TXR-4225

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

004275

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Review of Acute Toxicity and Mutagenicity Data on Butylate (EPA Reg. # 476-2156); Action Nos. 476-2000/2001/2024/2049/2103/2132/2156/2180/2208/2212/2213/2214; Caswell # 434A

TO: Robert Taylor, Product Manager 25
Registration Division (TS-767)

THRU: Christine F. Chaisson, Ph.D, Section Head C. F. Chaisson 2/1/85
Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: Chad B. Sandusky, Ph.D Chad B Sandusky 2/1/85
and
Margaret L. Jones, M.S. Margaret L Jones Feb. 1, 1985
Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769)

Action requested: Stauffer Chemical Company has submitted the following data for review in response to the Butylate Registration Standard, 1983. Mutagenicity and acute toxicity studies on the technical chemical were listed as data gaps in the standard.

1. Acute Oral Toxicity in Rats
   (Lab. Report Nos. T-6376, T-6460)
2. Acute Dermal Toxicity in Rabbits
   (Lab. Report Nos. T-6376, T-6460)
3. Primary Dermal Irritation in Rabbits
   (Lab. Report Nos. T-6376, T-6460)
4. Primary Occular Irritation in Rabbits
   (Lab. Report Nos. T-6376, T-6460)
5. Acute Inhalation Toxicity in Rats
   (Lab. Report Nos. T-6376, T-6460, T-6134)
6. Acute Intraperitoneal Toxicity
   (Lab. Report No. T-6009)
7. Acute Delayed Neurotoxicity
   (Lab. Report No. T-6801)
8. Mutagenicity
   (Lab. Report No. T-6310)

Individual reviews of each of the above studies are attached. The results of studies 1 - 6 are summarized in the attached table.

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Discussion and Recommendations: 004275

1. These acute data (studies 1 - 6 above) are sufficient to establish the following toxicity categories for Technical Butylate:

Acute Oral: III, LD_{50} 3500 mg/kg - 4850 mg/kg - Rats

Acute Dermal: IV, LD_{50} >5000 mg/kg - Rabbits

Acute Inhalation: III, LD_{50} >5 mg/l - Rats

Primary Dermal Irritation: IV, mild to moderate irritation reversible within 72 hours at .5 ml. - Rats

Acute Intraperitoneal: LD_{50} = 1050 mg/kg - Rats

2. The attached table shows two cases (footnotes 1 and 2) in which studies of the same type considered separately were judged supplementary. However, the combination of information contained in each separate supplementary study resulted in a Core Minimum classification for the particular toxicity category. The two separate categories in which more than one study was combined are Acute Dermal Toxicity and Primary Ocular Irritation.

3. Butylate did not produce TOCP-like delayed neurotoxicity in adult hens treated with 9.3 g/kg. This study is classified as Core Guideline.

4. The Mutagenicity study was unacceptable. No conclusions can be drawn from these studies (S. typhimurium or S. cerevisiae) because: 1) it could not be confirmed that the dose range used was high enough; 2) what materials and methods were used to assay mutations in S. cerevisiae strain D4; and 3) whether the results obtained were reproducible.

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Summary of Acute Toxicity and Mutagenicity Studies on Butylate,
Accession No. 254690; Vol 2 of 3

Study Type	Species/Sex	Results	Toxicity Category	Core Classification
Acute Oral Toxicity T-6376	Rat/M	LD_{50}=3500 mg/kg	III	Minimum
	Rat/F	LD_{50}=3970 mg/kg	III	Minimum
Acute Oral Toxicity T-6460	Rat/M	LD_{50}=4850 mg/kg	III	Minimum
	Rat/F	LD_{50}=4785 mg/kg	III	Minimum
Acute Dermal Toxicity T-6376	Rabbits/M&F	LD_{50}>5000 mg/kg	Not determined[1]	Supplementary[1]
Acute Dermal Toxicity T-6460	Rabbits/M&F	LD_{50}>5000 mg/kg	Not determined[1]	Supplementary[1]
Primary Dermal Irritation T-6376	Rabbits/M&F	Moderate-mild irritation reversible within 72 hrs. at .5ml	IV	Minimum
Primary Dermal Irritation T-6460	Rabbits/M&F	Moderate-mild irritation reversible within 72 hrs. at .5ml	IV	Minimum
Primary Occular Irritation T-6376	Rabbits/M&F	No irritation at 0.1 ml.	Not determined[2]	Supplementary[2]
Primary Occular Irritation T-6460	Rabbits/M&F	No irritation at 0.1 ml.	Not determined[2]	Supplementary[2]
Acute Inhalation Toxicity T-6376	Rats/M&F	LD_{50}>5 mg/l	III	Minimum
Acute Inhalation Toxicity T-6460	Rats/M&F	LD_{50}>5 mg/l	III	Minimum
Acute Inhalation Toxicity T-6134	Rats/M&F	LD_{50}>14 mg/l	Not determined	Supplementary
Acute Intraperitoneal Tox. T-6009	Rats/M&F	LD_{50} = 1050 mg/kg	N/A	N/A

1. The combined results of these two tests permits placement in Tox. Category III and in Core Minimum Classification.
2. The combined results of these two tests permits placement in Tox. Category IV and in Core Minimum Classification.

