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DATE: 11/11/77

SUBJECT: EPA 476-2107, 6(a)(2) Submission of Data on Ordram (8-E g#/gal E

FROM: R.B.Jaeger
TB

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TO: Henry Jacoby
PM 24

Data submitted on Ordram is reviewed as follows:

1. "Suppression of Fertility In Male Rats, Ordram Technical, Final Report", Litton Bionetics, Inc., LBI Project No. 2021, dated 10/29/76, submitted by Stauffer Chem. Co. 8/19/77 (Acc#231329).

a. Protocol:

Substance Tested: Ordram Technical

Species: Charles River Rats (CRL:COPS CD(SD)ER)

Sex and Age: Male, 100 days of age

Number of Animals: as below -

Group No.	No. of Male rats	Dose (mg/kg/day)
1	20	0
2	10	0.2
3	10	1.0
4	20	5.0* (in diet) 2.0* (by gavage)
5	10	0
6	10	5.0

*Note; laboratory miscalculated the amount for gavage and instead of 5.0 administered only 2.0 mg/kg/day.

Conduct of Test:

Design: Diets prepared by blending the amounts of test material necessary to provide the daily intake of doses, indicated above, to the basal diet of Purina Laboratory Chow. Male rats in groups 2,3 and 4 received these diets for nine days; then the test material, suspended in corn oil, was administered by gavage for five days during mating. The males of group 4 which were retained

for recovery matings continued to receive the test material in the diet during this 5-day period. Group 6 rats were provided the test diet for 14 days. The control groups (1 and 5) received diets containing basal diet throughout the study, and Group 1 males used for the first mating were gavaged with 10 mg/kg of corn oil during the same period treated groups received test material by gavage.

On days 10 to 14 after the initiation of treatment 10 male rats of Groups 1 through 4 were paired for mating with 2 females of the same strain and source of the males. A similar pairing occured in Group 5 and 6 immediately after treatment ceased, and in Groups 1 and 4 at 2 and 4 weeks after treatment had been stopped.

Females inspected daily during mating for signs of copulation (copulatory plug or presence of sperm in the vagina). At 13 day. after removal from the mating cage, the females were killed. The number of live and dead fetuses, resorption sites, and corpora lutea were counted.

After the mating period, the male rats were killed, the testes removed and weighed, sperm samples obtained and evaluated, and the testes, seminal vessicles, coagulation gland, and prostate were fixed in 10% neutral formalin and submitted for histopath. evaluation.

Schematic of the above experimental design is believed to be as follows:

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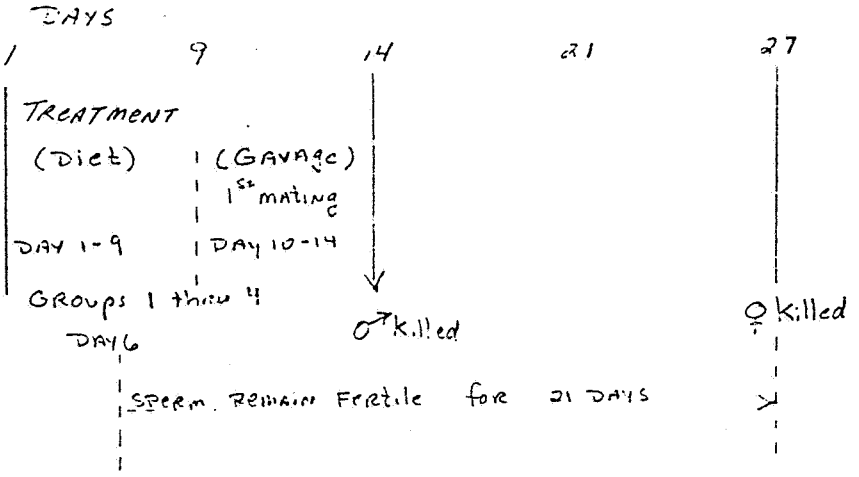
#1



