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

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JUN 26 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Butylate - Interim and Final Reports of a Two Generation Reproduction Study in Rats submitted by Stauffer Chemical Company. Accession Nos. 260958-260959 and 263612-263614.
Tox. Chem. No.: 434A

To: Robert Taylor
Product Manager #25
Registration Division (TS-767C)

From: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 6/25/87
Section Head, Section VI
Toxicology Branch/HED (TS-769C)

Thru: Theodore M. Farber, Ph.D., Chief
Toxicology Branch/HED (TS-769C)

Action Requested: Review of interim and final reports of a two generation reproduction study in rats.

Conclusions: Systemic NOEL = 200 ppm
Systemic LEL = 1000 ppm based upon decreased food consumption for P_1 males, decreased body weights of dams during gestation (P_1, first mating) and during lactation (P_1, all matings) and increased relative liver weights in P_0 females.

Reproductive NOEL = 200 ppm
Reproductive LEL = 1000 ppm based upon decreased pup weights in the F_{2A} generation on days 14 and 21 and decreased absolute brain weights of the F_{1B} male weanlings.
Developmental NOEL = 1000 ppm
Developmental LEL = 4000 ppm based upon an increased incidence of dilated renal pelvis and retinal folds in the F_{1B} generation.

Other systemic and reproductive effects seen at 4000 ppm (HDT) that were considered to be treatment-related included:
1. decreased body weights and food consumption compared to the controls for parental animals (P_0 and P_1);
2. decreased hematocrit in P_0 males and females and decreased hemoglobin levels in P_0 and P_1 females;
3. increased relative liver weights in P_0 males and females

and P_1 females;
4. increased incidence of hepatocyte vacuolation in P_1 males;
5. decreased litter size in F_{1A}, F_{1B} and F_{2B} generations;
6. decreased number of live pups on day 21 of F_{1A} and F_{1B} generations;
7. decreased absolute kidney and brain weights of F_{1B} males and females and kidney weights of F_{2C} males and increased relative liver weights of males and females of the F_{2C} generation.

Core Classification: Minimum

Reviewed by: Judith W. Hauswirth, Ph.D., Section Head Judith W. Hauswirth 6/25/87
Section VI, Tox. Branch (TS-769C)
Secondary Reviewer:

DATA EVALUATION REPORT

STUDY TYPE: 2-Generation Reproduction Study TOX. CHEM. NO: 434A

ACCESSION NO.: 263612-263614 MRID NO.:

TEST MATERIAL: Butylate

SYNONYMS: Sutan

STUDY NO.: T-11940

SPONSOR: Stauffer Chemical Company

TESTING FACILITY: Stauffer Chemical Company
Environmental Health Center
Farmington, CT

TITLE OF REPORT: A Two-Generation Reproduction Study in Rats with Sutan

AUTHORS: JL Minor, VM Nelson, JM Killinger and DON Taylor

REPORT ISSUED: June 18, 1986

CONCLUSION: Systemic NOEL = 200 ppm
Systemic LEL = 1000 ppm based upon decreased food consumption of P_1 males, decreased body weights of dams during gestation (P_1, first mating) and during lactation (P_1, all matings), and increased relative liver weights in P_0 females.

Reproductive NOEL = 200 ppm
Reproductive LEL = 1000 ppm based upon decreased pup weights in the $F2_A$ generation on days 14 and 21 and decreased absolute brain weights of the F_{1B} male weanlings.

Developmental NOEL = 1000 ppm
Developmental LEL = 4000 ppm based upon an increased incidence of dilated renal pelvis and retinal folds in the F_{1B} generation.

Other systemic and reproductive effects at 4000 ppm (HDT) that were considered to be treatment related included:
1. decreased body weights and food consumption compared to controls in parental animals (P_0 and P_1);

2. decreased hematocrit in P_0 males and females and decreased hemoglobin levels in P_0 and P_1 females;
3. increased relative liver weights in P_0 males and females and in P_1 females;
4. increased incidence of hepatocyte vacuolation in P_1 males;
5. decreased litter size in F_{1A}, F_{1B} and F_{2B} generations;
6. decreased number of live pups on day 21 of F_{1A} and F_{1B} generations;
7. decreased absolute kidney and brain weights of F_{1B} males and females and kidney weights of F_{2C} males and increased relative liver weights of males and females of the F_{2C} generation.