Tox Chem No. 434A Dalapon

File Last Updated 1/31/85

Current Date 1/22/85

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute Oral LD_{50}- rats Stauffer Chem. Co. # T-6376; 4/4/79 Lot # WRC-5251-40-1	Technical (97.7%)	254690	LD_{50} = 3500 mg/kg/male rat = 3970 mg/kg/female rat gavage; depression, tremors, salivation, shallow breathing	III	Minimum
Acute Oral LD_{50}- rats Stauffer Chem. Co. # T-6460; 12/15/81 Lot # GGC-0301	Technical (98.0%)	254690	LD_{50} = 4850 mg/kg/male rat = 4785 mg/kg/female rat gavage; dep., saliv., trem., sh.br.	III	Minimum
Acute Dermal LD_{50}- rabbits Stauffer Chem. Co. # T-6376; 4/4/79 Lot # WRC-5251-40-1	Technical (97.7%)	254690	LD_{50} > 5000 mg/kg depression, mild erythema	Not det.*	Suppl.*
Acute Dermal LD_{50}- rabbits Stauffer Chem. Co. # T-6460; 12/15/81 Lot # GGC-0301	Technical (98.0%)	254690	LD_{50} > 5000 mg/kg erythema, edema * combined; LD_{50} > 5000 mg/kg	Not det.* III	Suppl.* Minimum
Primary Dermal Irrit.- rabbits Stauffer Chem. Co. # T-6376; 4/4/79 Lot # WRC-5251-40-1	Technical (97.7%)	254690	Moderate to mild irritation, reversible within 72 hours @ 0.5 ml.; erythema, edema	IV	Minimum
Primary Dermal Irrit.- rabbits Stauffer Chem. Co. # T-6460; 12/15/81 Lot # GGC-0301	Technical (98.0%)	254690	Moderate to mild irritation, reversible within 72 hours @ 0.5 ml.; erythema	IV	Minimum
Primary Ocular Irrit.- rabbits Stauffer Chem. Co. # T-6376; 4/4/79 Lot # WRC-5251-40-1	Technical (97.7%)	254690	No iritation at 0.1 ml.	Not det.**	Suppl.**
Primary Ocular Irrit.- rabbits Stauffer Chem. Co. # T-6460; 12/15/81 Lot # GGC-0301	Technical (98.0%)	254690	No irritation at 0.1 ml. ** combined; No irritation	Not det.** IV	Suppl.** Minimum

Tox Chem No. 434A

File Last Updated 1/31/85

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Study/Lab/Study #/Date	Material	EPA Accession No.	Results: C LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute Inhalation-rats Stauffer Chem. Co. # T-6376; 4/4/79 Lot # WRC 5251-40-1	Technical (97.7%)	254690	LD_{50} > 5 mg/l; 4 hrs.; lethargy, lowered body weight in males	III	Minimum
Acute Inhalation-rats Stauffer Chem. Co. # T-6460; 12/15/81 Lot # GGC-0301	Technical (98.0%)	254690	LD_{50} > 5 mg/l; 4 hrs.; lethargy, lowered body weight in females	III	Minimum
Acute inhalation-rats Stauffer Chem. Co. #-T-6134; 12/19/77 Lot # ZGC 0801	Sutan + 6.7E	254690	LD_{50} > 14 mg/l; 4 hrs.; increase in activity, eye squint, dyspnea, salivation, lacrimation, nasal discharge	Not det.	Suppl.
Acute Intraperitoneal-rats Stauffer Chem. Co. # T-6009; 11/8/77 Lot # AEJ 1501	Technical (97.8%)	254690	LD_{50} = 1050 mg/kg, male and female rats; convulsions, depression, salivation, exophthalmus, blood-like lacrimation	N/A	N/A
Acute delayed neurotoxicity- hens Richmond Toxicology Lab. Study # T-6801; 10/1/80; Lot # GHE 2501	Technical (98.97%)	254690	Butylate did not produce delayed neurotoxicity in hens at 9.3 g/kg Some pharmacotoxic signs were observed, e.g., diarrhea, transient motor incoordination, feather loss, and non vocal behavior. TOCP (500 mg/kg) produced walking and standing disorders; also axonal degeneration in brain and spinal cord and swelling and degeneration of sciatic nerve.	N/A	Guideline
Ames Assay (Salmonella typhimurium, TA 1535, 37, 38, TA 98, TA 100; Saccharomyces cerevisiae D4) Litton Bionetics Study # T-6310; 10/27/77 Lot # 66C-0301	Technical ~~(GGC-0301)~~ (unspecified)	254690	No conclusions can be drawn due to: 1) dose range may not be high enough; 2) materials/methods underreported; 3) no evidence of reproducibility	N/A	Unacceptable

