 14 were significantly decreased compared with controls (11, 25 and 22% respectively). Necropsy findings included red discolored lungs in 2 mice. No other gross lesions were observed.

Three of 4 mice that died were examined histopathologically. Moderately severe testicular alterations were found in the 2 males that died on the 5th day after exposure. Furthermore, four of the 6 mice killed after the 14-day test period had slight to marked testicular germinal cell necrosis while the other 2 mice had no apparent exposure-related testicular damage.

5. All 10 mice survived the inhalation of 1.1 mg/l of Ordram aerosol for 4 hours. Toxic signs included depression and ptosis. All mice appeared normal by day 2. Body weights measured on days 3, 7 and 14 were significantly decreased compared to controls (10, 8 and 10% respectively). At necropsy, no gross lesions were observed. There were no testicular lesions with severity pattern which might be considered as an exposure-related effect.

6. Because of the unexpected mortality observed in the initial 0.23 mg/l exposure group, a second group of males was exposed to this level. The exposure levels were designated as 0.23a and 0.23b mg/l, respectively.

   a. Inhalation of 0.23a mg/l for 4 hours: 5 of 10 mice were killed. All deaths occurred on days 1 and 2. Toxic signs included depression, dyspnea, ataxia, ptosis, ruffled fur, emaciation, pale extremities, red stains around the eyes and a swollen muzzle. All survivors appeared normal by day 14. Body weights measured on days 3, 7 and 14 were significantly decreased compared to controls (11, 23 and 14%, respectively).

   Necropsy findings included red discolored lungs in 5 mice, small testes in 1 mouse and a slightly purple discolored left testes of 1 mouse. However, no testicular lesions were observed with an incidence or severity pattern suggestive of an exposure-related effect.

   b. Inhalation of 0.23b mg/l for 4 hours: All 10 mice survived. Slight depression was the only toxic sign observed. All mice appeared normal by day 2. Body weights measured on days 3, 7 and 14 were significantly decreased compared to controls. At necropsy, no gross lesions were noted. There were no testicular lesions with an incidence or severity pattern suggestive of an exposure-related effect.

   c. No explanation was offered for the possible reasons concerning the different results obtained at the afore-mentioned inhalation levels of 0.23a and 0.23b mg/l for 4 hours.

7. All 10 mice survived inhalation of 0.09 mg/l of Ordram aerosol and vapor for 4 hours. Moderate depression was the only toxic sign observed. All mice appeared normal by day 1. Body weights measured on day 3 were significantly decreased compared to controls (7%). At necropsy, no gross lesions were noted. There were no testicular lesions which might be considered as exposure-related.

8. All 10 mice survived inhalation of 0.034 mg/l of Ordram aerosol and vapor for 4 hours. Toxic signs included depression, prostration and transient hindleg weakness. All mice appeared normal by day 5. At necropsy, no gross lesions were observed. There were no testicular lesions observed and suggestive of an exposure-related effect.

C. LC_{50} data: The acute LC_{50} of Ordram Technical in male mice was determined to be 2.1 (1.9-2.3) mg/l.

Conclusions

1. Toxic signs included depression, prostration, ataxia, dyspnea, hindleg weakness, ptosis and red stains around the eyes. These signs generally disappeared by day 6. However, signs that were apparently exposure-related in intensity and duration included prostration, ataxia and dyspnea.

2. The LC_{50} was determined to be 2.1 (1.9 - 2.3) mg/l (minimum data).

3. Gross testicular changes were seen in a few mice and included apparent atrophy, purple discoloration, and prominent vessels on the surface of the testes.

4. No effect on relative or absolute testes weights was measured in mice exposed to 1.8 mg/l or less (absolute testes weights in the 2.0 mg/l exposure group were significantly decreased with $p<0.05$).

5. Testicular lesions including variably severe necrosis of testicular germinal cells were seen in several male mice of exposure groups 1.8 and 2.0 mg/l but were not present in any exposure-related patterns in mice exposed to concentrations of Ordram Technical 1.1 mg/l or less.

6. At necropsy, reddened lungs were the most prominent finding.

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Review of the Submitted Results - Review 5.

Dose and Time Relationships on Coagulation Parameters, Hematology and Blood Chemistry in Male and Female Rats Fed Ordram in the Diet - Stauffer Toxicology Report T-6216 (by Jean Scholler of Stauffer Chemical Company, Richmond, California).

