3-13-88

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

004338

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Review of a Teratology Study in Rats with Butylate, Study # T-11713; Action # 476-2000

TO: Robert Taylor
Product Manager (25)
Registration Division (TS-769)

THRU: Robert P. Zendzian, Ph.D. 3/13/85
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Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: Chad B. Sandusky, Ph.D. Chad B Sandusky 3/6/85
Pharmacologist
Toxicology Branch
Hazard Evaluation Division (TS-769)

Compound: Butylate (Sutan®) Tox. Chem.#: 434A

Registration #: 476-2000 Registrant: Stauffer

Accession #: 251187

Action Requested:

Stauffer Chemical Company has submitted for review a "Teratology Study in CD Rats with SUTAN Technical (butylate)", study #T-11713, accession # 251187.

Conclusions:

Technical butylate was not teratogenic in rats at doses as high as 1000 mg/kg/day even in the presence of maternal toxicity.

However, there were embryotoxic and fetotoxic effects observed, but only at doses which also produced maternal toxic effects. These data were CORE Minimum and meet the requirements for a teratology study in this species.

Background:

These data constitute the only currently available CORE Minimum teratology study on butylate. A previously reviewed teratology study (1) in mice was CORE Supplementary due to the lack of any effects even at the highest dose tested (24 mg/kg/day).

Discussion:

The oral administration of butylate technical (40, 400 and 1000 mg/kg/day to pregnant Sprague-Dawley CD rats during major organogenesis and continuing into late organogensis (gestation days 6-20) did not produce teratogenic effects under the conditions of this study. Dose-related fetotoxic effects (decreased fetal body weight, an increased incidence of misaligned sternebrae, and delayed ossification) were observed, but only at doses (400 and 1000 mg/kg) that also produced maternal toxic effects. No adverse effects were seen in mothers or offspring at the low-dose (40 mg/kg).

Based on findings such as decreased body weight, body weight gain, and food consumption, in addition to an increase in relative liver weight, 400 mg/kg/day was the LOEL for maternal toxicity and 40 mg/kg/day was the NOEL. The embryotoxicity LOEL is 1000 mg/kg/day and the NOEL is 400 mg/kg/day, based on the increase in early resorptions. The fetotoxicity of butylate technical, as evidenced by lower fetal body weight and an increase in misaligned sternebrae and fetal hematomas, substantiates an LOEL of 400 mg/kg/day and a NOEL of 40 mg/kg/day. No effects on reproduction were observed at the highest dose (1000 mg/kg/day).

Core Classification: Minimum data.

The deficiencies noted in this study are given below:

1) Absence of individual fetal body weight

2) No counts were given for the total number of fetuses and the number of males in each litter.

3) Uteri of nonpregnant dams were not adequately examined for early implantation losses.

4) The method(s) for soft tissue examination was neither cited nor sufficiently described.

DER:

The following DER is attached:

Downs, J.R. and Greci, L.K. A teratology study in CD rats with SUTAN technical. (Unpublished report No. T-11713 prepared and submitted by Stauffer Chemical Company, Farmington, CT; dated August 22, 1983.) Accession # 251187.

References:

1) R-1910 (butylate) Safety Evaluation by Teratological Study in the Mouse, Stauffer Chemical Company, 4/26/67 by Woodard Research Corp., MRID #00026311; Reviewed in Butylate Registration Standard, 7/6/83.

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EPA: 68-01-6561
TASK: 20
February 12, 1985

DATA EVALUATION RECORD

SUTAN

Teratogenicity Study in Rats

CITATION: Downs, J.R. and Greci, L.K. A teratology study in CD rats with SUTAN technical. (Unpublished report No. T-11713 prepared and submitted by Stauffer Chemical Company, Farmington, CT; dated August 22, 1983.)

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DATA EVALUATION RECORD

STUDY TYPE: Teratogenicity in rats.

CITATION: Downs, J.R. and Greci, L.K. A teratology study in CD rats with SUTAN technical. (Unpublished report No. T-11713 prepared and submitted by Stauffer Chemical Company, Farmington, CT; dated August 22, 1983.)

ACCESSION NUMBER: 251187.

LABORATORY: Stauffer Chemical Company, Environmental Health Center, 400 Farmington Avenue, Farmington, CT 06032.

QUALITY ASSURANCE STATEMENT: Present, signed, but not dated (report inspection dates August 8-10, 1983).

TEST MATERIAL: SUTAN (Lot # EHC-0469-19) was 98.2% pure. The stability of SUTAN in corn oil was confirmed by pre-test data to be 35 days at ambient room temperature.