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Study Type: Acute Oral Toxicity in Rats

Accession No: 254690, Vol. 2 of 3; Butylate Registration Standard Toxicology Data; Stauffer Chemical Co., Sept 10, 1984; Appendix I, Sec. A. Toxicology Laboratory Report T-6376.

Contracting Lab: Stauffer Chemical Co.

Sponsor: Same

Date: April 4, 1979

Test Chemical: Sutan Technical, 97.7% pure (Butylate), Lot # WRC 5251-40-1.

Experimental Protocol:

Eight groups of ten adult Sprague-Dawley albino rats of each sex were used to test the substance at each dose level. The weight of males ranged from 149 to 234 grams and the weight of females ranged from 140 to 192 grams.

The test material was administered in a single dose of 10 ml/kg volume to body weight ratio using a stomach tube and the vehicle of the test substance was corn oil. Thirty control animals from each sex were administered corn oil at the time of dosing. The doses were 5000 mg/kg, 4456 mg/kg, 3972 mg/kg, 3540 mg/kg, 3155 mg/kg, 2506 mg/kg, 1991 mg/kg, and 1583 mg/kg for both male and female groups.

Before treatment, the animals were fasted for 24 hours. After treatment, the animals were observed for 14 days for signs of toxicity and mortality. These signs were recorded. However, individual weights of animals were not recorded. Necropsies were performed on animals that died during the study and on survivors at 14 days.

The method of Litchfield and Wilcoxon was used to calculate the LD_{50} value, the slope of the curve,and the 95% confidence interval.

Results:

The LD_{50} was 3500 mg/kg (95% confidence interval= 3125-3920 mg/kg) for males and 3970 mg/kg (95% C.I.= 3452-4566 mg/kg) for females. Table I shows the doses and mortality by sex.

Males: The signs of toxicity in affected animals included depression, salivation, stained fur, shallow breathing, tremors, and pale ears and eyes. Each of these signs was present in all animals at the high dose where 100 % mortality was observed. At lower doses, similar toxic signs were present in lesser severity and fewer animals died, as shown in Table I. The longest time to return to normal was 8 days at 4456 mg/kg and times generally decreased with doses, as shown in Table I.

At necropsy, animals in the high dose group showed apparent hemorrhages in the intestines and thoracic cavity and showed dark red lungs, pale livers and dark red fluid in the intestines. Animals dying in the course of the study also showed the above abnormalities. Surviving animals showed none of the above at necropsy.

In the control group, treated with corn oil, no signs of toxicity were observed during the 14-day test period and no unusual observations were made at necropsy.

Females: The signs of toxicity were depression, tremors, and stained fur and death of the entire high dose group (5000 mg/kg) occurred in three days. Other signs observed at lower doses were salivation, and shallow breathing. The longest time to return to normal was 6 days at 4456 mg/kg, and times decreased with doses, as shown in Table I.

At necropsy, one animal showed darkened intestines. The remaining animals which died during the course of the test showed "apparent hemorrhages", pale livers and dark fluid in the intestines with some variation in severity between dose groups. Those sacrificed at the end showed no gross abnormalities at necropsy.

No signs of toxicity or abnormalities at necropsy were observed in the control group.

Conclusions:

Sutan Technical produces a definite pattern of toxicological effects in Sprague-Dawley albino rats at high doses. Males appeared to be slightly more sensitive to the effects of Sutan Technical, showing a greater incidence of toxicity at each given dose, compared to females. This difference does not appear significant.

The test was performed over a 14 day period at the following doses in both males and females: 5000 mg/kg, 4456 mg/kg, 3972 mg/kg, 3540 mg/kg, 3155 mg/kg, 2506 mg/kg, 1991 mg/kg, 1583 mg/kg. The LD_{50} value in males was 3500 mg/kg (Tox. Category III), (95% confidence interval = 3125-3920). In females the LD_{50} value was 3970 mg/kg (Tox. Category III), (95% C.I. = 3452-4566).

Toxicity Category: III

Core Classification: Minimum

1. Individual weights of the animals at the start of and during the test were not reported.
2. The time for symptoms to appear and the description of the onset of symptoms were not found in the test report.