Core Classification: Minimum

A. MATERIALS:

1. Test compound: Sutan Technical. Description - Lots #EHC-0586-23 and EHC 0469-19, an amber liquid, purity 99.2%.

2. Test animals: Species: rat; Strain: CrlCD®(SD)BR from Charles River Breeding Laboratories; Age: 38 days; Weight: 230±10 g males and 161±12 g females; Source: Charles River Breeding Laboratories in Kingston, New York; animals were quarantined for 11 days.

B. STUDY DESIGN:

1. Animal assignment: Twenty-five males and 25 females were assigned to one of four groups according to body weights such that mean body weights would be similar for each group.

2. Experimental design: Animals were placed on the control and test diets at 49 days of age. After ingestion of the diets for 63 days, animals were mated in a one to one ratio. Mating of animals was random. Two matings were conducted for the first generation, producing the F_{1A} and F_{1B} litters. Three matings were conducted for the second generation, producing the F_{2A}, F_{2B} and F_{2C} litters. The third mating was done since during the second mating animals were unintentionally exposed to continuous light. P_0 males and females were sacrificed and necropsied at 211-215 and 238243 days of age, respectively. P_1 males and females were sacrificed and necropsied at approximately 270 and 300 days of age, respectively. F_{1A} pups were discarded after weaning. F_{1B} pups were culled to 4 males and 4 females on day 4 postpartum. Culled pups were necropsied. Weanlings not used as parents for the second generation were necropsied. F_{2A} pups were weaned and discarded. F_{2B} and F_{2C} pups were handled in the same manner as the F_{1B} pups.

3. Test diet: Sutan was mixed with Purina Certified Rodent Meal #5002. The concentrations of Sutan used were 0, 200, 1000 and 4000 ppm. According to the report:

> The test diets were prepared three times during the first month of the study and were analyzed for concentration and homogeneity. Test diets were prepared every 17-27 days thereafter with

a blend analyzed each month. Blended diets were maintained at ambient temperature.

Mean actual levels were determined to be 207, 1065 and 4230 ppm.

4. Statistics: Body weight, feed intake, pup weight and organ weight data were tested for significance using one-way analysis of variance and Dunnett's procedure. Clinical observations, necropsy findings, reproductive counts and weanling findings were analyzed using the Fisher exact probability test using Bonferroni correction for multiple comparisons. Litter data were tested with a nonparametric rank test. Differences were not considered significant if $p > 0.05$.

5. A signed quality assurance statement was included with the study report.

C. METHODS, RESULTS, and DISCUSSION:

1. Parental Animals:

a. Observations: Animals were observed daily for signs of toxicity. A statistically significant increase in dehydration was seen in high dose P_0 females (0/25 control, 1/25 low dose, 0/25 mid dose and 6/25 high dose). Dehydration was seen in P_0 males and P_1 males and females but was distributed among the dose groups in an unrelated manner. A statistically significant increase in the incidence of ear scabs was seen in high dose P_1 males; however, in P_0 males and females and P_1 females, it occurred to similar degree in all dose groups. Neither of these is considered by this reviewer to be treatment related, since they occurred in other parental animals and generations at all dosage levels. Other non-dose-related observations were hair loss, swollen ears and chromorhinorrhea.

b. Body weights: Body weights were determined weekly. During lactation body weights of the dams were recorded on days 0, 4, 7, 14 and 21 of lactation. Selected body weight data can be found in the following table.