Experimental:

(1) The test material used in this study was a brown liquid Technical Ordram (Lot No: 4816-13-328). Appropriate amounts of Technical Ordram (adjusted for purity) were first premixed with volumes of corn oil (1% by weight of the total diet). The test material-corn oil premixes were then added to a small amount of the basal diet (Purina Laboratory Chow, a commercial laboratory ration) and mixed in a Hobart blender. These mixtures were then added to the required amounts of basal diet and thoroughly mixed in a twin-shell Patterson-Kelly blender. This final diet provided the appropriate dietary concentrations of Ordram. Fresh diets were prepared weekly. The control diet, prepared in the same manner as the test diet, was a mixture of the basal diet and 1% by weight corn oil.

Samples of the control and test diet prepared were taken at periodic intervals and were chemically analyzed for the actual concentrations of the test material in the diets.

(2) Two hundred and fifty male and the same number of the female Sprague-Dawley derived rats were purchased from Simonsen Labs., Gilroy, California. The rats were approximately 6 weeks of age at the initiation of the test. The rats were assigned in a random fashion to t following control and test groups:

Group	Sex	Dietary Dose Level (mg/kg/day)	No. of Rats
A	F	Control	40
B	F	5	25
C	F	20	40
D	F	80	45
E	F	160	50
F	M	Control	40
G	M	5	25
H	M	20	40
I	M	80	45
J	M	160	50

(3) Body weights and food consumption were recorded weekly. Observations of gross signs of toxicity, pharmacologic effects, and mortality were taken twice daily.

(4) A few clinical studies were performed during the course of the test using standard laboratory procedures.

a. Hematology: Hematocrit, Erythrocyte Count and Total Leucocyte Count.

b. Clotting parameters: Prothromlin Time, Activated Partial Thromboplastin Time, Factor V, Factor VII, and Factor X.

c. Clinical Blood Chemistry: Blood Urea Nitrogen, Plasma Glutamic Pyruvic Transaminase and Total Protein.

(5) Five randomly chosen rats were sacrificed at various intervals (Interim sacrifices) for the determination of the above-mentioned clinical studies.

Results

(1) It is noted in the following that the only significant difference (clinical observations) between the control and the rats treated with Ordram was observed at the 80 and 160 mg/kg dose levels in which weakness in the hind legs was observed.

Females - 80 mg	7/40	slight to moderate with the majority showing
Females - 160 mg	13/50	recovery prior to termination
Males - 80 mg	20/40	slight to moderate
Males - 160 mg	44/50	moderate to severe with all rats showing improvement prior to termination.

(2) No significant differences in body weights were observed between the controls and the 5 and 20 mg/kg dose levels for both male and female rats. However, at dose levels of 80 and 160 mg/kg a moderate to servere weight gain depression in both sexes, -27% and -40% for

the males and -16% and -37% for the females were noted respectively.

(3) The amount of Ordram-intake from the diet should be theoretically proportional to the amount of food (test diet) consumption recorded.

It is practically difficult to record the accurate amount of food consumed by the individual animals because of the unusual habits of these "sloppy" eaters, although food consumption was not severely depressed. For this reason, the actual amount of Ordram-intake was variable, especially at the high doses as the following:

Theoretical:	Actual Dose Levels[a] - mg/kg/day Actual Days of Experiment		
Females	0	6	12
5	3.3	3.8	6.1
20	16	15	25
80	56	86	86
160	96	148	234
Males			
5	3.9	4.4	5.3
20	17	18	20
80	74	96	80
160	172	154	180

[a] Ordram concentration (mg/hg of chow)[b] x Mean Food Consumption (kg/rat/day) Mean Body Weight (hg/rat).

[b] Based on actual analyses of feed.

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(4) No significant alterations were observed in the hematological nor the blood biochemical parameters examined. At the 160 mg/kg dose level,

both the male and female rats showed a slight coagulopathy as indicated by a slight increase in the activated partial thromboplastin time (APTT) and increase in Factor X at day 4. These values for the females returned to normal by day 12 of the test. The APTT for the males was normal at day 21 whereas Factor X did not return to normal level until termination at day 21.