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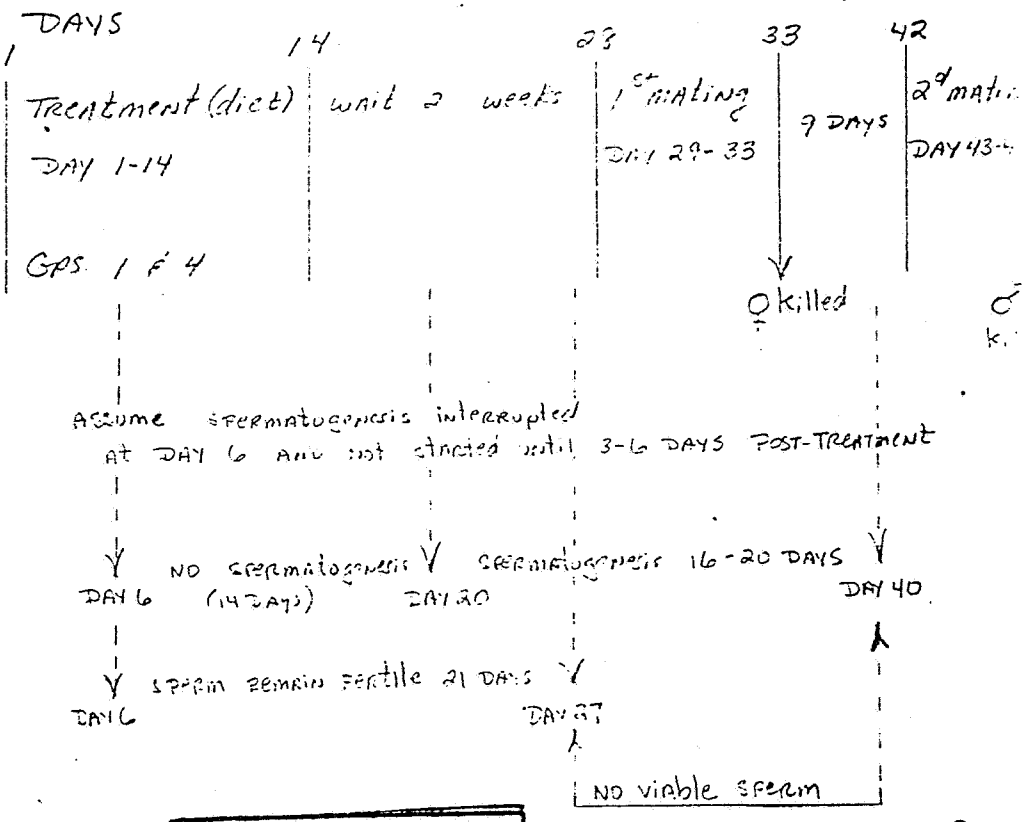

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b. Results:

-No significant difference in body weight and food consumption from treatment and control groups.

-Pathology was unremarkable, however, overall pathology reported only for male and female rats in "first mating" groups. Histologic exam of all male rats, but confined to testes, epididymis, prostate and accessary genital gland.

-Slight increase in agglutination of sperm from 1.4 for GpI to 1.9 for GpIV. Semen collected from rats killed after treatment ("first mating" males).

-Testes to body weight increased from GpI to GpIV :

Gp I	0.7362
Gp II	0.7437
GP III	0.7722
GpIV	0.8044*

*Note: Male #3172 excluded because of low testicular weight (0,3566 It would appear that only one testicle was weighed or that the animal possessed only one testicle. It is to be noted, also, the same male produced no viable fetuses and no sperm was found in either of 2 females mated.

-Testes to body weight for GpI and GpIV after 4 week recovery period:

GpI	0.7112
GPIV	0.6[illegible]1

Also noted was increased agglutination and abnormalities, and decrease sperm concentration for GpIV.