Selected Parental Body Weight Data

Mean Body Weights (g)

	Day									
	Males P_0					Females P_0				
Dose (mg/kg)	0	56	119	161	Δ^1	0	56	168	188/189	Δ^2
0	230	491	598	640	13	161	267	325	339	4.9
200	230	489	600	640	15	160	262	317	330	3.5
1000	229	474	585	627	9.9	160	262	315	326	-1.4*
4000	229	442*	531*	567*	7.1*	159	232*	296*	392*	-2.2*

	Females P_0 (Gestation, 1st mating)					Females P_0 (Lactation, 1st mating)		
	0	6	13	20	Δ^3	0	7	21
0	272	301	325	397	72	316	334	315
200	269	295	318	391	73	308	328	307
1000	266	294	318	391	73	309	327	310
4000	234*	260*	282*	345	63*	271*	290*	290*

	Males P_1					Females P_1				
	0	56	182	230/231	Δ^4	0	56	238	258/259	Δ^5
0	177	490	649	677	7.5	147	282	367	380	2.7
200	174	495	655	682	7.4	141	275	364	372	1.1
1000	163*	470	629	657	6.5	137*	268	355	367	2.2
4000	146*	437*	588*	611*	4.6	123*	235*	314*	308*	-2.5*

	Females P_1 (Gestation, 1st mating)					Females P_1 (Lactation, 1st mating)			
	0	6	13	20	Δ^6	0	7	14	21
0	289	315	346	403	67	323	349	344	323
200	279	304	331	402	71	315	333	333	316
1000	273	294	320*	385*	65	304	320*	325*	306*
4000	241*	261*	288*	346*	59*	267*	285*	291*	288*

* $p<0.05$
1 delta change between days 154-161
2 delta change between days 182/183-189
3 delta change between days 13-20
4 delta change between days 224-230/231
5 delta change between days 252-258/259
6 delta change between days 13-20

P_0 Generation:

In the first mating of the P_0 parents, the mean body weights of the 4000 ppm group were significantly lower than those of the control group for males and females. The same was true for the second mating of this generation.

P_1 Generation:

Body weights were statistically significantly depressed when compared to the control group for males and females in the 4000 ppm dose group for each of the three matings. For females, body weights were also significantly depressed during gestation and lactation at 1000 ppm butylate for each of the three matings.

c. Food Consumption: Food consumption was determined weekly for

each animal, but was not recorded during cohabitation. Food consumption was recorded for the following periods during lactation for the dams: 0-4, 4-7, 7-14 and 14-21 days.

P_o Generation:

Food consumption was statistically significantly decreased at the high dose (4000 ppm) for males and females at most of the reported time intervals. During gestation and lactation, food consumption was also significantly depressed in the high dose females.

P_1 Generation:

Food consumption was statistically significantly decreased at the high dose (4000 ppm) for males and females at most of the reported time intervals. This was also true for the females during gestation and lactation. Food consumption was also significantly depressed in the males of the 1000 ppm group during the following intervals: 14-23, 84-98 and 217-230/231 days. During gestation and lactation food consumption was significantly decreased sporadically in females at 1000 ppm.

d. Hematology:

Blood was collected at termination from all parental animals. The following tests were performed:

Hematocrit (if <37% then reticulocyte count was done)
Hemoglobin
Total erythrocyte count
Total leukocyte count
Total platelet count
Prothrombin time
Activated partial thromboplastin time

Hematocrit and hemoglobin values for parental animals are shown in the following table.

Parental Hemoglobin and Hematocrit Values

Generation:	P_o				P_1			
	Males		Females		Males		Females	
Dose (ppm)	Hct.	Hbg.	Hct.	Hbg.	Hct.	Hbg.	Hct.	Hbg.
0	42.5	14.1	45.0	15.0	42.5	15.8	42.0	16.5
200	42.2	14.0	44.2	14.8	41.7	15.7	43.1	16.4
1000	41.4	14.1	44.7	15.1	40.9*	15.6	41.8	16.1
4000	40.7*	13.9	43.1*	14.1*	42.3	15.4	41.5	15.5*

*$p<0.05$

The hematocrit of the high dose P_o males and females statistically significantly decreased. Hemoglobin levels were also significantly decreased in P_o and P_1 females at the highest dose tested (HDT). The statistically significant decrease in hematocrit seen in the P_1 males at the mid dose only was not

considered to be treatment-related. All other parameters were unaffected by treatment.

e. Sacrifice and Pathology:

All parental animals were subjected to gross pathological examination. The following organs were weighed:

liver	adrenals
kidneys	thymus
heart	spleen
brain	gonads

The following tissues were collected for microscopic examination:

lungs	spleen
heart	pancreas
thymus	liver
kidneys	urethra
urinary bladder	bulbo-urethral glands
testes	oviducts
epididymides	ovaries
prostate	vagina
spermatic cords	cervix
seminal vesicles	uterus
coagulating glands	pituitary
ampullary gland	thyroid
parathyroid	adrenals
brain	eyes
Hardarian gland	gross lesions

The kidneys in both sexes and the ampullary glands in males were not examined. In parental males and females liver and reproductive organs from the control and high dose groups were examined microscopically.