PROCEDURES:

1. Sexually mature male and female CD (Sprague-Dawley) rats were received from Charles River Breeding Laboratories, Kingston, New York. During the 17 day acclimation period, each rat was identified with a quarantine number on a cage card; in addition each rat was examined for general clinical health. Animals deemed unhealthy or not within the normal body weight range were not used in the study.

   Animals were individually housed in suspended polycarbonate cages with hardwood chip bedding during the quarantine and treatment periods. The environment was maintained at 65 to 75° F with 40% to 60% relative humidity, approximately 15 air changes an hour, and a 12/12 hour light/dark cycle. Purina Certified Rodent Chow #5002 and water were available ad libitum.

   For mating, one male was housed overnight in a breeding cage with one or two females. The distinction between females was made by ear punch. The day sperm were detected in vaginal smears or a copulation plug was present denoted gestation day 0.

The post-coitum females were distributed to four treatment groups by: 1) the date of mating, 2) the male used for mating, and 3) the gestation day 0 body weight. Animals were then ear tagged for identification. The control, low- and mid-dose groups were comprised of 26 females each, while the high dose group consisted of 28 females.

2. Test material mixtures in corn oil were prepared at the start of the study by a dilution method ("wt/vol, then vol/vol dilutions") to establish concentrations of 0, 8, 80, and 200 mg/ml. Aliquots of the dosing solutions were analyzed for test material concentrations on the second day of the dosing period.

3. The test material was administered daily by oral intubation at 5.0 ml/kg on each of gestation days 6 through 20 at dose levels of 0 (vehicle), 40, 400 or 1000 mg/kg/day. All animals were dosed at a volume of 5 ml/kg, based on the female's gestation day 6 body weight.

   The dose levels were selected on the basis of preliminary studies; however, only acute oral toxicity data were available. These results indicated toxic effects including mortality in male and female rats at dose levels greater than 2500 mg/kg.

4. Observations for clinical signs of toxicity were recorded at least once daily. Body weights were measured on gestation days 0, 6, 7, 9, 12, 16, and 21. Food consumption data were collected for gestation days 0-6, 6-9, 9-12, 12-16, and 16-21.

   Dams were sacrificed on gestation day 21 by carbon dioxide asphyxiation and examined internally for gross lesions and the liver weights recorded. The uterus (with cervix) and ovaries were removed and weighed and the corpora lutea counted. The uterus was opened and the numbers and distributions of live and dead fetuses, early resorptions (only placenta was evident), mid resorptions (both placenta and embryonic tissues without recognizable features were present) and late resorptions (the conceptuses showed either external degenerative changes or an arrested state of development) were recorded. Although not indicated in the text or protocol, from the reported data it was determined that the number of implantation sites was also recorded. However, the uteri of apparently nongravid females were not checked for very early implantation loss by animonium sulfide staining. The fetuses were individually sexed, weighed, and examined for external abnormalities. Live pups were sacrificed by intrathoracic injection of sodium pentobarbital. One half of the fetuses were decapitated. The heads were placed in Bouin's solution for fixation and subsequent cephalic examination; the trunks were examined internally by an unspecified method for soft-tissue abnormalities. The remaining half of the fetuses were "eviscerated, stained and examined for skeletal anomalies."

The authors used a system of ranking fetal anomalies on a scale of 1 to 4. Rank 1 was defined as "variants which occur regularly in the control population or are thought to be artifacts." Rank 2 was described as "variants which are increased by known teratogens or for which background data were not known" but which do not "impair normal life processes." Rank 3 represents effects that result in impairment of "normal physiological processes but are not lethal." Rank 4 corresponds to "frank malformations with lethal consequences."

5. The Fisher exact probability test was performed to compare incidences of clinical signs of toxicity in dosed dams as compared to controls and for comparisons of fetal external, soft-tissue and skeletal anomalies. Differences in maternal body weight and body weight change, net maternal body weight and change, uterine weight, relative and absolute liver weight, food consumption, fetal body weight, and litter data were analyzed statistically using the Mann-Whitney U test (two tailed, $p \leq 0.05$).

   Unless otherwise stated, use of the word "significant" in this evaluation implies a statistical connotation, with $p \leq 0.05$ as reported by the study authors.

RESULTS:

Clinical Observations, Necropsy Findings and Mortalities: Toxic effects were observed at the high dose (1000 mg/kg), as evidenced by inactivity and excessive salivation. A significant increase in the incidence of salivation was also seen in the mid-dose (400 mg/kg) rats. The low-dose (40 mg/kg) dams exhibited a significant increase in alopecia, observed in all three dosed groups but not in the controls; the incidence of other clinical signs were not increased in comparison with the vehicle controls. Additionally, three (11.1%) of the high-dose females died, apparently of compound-related toxicity, and a fourth died of spinal trauma incurred during dosing.