-Two week recovery period for GpI and GpIV produced 0/10 in GpI and 5/10 males in GpIV which did not produce viable fetuses. In GpIV, male #31[illegible]0 produced no sperm in either of 2 females mated. Consequently, the viable fetuses were significantly reduced :

GpI 9.6
Gp IV 9.4

-4 week recovery period for GpI and GpIV demonstrated reversibility of above results. All males produced vaible fetuses except male # 3006 in GpI, no sperm found. Viable fetuses:

GpI 9.9
GpIV 10.4

-Increased number sperm abnormalities in GpsIV and VI as compared to Gps I and V :

GPI	31%	GpIV	42%
GpV	2[illegible]	GpVI	50.2%

Also noted were increases in agglutination:

GpI	0.8	GpIV	1.8
GpV	1.1	GpVI	1.7

Similarly, GpVI demonstrated a reduced sperm motility over other groups (45%)

-In the following chart two things are noted:

Gp #	Implantation Sites	Viable Fetuses	Resorptions
I	204	187 (91%)	17 (8.3%)
II	183	163 (89%)	20 (10.9%)
III	139	123 (88%)	16 (11.5%)
IV	160	140 (87.5%)	20 (12.5%)

(1) When you compare the number of viable fetuses to the number of implantation sites a dose response is noted (slight) in that the % of viable fetuses decreases as dose increases (non-pregnant females excluded).

(2) Conversely, when you compare the number of resorption sites to the number of implantation sites a similar dose response is noted, in that the % of resorptions increase as dose incr (non-pregnant females excluded).

-Under similar circumstances as above, male rats were allowed 2 weeks and 4 week recovery periods before mating. The following was noted:

	Gp #	Implantation Sites	Viable Fetuses	Resorptions
2 wk	I	181	144 (79%)	37 (21%)
	IV	60	54 (90%)	6 (10%)
4 wk	I	165	138 (83%)	27 (17%)
	IV	159	135 (84%)	25 (16%)

The number of implantation sites is significantly reduced in GpIV but the number of viable fetuses was extremely good (90%). Four males in GpIV produced no litters and two other males were successful in only 50% of their matings. At 4 weeks the symptoms were reversible to an extent, in that 4 out of the same 6 males still failed to produce litters 50% of the time (1/2 of females mated) - two similar findings in 4/5 males in GpI

c. Conclusions:

Study is considered SUPPLEMENTARY Data. Problems noted:

(1) The same males were not used throughout the study, i.e. one set of males for the first mating and a complete separate set of males for the so-called second mating.

(2) Too few matings for the males. Also, the matings did not cover an adequate span of the reproductive cycle (spermatogenesis) of the male. Dominant lethal evaluations should involve at least five and sometimes seven separate paired matings for one group males. Reproductive mating regimen would be as follows:

1st mating	while on trea ment (day 10)
2nd mating	day 21 of experiment
3rd mating	" 27
4th "	" 32
5th "	" 40
6th "	" 45
7th "	" 52

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2. "Three Generation Reproduction Study in Rats", Woodard Research Corp., dated 6/3/77, submitted by Stauffer Chem. Co. 8/19/77 (Acc# 231331).

a. Protocol:

Substance Tested: Ordram Technical

Species: Charles River CD rats from Sprague Dawley descendents

Sex and Age: Male and female 100 days of age (1st mating)

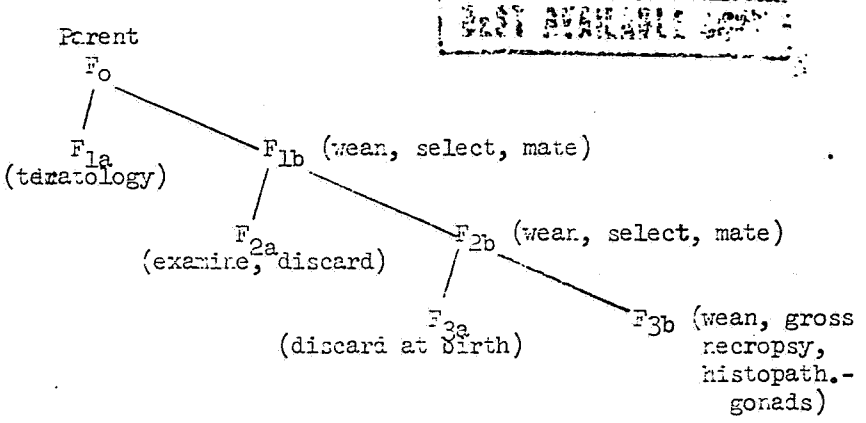
Number of Animals: 25 Male and 25 Female per each of 4 groups (0, 0.063, 0.2, 0.63 mg/kg/day)