1) Organ weights: The following statistically significant changes were noted at 4000 ppm:

- P_0 males - absolute spleen and thymus weights decreased; relative brain, testes, kidney and liver weights were increased;
- P_0 females - relative kidney, brain and liver weights were increased;
- P_1 males - absolute brain and spleen weights were decreased; relative testes weights were increased;
- P_1 females - absolute brain, kidney and thymus weights were decreased; relative brain, heart, spleen and liver weights were increased.

At 1000 ppm in the P_0 females relative liver weights were also increased.

2) Gross necropsy: No treatment related effects were seen in either generation.

3) Histopathology: No treatment-related effects were seen in the P_0 males or the P_0 and P_1 females. In the P_1 males there was a statistically significant increase in vacuolation of hepatocytes at 4000 ppm (3/25 control vs 9/25 high dose). The degree of severity of this lesion was very mild and presumably, therefore, the low and mid dose groups were not examined for its presence.

2. Reproductive Effects

a. Pup Weights: Mean pup weights per litter were recorded on lactation days 0, 4, 7, 14 and 21. Overall mean pup weights for each dosage group and each generation are shown in the following table.

Mean Pup Weights (g)

F_{1A} Generation			Day		
Dosage Group (ppm)	0	4	7	14	21
0	6.2	9.6	14.3	26.6	44.3
200	6.2	9.8	14.5	26.5	43.8
1000	6.1	9.6	14.1	26.3	42.4
4000	6.0	9.2	13.1*	23.6*	37.1*
F_{1B} Generation					
0	6.1	9.7	16.6	34.7	56.9
200	6.1	9.8	16.6	34.2	55.9
1000	6.0	9.8	16.1	33.6	54.2
4000	5.9	9.2	14.7*	30.1*	49.1*
F_{2A} Generation					
0	6.2	10.3	15.0	27.8	45.4
200	6.2	10.1	14.2	26.4	43.1
1000	6.0	9.9	14.1	25.6	40.4
4000	5.6*	8.9*	12.4*	22.5*	35.7*
F_{2B} Generation	0	4	7	14	21
0	6.4	10.8	17.2	34.2	56.0
200	6.8	11.0	17.4	33.8	57.6
1000	6.6	11.1	17.0	32.4	53.2
4000	6.4	10.8	16.0	29.2*	46.9*
F_{2C} Generation					
0	6.5	10.1	16.4	34.7	57.4
200	6.6	10.5	16.9	35.1	58.6
1000	6.3	10.2	16.4	32.8	55.3
4000	6.1	9.8	15.2	30.1*	48.8*

* $p<0.05$

Mean pup weights were statistically significantly decreased at various time intervals when compared to the control group for the high dose group of each generation and mating, and for the mid dose group for the F_{2A} generation.

b. Litter Size: Litter size was determined from the total live pups on day 4 plus all pups either dead or missing through day 4. The following table contains the litter size data for each generation and mating. This parameter was referred to as <u>total born</u> in the report.

Litter Size

	Generation				
Dosage (ppm)	F_{1A}	F_{1B}	F_{2A}	F_{2B}	F_{2C}
0	14.5	15.5	13.9	11.5	13.0
200	14.3	14.7	13.8	8.6	11.4
1000	14.4	15.3	13.7	10.5	12.8
4000	12.3*	13.6	12.9*	8.3*	10.8

* $p<0.05$

Total born was statistically significantly decreased when compared to the control group for the F_{1A}, F_{2A} and F_{2B} generations at the HDT.

c. Survival Indices: The following survival indices were determined: liveborn index, viability index, lactation index, survival indices on Day 4, day 7, Day 14 and Day 21. Each of these indices was unaffected by treatment. (See Appendix 1 taken from the report.)