(5) No mortalities were observed in any of the rats at any dose level. There were no abnormalities noted at autopsy in the females at any dose level and at any time intervals. In the case of male rats, the only abnormality observed at autopsy was a slight to moderate hemorrhage in the testes at the dose level of 160 mg/kg/day. This was noted in 4/5 males at day 4 but in only 1/5 males at day 12 and in none of these male rats at day 21. This indicated that in spite of continued treatment, the tendency to hemorrhage is reversible. These findings corroborate the reversal of the coagulopathy observed in the clotting parameters.

Discussions

(1) Coagulopathy produced in male and female rats at a dose level of 160 mg/kg was mild and reversible as evidenced by slight increase in APTT and by slight increase in factor X at day 4 (Note: The submitted report stated that "slight decrease in factor X showed up at day 4" was an error). These values returned to normal by day 12 in the females and day 21 in the males. It appeared that 20 mg/kg was the NOEL for the males and 80 mg/kg was the NOEL for the females.

(2) The only adverse gross observations recorded were (a) a mild to severe hindleg weakness which was dose-related (at both 80 and 160 mg/kg dose levels) and reversible or improvable prior to termination and (b) a mild to severe weight-gain depression in both sexes at 20, 80 and 160 mg/kg dose levels.

(3) The applicant stated in their submitted report that at autopsy slight to moderate hemorrhage in the testes was noted in 4/5 male rats at day 4 and in only 1/5 male rats at day 12 in the case of 160 mg/kg/day dose level and that this abnormality disappeared at day 21. No such experimental records were provided in the application to support the foregoing statements.

Review of the Submitted Results - Review 6.

SUBJECT: EPA Registration No. 476-2107. The Effect of Ordram on Nonhuman Primate Sperm Production - Stauffer Toxicology Report T-10714

(By J. M. Killinger, S. L. Wilcznski, et. al. of Stauffer Chemical Company, Environmental Health Center, Farmington, Connecticut 06032; Stauffer Toxicology Report T-10714 of December 16, 1981.)

This study was intended to determine the effect of Ordram on nonhuman primate sperm production and serum reproductive hormone levels.

I. Experimental

(1) The Ordram Technical (Lot #CGB-1802; Code #EHC-0009-46; expiration date 9/87) was used as the test material (an amber liquid with 98.6% purity and a pH of 8.0).

The dose level selection of vehicle control, 0.2, 10.0, and 50.0 mg/kg/day of Ordram was based on the results of a preliminary range-finding study in monkeys (T-10709).

The dose solutions were prepared by mixing technical Ordram with food grade Mazola corn oil (Lot #NOV 1181A, expiration date 11/81) from Best Foods in Englewood Cliffs, New Jersey. The dose solutions were prepared in corn oil once for the study and analyzed for concentration initially and then every three to five weeks throughout the study. The final analysis was approximately four months after its preparation. Samples of the dose solutions and test substance were retained in the EHC archives.

(2) Thirty adult male Macaca fascicularis (crab-eating or cynomolgus macaques) monkeys were obtained from Charles River Research Primates, Inc., Port Washington, New York. Twenty-eight animals were selected for the study on the basis of general good health and ability to produce sperm samples of adequate quantity and quality. The animals were assigned to the aforementioned four dose groups (7 each group), namely 0 (vehicle control), 0.2, 10.0, and 50.0 mg/kg/day of Ordram in accordance with their weight. It was 3.5 to 6.0 kg at their arrival.

(3) The animals received 1 ml/kg of the appropriate dose solution based on their weekly body weight. The animals were dosed orally five days per week for 12 weeks using a nasal gastric tube. During the treatment period, the animals were weighed weekly and observed twice daily for overt signs of toxicity.

(4) During treatment, blood samples were collected every four weeks from the femoral vein or artery after the animals were anesthetized with ketamine. The blood samples were analyzed for clinical chemistry, hematology, clotting time, and reproductive hormone values.