Conduct of Test:

Design: At 100 days of age, male and female of the F_o generation were paired for mating by placing a female in a respective male's cage for a period of 10 days. One half of the resulting F_{1a} pups were fixed in Bouin's fixative and subsequently examined for visceral abnormalities; the remaining half of the F_{1a} pups were cleared, stained with Alizarin Red and examined for skeletal abnormalities. Approximately 2 weeks after the birth of the F_{1a} pups, the F_o rats were again bred to produce F_{1b} litters. Following weaning of F_{1b} litters the offspring were placed on their respective diet levels until they reached 100 days of age and groups constituted from representative offspring were mated twice producing F_{2a} and F_{2b} litters. The F_{2a} litters were carried through lactation before being examined and discarded. The F_{2b} litters were mated after 85 days to produce F_{3a} and F_{3b} litters. Living

pups from F_{3a} litters were discarded at birth; F_{3b} pups continue through weaning, subjected to gross necropsy and representative tissues saved in formalin.

The scheme is as follows:



Observations:

Food consumption, body weight, F_{3b} organ weight

Histopathology:

F_{3b} pups only: heart, liver, kidneys, spleen, adrenals, thyroid, gonads and bone marrow. Stained with hematoxylin and eosin.

b. Results:

-Reproductive Performance, table on page A-1 - reduced number of females bred at 0.63 mg/kg/day; however, there is no apparent adverse effect on the number of litters (%) nor on the mean litter size that can be associated with compound administration.

NOTE: there are two typographical errors in this table-(1) footnot "2" is not defined; there are two footnotes designated as "1/" at the bottom of the page and it is assumed the second of these footnotes (1/) is supposed to be 2/.

(2) Also, mean litter size for group F_{3a} (0.63 mg/kg/day)

should be 3.166, not 2.2 .

-Mean Weekly Body Weight - no significant difference related to compound administration.

NOTE: Typographical error on page A-18 - 0.062 mg/kg/day should be 0.063. Likewise, Group IV is reported to be 0.632, whereas on page A-1 it is 0.63. Which value is correct? Also noted is that page A-16 is for F_0 females (weeks 0-13) whereas page A-17 is for F_{1b} females (weeks 14-22). The same mistake occurs again on pages A-18 and A-19, A-20 and A-21, and A-22 and A-23. Animal identity number does not change however - assume typo error! Please clarify. Does week 0 correspond to weaning age or start of compound administration or both?

Fo —→

4-5 wks old —→

-Mean Weekly Food Consumption - no significant differences related to compound administration.

-Mean Absolute and Relative Organ Weights - males and females on high dose had reduced body weights (F_{3b} weanlings) with slightly reduced relative liver weights.

Relative heart wts. for females (high dose) were significantly reduced over other groups.

-Pathology - no differences noted among male or female of any group.

NOTE: Woodard Pathology Lab does not distinguish the difference between the sets of values reported for Gps I through IV. For instance, there are 3 separate sets of values, with corresponding separate rat identity numbers, for Control-Males in Gp I. What do they represent?

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c. Conclusions:

Study is considered SUPPLEMENTARY Data. Questions raised but not answered by study:

(1) What about litter weight?

(2) " " sex related toxicity, if any?

(3) " " general condition of offspring?

(4) Fertility index of females?

(5) Length of gestation period?

(6) Viability at birth, day 4 and weaning not reported or compared.

(7) Growth rate from birth to weaning not apparently reported.