d. Suckling Indices: Suckling indices were unaffected by treatment in the three generations for which they were determined (F_{1B}, F_{2B} and F_{2C}). (See Appendix 2 taken from the report.)

e. Fertility and Reproductive Behavior: The following parameters were determined for each mating of each generation: mating index and fertility index for males and females, gestation index, number of litters with <6 pups, and mean gestation length. None of these parameters were affected by treatment in a dose related manner. There was a rather dramatic decrease in the gestation index of the control group of the third mating in the second generation. Nine females showed signs of positive mating but did not produce litters. This brings into question the adequacy of this mating; however, since this is the third litter produced in this generation and in the first two the control groups were acceptable, this inadequacy in the study does not affect the overall integrity of the study. (see Appendix 3 taken from the report.)

f. External Observations of Pups during Lactation: No statistically significant findings were reported. At the high dose there were a few small pups.

g. Sacrifice and Necropsy of Weanlings: Five male and five female weanlings from the F_{1B}, F_{2B} and F_{2C} generations were necropsied as was done

for the parental animals. Weights of liver, heart, brain, kidneys and gonads of these animals were taken and recorded. According to the report, "pups culled on day 4 and those not selected on day 21 for either organ weight determinations or the next parental generation were necropsied". Furthermore, "heads of culled pups were fixed in Bouin's fixative and then examined by free-hand razor blade sectioning" and "throacic and abdominal viscera were examined.."

1) Organ Weights: The following statistically significant differences in organ weights were considered to be treatment-related: in the high dose, decreased absolute kidney and brain weights of F_{1B} males and females and kidney weights of F_{2C} males and increased relative liver weights of males and females of the F_{2C} generation and in the mid dose decreased absolute brain weight in the F_{1B} males. Selected organ weight data can be found in the following table.

Weanling Absolute Organ Weights (g)

	Organ	
F_{1B} male weanlings		
Dose (ppm)	kidney	brain
0	1.001	1.657
200	0.827*	1.554
1000	0.852*	1.528*
4000	0.763*	1.495*
F_{1B} female weanlings		
	kidney	brain
0	0.851	1.516
200	0.920	1.528
1000	0.819	1.501
4000	0.673*	1.400*

* $p<0.05$

Although absolute kidney weights were significantly reduced in all dosage groups for the males of the F_{1B} generation, only the effect at the high dose was considered to be treatment-related, since the effect at the low and mid dose was not dose-related, i.e. weights at mid dose > weights at low dose.

2) Gross Necropsy: Possible treatment-related effects were seen only in the F_{1B} generation. The incidence of selected gross lesions seen in this generation can be found in the following table.

Incidence of Pertinent Findings in F_{1B} Pups[1]

	Dosage Group (ppm)			
By Pup	0	200	1000	4000
ureter, dilated	18/165(11)[2]	9/133(7)	8/161(5)	10/119(8)
ureter, convoluted	17/165(10)	8/133(6)	11/161(7)	13/119(11)
kidney, dilated	11/165(7)	6/133(4.5)	8/161(5)	14/119(12)
retinal folds	12/165(7)	18/133(13.5)	8/161(49)	20/119(17)
By Litter				
ureter, dilated	11/23(48)	5/20(25)	6/23(26)	5/19(26)
ureter, convoluted	9/23(39)	5/20(25)	8/23(35)	9/19(47)
kidney, dilated	6/23(26)	3/20(15)	6/23(26)	10/19(53)
retinal folds	8/23(35)	11/20(35)	7/23(30)	10/19(53)

[1]incidence in pups culled on day 4
[2]percentage incidence

The report did not indicate that any of these lesions were induced to a statistically significant degree. There was a treatment-related increase in the incidence of retinal folds and dilated kidneys (renal pelvis) by both the number of pups affected and by litter at the HDT. An increase in these lesions was not seen in any other generation examined nor in a previously reviewed rat teratology study conducted on butylate in Sprague-Dawley rats which was acceptable. The study authors did not consider these lesions to have any "specific developmental or reproductive consequences". Nevertheless, since the significance of these lesions is questionable and not known by this reviewer, they were used for setting a developmental NOEL for this study.

Appendix 1

Pages 14-28 - *Access to FIFRA health and safety data is restricted under FIFRA