There were no differences between groups in findings at necropsy except in terms of organ weights. As shown in Table 1, uterine weights were significantly reduced and relative liver weights increased at the mid- and high- doses, while the absolute liver weight was significantly increased in the high-dose dams.

Body Weights and Food Consumption: As seen in the incidence of clinical findings, body weights were significantly decreased at the high and mid-doses, but not at the low dose (Table 1). Similar results were seen when data on weight change during the treatment period were

TABLE 1. Summary of Maternal Body and Organ Weights (g) for Rats Treated with SUTAN (Mean ± S.D.)
These data are from TABLE 6 of the original report.

	Dosage (mg/kg/day) 0(Vehicle)	40	400	1000
No. rats pregnant:	21	23[a]	21[a]	19
	Body weight			
Gestation Days				
0	236 ± 18	238 ± 18	239 ± 17	239 ± 16
6	270 ± 19	270 ± 17	270 ± 17	272 ± 15
7	265 ± 20	266 ± 19	260 ± 18	256 ± 15
9	273 ± 18	271 ± 18	264 ± 16	247 ± 16*
12	289 ± 19	286 ± 18	278 ± 15*	257 ± 23*
16	313 ± 19	310 ± 19	302 ± 19	281 ± 29
21	388 ± 22	372 ± 36	366 ± 22*	333 ± 43*
	Body weight change			
Gestation Days				
6-7	-5 ± 5	-4 ± 7	-10 ± 6*	-16 ± 6*
6-9	3 ± 5	1 ± 8	-6 ± 7*	-26 ± 10*
6-12	19 ± 8	15 ± 10	8 ± 7*	-15 ± 21*
6-16	43 ± 8	40 ± 9	33 ± 7*	9 ± 28*
16-21	74 ± 9	61 ± 27*	63 ± 9*	52 ± 21*
Net body weight	291 ± 21	284 ± 23	279 ± 17*	262 ± 27*
Net body weight change	20 ± 9	14 ± 18	9 ± 9*	-8 ± 25*
Total uterine weight	97 ± 10	88 ± 18	88 ± 10*	69 ± 32*
Liver weight	14 ± 1.5	14 ± 1.7	14.4 ± 1.6	16.6 ± 1.3*
Relative to final body weight (%)	3.6 ± 0.4	3.8 ± 0.4	3.9 ± 0.3*	5.1 ± 0.6*
Relative to net body weight(%)	4.8 ± 0.4	4.9 ± 0.5	5.2 ± 0.4*	6.4 ± 0.6*

[a]Does not include females delivering one week before scheduled termination, apparently due to inaccurate timing of pregnancy.

*Significantly different from control value ($p \leq 0.05$).

analyzed. Food consumption was significantly reduced throughout the dosing period for high-dose dams, and during most of this period for the mid-dose group, while the low-dose rats were unaffected in terms of food intake (Table 2).

Reproductive Indices: A summary of the reproductive indices and fetal weights is presented in Table 3. The numbers of corpora lutea, implantations, resorptions, and the living or dead fetuses, as well as the fetal sex ratios were reportedly unaffected by the test material. Two of 19 females (10.5%) in the 1,000 mg/kg (high-dose) group completely resorbed their litters. From the description of the uterine contents; it appeared that resorption occurred shortly after dosing commenced. The increased incidence of resorptions at the high dose was not statistically significant because the increase was entirely due to the contribution of the two totally resorbed litters. However, fetal body weights were significantly decreased in the mid- and high-dose groups.

Teratogenicity Evaluation: Major malformations were seen in two fetuses, one at 40 mg/kg described as having microencephaly with agnathia, arrhinencephaly, closely set eyes, and dislocated ear flaps, and one at 400 mg/kg with partial situs inversus without lobulation of the right lung and tricuspid value atresia. The authors concluded that the occurance of these major malformations was spontaneous and not attributable to the test material.