(8) Generally, insufficient data on litters -

needed: Gestation Index

$$\frac{(\#\text{ of litters w/ live pups}) \times 100}{\#\text{ of pregnancies}}$$

Fertility Index

$$\frac{(\#\text{ of pregnancies}) \times 100}{\#\text{ of matings}}$$

Viability Index

$$\frac{(\#\text{ of pups alive at 4 days}) \times 100}{\#\text{ of pups born alive}}$$

Lactation Index

$$\frac{(\#\text{ of pups alive at 21 days}) \times 100}{\#\text{ of pups alive at 4 days}}$$

(9) No indication that there were daily observations for evidence of copulatory plug in mated females. If pregnancy is not noted at the end of one week the pair of rats should have been changed.

(10) Pups from each generation were not autopsied for gross

abnormalities.

(11) Doses selected were signifidantly smaller than doses used in chronic feeding study (rat)

The Teratology evaluation is likewise SUPPLEMENTARY Data. Problems noted:

-Pups not taken by C-section

-Pups evaluated should be 2d litter of the 3rd generation

-Resorptions sites not reported

-# of corpora lutea not reported

-# of implantation sites not reported

-fetal weight not reported

-sex differences not distinguished

-# of litters examined not reported

-% of dead and resorbed fetuses per litter not reported

-total number of implantations per litter not reported

-average number of anomalous fetuses per litter not reported

NOTE: Addendum submitted in Acc# 231330 was conducted during the 104 week rat feeding experiment and deals with reproductive performance. Information is SUPPLEMENTARY Data for many of the same reasons as with the three generation reproduction study reviewed above. One thing which may be summarized is that "reproductive performance" was impaired while animals were on treatment. How, why and to what extent cannot be determined from the insufficient amount of data submitted.

3. "Ordram - Safety Evaluation by Repeated Oral Administration to Rats for 104 Weeks", Woodard Research Corp., dated 6/3/77, submitted by Stauffer Chem. Co. 8/19/77 (Acc#231327).

a. Protocol:

Substance Tested: Ordram Technical

Species: Fischer strain albino rats

Sex and Age: Male/female approx. 8 weeks of age

Number of Animals: 60 M and 60 F per each of 4 groups (0, 0.63, 2.0 and 6.32 mg/kg/day)

Conduct of Test:

Design: Animals originally started at 32, 16, and 8 mg/kg/day in the diet but these were decreased after 18 weeks to 6.32, 2.0, and 0.63 mg/kg/day because of 10% decrease in body wt. gain. Animals were housed individually and fed Purina Lab Chow. Animals weighed each week and the amount of food consumed the prior week was determined. After 13 months, 5M/5F rats per group were sacrificed. Again at 78-81 weeks another interim sacrifice of 5M/5F per group. Prior to necropsy the following determinations were made:

Urinalysis: appearance, albumin, sugar, pH, blood, microscopic exam of sediment

Hematology: hemoglobin, hematocrit, RBC, WBC, diff. leukocyte, platelets

Phenolsulfophthalein excretion.

Sulfobromophthalein retention.

At necropsy the following blood determinations were made: cholesterol, total protein, glucose, BUN, SAP, SGPT

Additionally, the following organ wts. were determined: heart, liver, kidneys, spleen, lungs, gonads,

adrenal, thyroid, pituitary, prostate or uterus, br Histopahtology of the following organs were also evaluated:

liver, adrenal, spleen, kidneys, testes, uterus

Finally, beginning week 104 surviving animals in all groups were sacrificed and the same determinations, a noted above, were conducted on all animals.

b. Results:

Survival:

	Male	Female	Sacrificed
GpI (0)	37/51	32/50	2M/10F
GpII (0.63)	37/50	33/50	10M/10F
GpIII (2.0)	35/50	42/50	"
GpIV (6.3)	40/50	37/50	"

Body Weight: Original feeding levels resulted in dose related of weight gain necessitating a decrease in the dose levels fed body weight near normal after 26 weeks but females still depre After one year there were no significant differences between treatment and control groups. During the second year the mean body weights of the high dose group decreased from control to about 93% of control (week 78) for both M and F. [illegible] weeks values were 97% for M and 101% for F.

Hematology: No significant difference between control and [illegible] groups at any of the times investigated.