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A. Clinical chemistry parameters measured:

Plasma and RBC cholinesterase activity
Serum glucose concentration
Serum blood urea nitrogen concentration
Serum creatinine concentration
Serum gamma glutamyl transferase activity
Serum alkaline phosphatase activity
Serum glutamic oxaloacetic transaminase activity
Serum glutamic pyruvic transaminase activity

B. Hematology parameters measured:

Total leukocyte concentration and differential leukocyte percent
Total erythrocyte concentration
Hemoglobin concentration
Hematocrit percent
Platelet concentration

C. Coagulation time measured:

Prothromlin time
Partial thromboplastin time
Stypven time

D. Reproductive hormone assays

The materials for LH and FSH radioimmunoassays were supplied by the National Institutes of Health. Rhesus pituitary gonadotropin was used as the reference preparation for both assays. Cynomolgus LH was used for iodination and anti-human chorionic gonadotropin was used as the primary antibody in the LH assay. Human FSH was used for iollination and anti-ovine FSH was used as the primary antibody in FSH assay. The testosterone was also assayed. Serum samples were used for assaying LH, FSH and testosterone by using the double antibody techniques.

(5) Sperm sample collection and analysis: Sperm samples were collected and analyzed weekly during the treatment period. The procedures of electro-ejaculation, using the rectal probe method after the animals were anesthetized with ketamine, have been used for collection of sperm samples. Sample volume was estimated, the ejaculate was diluted in a modified Ham's F-10 media, and motility was evaluated immediately. The diulted samples were kept at 37°C for one hour to allow the coagulation to liquefy and the sperm cell concentration and morphology were evaluated. The total sperm count was estimated from these obtained values accordingly.

(6) A recovery period was not set up because they found no treatment-related effects of Ordram on sperm production. The animals were not sacrificed either at the end of the study.

(7) The obtained data, such as body weight, pupil diameters, clinical chemistry values, hematology values, clotting times and reproductive hormone values, were analyzed by the one-way analysis of variance and Dunnett's t-test. Enumeration data from the sperm analysis parameters were analyzed by the Mann-Whitney U two-sample rank test. The fiducial limit of 0.05 was employed to delineate the critical level of significance in all statistical comparisons.

II. Results

A. Analyses of the dose solutions indicated that the test material was stable in corn oil at room temperature for four months. The concentration of the 0.2 mg/ml solution was within 15% of the designated concentration and the 10.0 and 50.0 mg/ml solutions were within 10% of the designated concentrations.

B. General Observations:

There were no significant differences in body weights between treated and control animals observed. There were no treatment-related clinical signs or changes in the parameters measured during physical examinations. There were no significant treatment-related changes in pupil diameter measurements. There were no animal mortalities noted during this study.

C. Blood sample anaylses:

1. Clinical Chemistry - There was a treatment-related decrease in plasm cholinesterase activity in the 50 mg/kg dose group with $p<0.05$ significantly different from control. No other treatment-related changes in clinical chemistry parameters were noted.

2. Hematology - No significant treatment-changes in hematology or coagulation factors were observed.

3. Clotting times - There were no significant treatment-related effects on the clotting times.

4. Reproductive hormones - There were no treatment-related changes in the levels of hormones measured in this study.

D. Sperm sample analyses:

1. Sperm motility - There were no treatment-related decreases in sperm motility. The percentage of the motile sperms in the 0.2 mg/kg dose group was observed to be significantly lower than the control value during the 9th week of treatment. However, this was not significantly different from its pretreatment values.

2. Abnormal sperm morphology - The percentage of the abnormal sperms in the 0.2 mg/kg dose group was significantly higher than the control value during the second week of treatment. Again, this value was not significantly different from its pretreatment values. Therefore, in general there were no treatment related increases in abnormal sperm morphology.

3. Ejaculate volume of sperms - There were no treatment-related changes in the ejaculate volume of sperms.

4. Sperm cell concentration - Three values appeared in the 0.2 mg/kg dose group that were significantly lower than their corresponding control values during the first, second, and ninth weeks of treatment. However, these values during treatment were not significantly different from the pretreatment values for the 0.2 mg/kg dose group. The sperm cell concentration was consistently lower in this 0.2 mg/kg dose group throughout the study, including the pretreatment period. Therefore, in general there were no treatment-related decreases in sperm cell concentration.

5. Total sperm count - The total sperm count from the 0.2 mg/kg dose group was relatively low during the pretreatment and throughout the study period. In general, no treatment-related decreases were observed in the total sperm count.

III. Discussion

1. The center of interest in this study was the possible effects of Ordram on the sperm production and reproductive hormone levels in the non human primates. In general, Ordram appeared having no treatment-related effects observed in sperm sample motility, morphology, volume, concentration, or total count. Also, there were no treatment-related changes in the reproductive hormones assayed.