Incidences of variations seen in more than one litter in any one of the four compared groups are shown in Table 4. Among externally visible anomalies, only the incidence of fetuses affected with hematomas of the thoracic spinal region was significantly increased, a finding in both the mid- and high-dose groups. The fetal incidences were within the range of percentages of affected historical control fetuses, but the incidence of affected litters in the mid-dose group (27.3%) was slightly greater than the incidence of affected historical control litters (25%). No significant increases were reported for soft tissue defects, but the incidence of misaligned sternebrae was increased in the mid- and high-dose groups (Table 4). The percentages of affected litters in mid- (40.9%) and high- (37.4%) dose groups were outside the historical control range (29.2%). This was also true for the percentage of affected fetuses, with 7.79 and 10.34% for the mid- and high-dose groups, respectively, versus a high of 4.1% for the historical controls. In addition, the incidence of incomplete ossification of at least one of the first through fourth sternebrae was significantly increased at the high dose; the percentages for affected litters (29.4%) and fetuses (9.48%) were outside the historical control ranges (16.0 and 3.7%, respectively).

TABLE 2. Summary of Maternal Mean (± S.D.) Food Consumption (g/day) for Rats Treated with SUTAN
These data are from TABLE 6 of the original report.

	Dosage (mg/kg/day)			
	0(Vehicle)	40	400	1000
No. rats/group:	18	20	18	18
Gestation Days				
0-6	22 ± 2	23 ± 2	21 ± 3	22 ± 2
6-9	19 ± 2	18 ± 3	15 ± 3*	7 ± 5*
9-12	20 ± 3	21 ± 3	18 ± 2*	15 ± 6*
12-16	21 ± 2	21 ± 2	19 ± 2*	18 ± 4*
16-21	23 ± 2	22 ± 4	22 ± 2	20 ± 4*

*Significantly different from control value ($p \leq 0.05$).

TABLE 3. Summary of Reproductive Indices and Fetal Data for Rats Treated with SUTAN
These data are from TABLES 2 and 7 of the original report.

	Dosage (mg/kg/day)			
	0(Vehicle)	40	400	1000
Number of:				
Females dosed	26	26	25	27
Females pregnant[a]	21	25	23	23
Females dying	0	0	0	4[b]
Viable litters examined on day 21	21	24	22	17
Litters completely resorbed	0	0	0	2
Litters delivered early	0	1	1	0
Corpora lutea (mean)	15.4	16.3	15.3	14.8[c]
Implantations (mean)	15.3	14.8	14.8	14.8
Resorptions (mean):				
early	0.76	1.04	0.77	2.63
mid	0	0.13	0.09	0.11
late	0.05	0.04	0	0
total	0.81	1.21	0.86	2.74
Live fetuses (mean)[d]	14.5	13.5	14.0	13.5
Mean fetal body weight (g±S.D.)[e]	4.9±0.5	4.7±0.5	4.7±0.2*	4.2±0.5*

[a]One female each from the 0, 40 and 400 mg/kg groups was not pregnant because of mating with the same infertile male.

[b]One death reportedly due to physical trauma.

[c]Does not include a "0" value for the one female with a totally resorbed litter but no visible corpora lutea.

[d]No dead fetuses were observed. The high dose group mean does not include the 2 litters that were totally resorbed.

[e]Statistically analyzed by Mann Whitney U nonparametric rank test.

*Significantly different from control value ($p < 0.05$).

TABLE 4. Summary of the Most Frequently Observed Fetal Anomalies in Sprague Dawley CD Rats Given Sutan Technical
These data are from TABLES 8 & 10 of the original report.

	(mg/kg)				
	0	40	400	1000	Historical Control[a]
Total Number					
Fetuses	305	325	307	229	
Litters	21	24	22	16	
Observation					
Hematoma					
-all locations					
Fetuses (%)	4(1)	8(2)	11(4)	9(4)	(2.9)
Litters (%)	4(19)	8(33)	9(41)	6(35)	(28)
-mid thoracic spinal region					
Fetuses	1	4	7*	6*	(1.7)(0-3.1)
Litters (%)	1(5)	4(17)	6(27)	3(19)	(14)(0-25)
Misaligned sternebrae					
Fetuses affected	4	7	12*	12*	1.5(0-4.1)
examined	153	159	154	116	
Litters (%)	3(14)	6(25)	9*(41)	6(38)	9.5(0-29.2)
Sternebrae incompletely ossified[b]					
Fetuses affected	1	4	5	11*	(0-3.7)
examined	153	159	154	116	
Litters (%)	1(5)	4(17)	1(4)	5(31)	(0-16)

[a] Cumulative historical control data for CD rats used in teratology studies at the Stauffer Environmental Health Center; data were presented in the report as Appendix XII. The values are mean percent incidence and/or range.

[b] Other than the fifth sternebrae.

* Significantly different from control value ($p \leq 0.05$).