Clinical Chemistry: No dose related compound effects were evi[illegible] in the parameters measured.

Urinalysis: Unremarkable.

Relative Organ Weights:

13 month interim sacrifice - increased adrenal wt. in high males; females unremarkable.

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81 week interim sacrifice - males: dose related increase in heart wt. (slight); dose related increase in gonad wt. (moderate); dose-related increase in adrenal wt (significant increase thyroid wt. at high dose. Females: dose related increase liver wt.; dose related increase gonad wt. (sig.); increase thyroid and pituitary wt. in treatment groups.

Terminal sacrifice - males: decrease body wt.; increase gonad wt dose related increase adrenal wt.; dose related increase thyroid wt.; females;: slightly decreased adrenal wt.;, pituitary wt. increased at low dose.

Histopathology:

13 month - dose related in degree of pigment (hemosiderin) found in spleen; more incidence in 2 mg/kg/day group, but more pronounced in high dose group; primarily females. No other dose related incidences.

81 week - MTD, 1/2MTD, 1/4MTD correspond to doses used for the first 13 weeks - not the durationof the study! Did the laboratory change their testing protocol? Please clarify. These do not correspond to doses used throughout the study. No dose related effects evident.

Terminal - data is for 104 week feeding of 32, 16, and 8 mg/kg/d which supposedly was discontinued at 18 weeks due to decrea. body wt. gain. Where is data on 6.32, 2 and 0.63 mg/kg/day groups? The animal numbers are the same, however. Please clarify. Histopathology was unremarkable in that there was no differences between control and treatment groups either in severity or incidence of lesions.

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c. Conclusions:

Study is considered CORE:Minimum Data.

However, the registrant must kindly clarify the misrepresentation of informaton noted above in the pathology report. As noted, the animal numbers did not change but the reporting of dases was significantly changed (32 vs 6.32 mg/kg/day!)

A NEL will be determined when information requested above is received.

4. "Ordram - Repeated Oral Administration to Mice for Lifetime", Woodard Research Corp., dated 6/3/77, submitted by Stauffer Chem. Co. 8/19/77 (Acc#231323).

a. Protocol:

Substance Tested: Ordram Technical

Species: CAF_1 Hybrid mice (Gps I thru IV) and CAF_1 generation produced by breeding A/J females with BALB/cJ males (Gps V thru VIII)

Sex and Age: M/F approx. 4 weeks of age (CAF_1 Hybrid [illegible])

Number of Animals: 20M and 20F per each of 4 groups (0, [illegible], 14.4 mg/kg/day) for 18 months feeding portion of study; reproduction portion contained the same number of M/F per group resulting in the following number of offspring placed in treatment gr

Gp V	43M/46F
Gp VI	36M/31F
Gp VII	36M/28F
Gp VIII	37M/28F

NOTE: these figures are not consistent throughout study and result in confliction in number of animals reported which ultimately reduces the credability of the study, not to mention any dose related effect on survivability.

Conclusions:

Study is considered INVALID because of numerous [illegible]ies in the reporting of data. Uterus and ovary weights, for example,

are indicated to comprise 27 to 30% of the total body wt.! This is not a likely possibility, at best. Furthermore, values are reported in Organ to Body Wt. Ratios Tables for animals which died after only 17 weeks on test; conversely, animals surviving to termination of study are omited with no explanation given. Furhhermore, histopathology of Group IIfemales nos. 1584 and 1585 (sited above as having uterus/ovary wts. of 27 to 30% of total body wt.) were listed as "not remarkable" or "no section taken". Likewise, histopathology of Group I femalesno. 1586 (lung wt. 3.15% of total body wt.) listed "moderately severe/high focal mineralization oflung" as the only involvement of the lung - No tissue mass found. These few histopathology examples above would suspect if nothing other than the fact that their size alone would necessitate definite structural changes in the tissue. Clarification or explanation of these findings was not reported. The same value is reported differently in different tables or in summarizations.

Robert B. Jaeger, Physiologist
Toxicology Branch

11/22/77