DISCUSSION:

The authors concluded that except for misaligned sternebrae, the incidences of anomalies were within the background control ranges and that these variations are common anomalies of questionable teratological significance. The misaligned sternebrae were not considred a compound-related effect because the incidence was "not indicative of a dose-response litter effect". Overall, the authors concluded that both the dam and conceptus are "equally at risk to the toxic effects of SUTAN", it does not selectively alter fetal development, and is not teratogenic.

Our assessment also concludes that SUTAN was not teratogenic to rats under the conditions of this study, but we disagree with the authors in that the increased incidence of hematoma and misaligned sternebrae are clearly compound-related effects.

Oral administration of SUTAN to rats resulted in maternal toxicity at the high dose (1000 mg/kg), as evidenced by maternal salivation and inactivity, maternal mortality, changes in absolute or relative organ and body weights and reduced food consumption and body weight gains. Toxicity was also shown at the mid-dose (400 mg/kg) by increased salivation, reduced body and uterine weights, body weight gain, and food consumption, as well as an increased liver-to-body weight ratio. No relevant signs of toxicity were seen in low-dose (40 mg/kg) females. Although the low dose group exhibited an increased incidence of alopecia, the phenomenon was apparently not compound-related and was short in duration. These data indicated that the doses used were sufficient to achieve appropriate test sensitivity for teratogenicity.

Although no statistically significant effects were seen on prenatal survival in the dosed groups, two females in the high dose group totally resorbed their litters. These findings may have been compound-related, since total resorption is relatively rare in control rats, and no mention was made of such events in Stauffer's historical control data. No other measures of reproductive success appeared to have been altered by treatment with SUTAN, and the totally resorbed litters were likely to have been secondary to maternal stress.

No increased incidences of either gross external soft tissue, or skeletal malformations were reported following SUTAN treatment, indicating a lack of teratogenicity under the test conditions employed. However, signs of prenatal toxicity were observed and, among others, included decreased fetal weights and an increase in the incidence of misaligned sternebrae at maternal doses of 400 mg/kg/day and above. There was also an increase in the incidence of incompletely ossified sternebrae in the high dose fetuses. Such effects are likely to have been compound-related, but are considered signs of fetotoxicity and developmental delay rather than teratogenicity, it is not known whether these are direct effects on the conceptus or are merely

maternally-mediated toxic effects. Similarly, the incidences of hematomas seen on fetuses from the 400 and 1000 mg/kg dosage groups suggest fetotoxicity, but their toxicological significance is unknown and presumed to be minor.

Deficiencies in the final report were noted during our evaluation. These included: 1) no individual fetal body weights were presented; 2) individual animal data were not given for neither the numbers of males nor the total numbers of females in each litter of each dose group of the fetal report; 3) the uteri of dams presumed to be nonpregnant were not stained with ammonium sulfide (or some other method) to confirm the absence of implantations; 4) the method(s) used for examination of soft tissue was neither cited nor sufficiently described: and 5) continuous variables such as body weight and food consumption, were analyzed by a nonparametric procedure (Mann-Whitney U). However, these deficiencies compromise neither the study conclusions nor those of our evaluation.

CONCLUSIONS:

The oral administration of SUTAN technical to pregnant Sprague-Dawley CD rats during major organogenesis and continuing into late organogenesis (gestation days 6-20) did not produce teratogenic effects under the conditions of this study. Dose-related fetotoxic effects (decreased fetal body weight, an increased incidence of misaligned stermebrae, and delayed assification) were observed, but only at doses (400 and 1000 mg/kg) that also produced maternal toxic effects. No adverse effects were seen in mothers or offspring at the low- dose (40 mg/kg).

Based on findings such as decreased body weight, body weight gain, and food consumption, in addition to an increase in relative liver weight, 400 mg/kg/day was the LOEL for maternal toxicity and 40 mg/kg/day was the NOEL. The embryotoxicity LOEL is 1000 mg/kg/day and the NOEL is 400 mg/kg/day, based on the increase in early resorptions. The fetotoxicity of SUTAN technical, as evidenced by lower fetal body weight and an increase in misaligned sternebrae and fetal hematomas, substantiates an LOEL of 400 mg/kg/day and a NOEL of 40 mg/kg/day. No effects on reproduction were observed at the highest dose (1000 mg/kg/day).

CORE CLASSIFICATION: Minimum data.

The deficiencies contributing to this core classification include:

1) Absence of individual fetal body weights.

2) No counts were given for the total number of fetuses and the number of males in each litter.

3) Uteri of nonpregnant dams were not adequately examined for early implantation losses.

4) The method(s) for soft tissue examination was neither cited nor sufficiently described.