Table I

Acute Oral Toxicity of Sutan Technical (Butylate, 97.7% pure)/Report No. T-6376

Mortality and Recovery in Male and Female Rats

Dose (mg/kg)	Males			Females		
	Mortality	Time to death	Return to normal	Mortality	Time to death	Return to normal
5000	10/10	days 1-5	-----	10/10	days 1-3	-----
4456	8/10	days 1-3	day 8	9/10	days 2-3	day 6
3972	6/10	days 1-3	day 10	4/10	days 1-2	day 6
3540	5/10	days 1-3	day 7	4/10	days 2-4	day 7
3155	2/20	days 1-2	day 6	2/10	day 2	day 5
2506	1/10	day 1	day 5	2/10	day 1	day 5
1991	1/10	day 1	day 5	0/10	no deaths	day 4
1583	0/10	no deaths	day 2	0/10	no deaths	day 2

LD_{50} = 3500 mg/kg (95% Confidence Interval = 3125-3920)

LD_{50} = 3970 mg/kg (95% Confidence Interval = 3452-4566)

Study Type: Acute Oral Toxicity in Rats

Accession Number: 254690; Vol 2 of 3; Butylate Registration Standard Toxicology Data; Stauffer Chemical Co.; Sept. 10, 1984; Appendix 2; Sec A; Toxicology Laboratory Report No. T- 6460.

Contracting Lab: Stauffer Chemical Co.

Sponsor: Same

Date: December 15, 1981

Test Chemical: Sutan Technical, 98.0% pure, (Butylate), a pale yellow liquid. The vehicle for the test substance was "Tween 80 and water".

Experimental Protocol:

Male Rat Acute Oral Toxicity: Twelve dosage groups of at least 10 Sprague-Dawley male albino rats each were administered the test substance by gavage in a single dose of volume to body weight ratio of 10.0 ml/kg. Seventy five controls were treated with the vehicle, Tween 80 and water, at the time of original dosing. Prior to treatment, the animals were fasted for 16-18 hours. After treatment, the observation period continued for 14 days. During the observation period, the rats were seen at least twice daily and once daily on weekends and holidays. Observations included clinical signs of toxicity, the days on which deaths occurred for each dose group, and the day on which survivors returned to normal appearance.

Animals that died during the test were submitted to necropsy as were all survivors at 14 days. the method of Litchfield and Wilcoxon (1949) was used to calculate the LD_{50} level and the 95% confidence intervals.

Female

Rat Acute Oral Toxicity: Ten groups of at least 10 Sprague-Dawley female albino rats each were administered Sutan Technical in a single dose of 10.0 mg/kg volume-to-body weight ratio. Seventy controls were administered the vehicle, Tween 80 and water, at the time of original dosing. Prior to treatment, the animals were fasted for 16-18 hours. After treatment, the period of observation continued for 14 days. During the observation period the rats were seen at least twice daily on weekdays and once daily on weekends and holidays. Observations included clinical signs of toxicity, the days on which deaths occurred for each dose group and the day on which survivors returned to normal.

Animals that died during the observation period and all survivors at 14 days were submitted to necropsy. As for males, the method of Litchfield and Wilcoxon (1949) was used calculate the LD_{50} and the 95% mortality.

Results:

Table I shows the dose levels and mortality at each dose as well as the period in which deaths occurred and the time for survivors to return to normal. The LD_{50} for male rats was 4850 mg/kg (95% Confidence Interval= 4657-5050). The LD_{50} for female rats was 4785 mg/kg (95% C.I. = 4051-5655).

Males: Signs of toxicity included depression, salivation ruffled fur, red stains on the face, yellow staining in the ano-genital region, ataxia, chromodacryorrhea, tremors and convulsions at the high dose. Signs decreased in severity as the dose administered decreased. At lower doses some additional notable symptoms were anorexia at 4613 mg/kg, rapid respiration and exaggerated startle response (hyperreactivity) at 4571 mg/kg, labored respiration at 4406 mg/kg.

Necropsy of animals dying during the testing period revealed pale testes, dark-edged livers, bloated intestines filled with red-yellow fluid, bloated stomachs and yellow gastrointestinal tracts in 20 animals at 5000 mg/kg. Seven animals showed no signs of abnormality. At 4786 mg/kg, similar observations were made in addition to pale lungs at necropsy in the animals dying during the test. In the nineteen survivors no lesions were seen. In the remaining dosage groups, except 2780 mg/kg and 2208 mg/kg, for which results were not reported, animals which died during the test showed lesions similar to those above and the survivors revealed rats with no lesions.

The only sign of toxicity in the control group was mild depression in 10 of the 75 animals dosed with Tween 80 and water. Necropsy of the control animals revealed no abnormalities.

Females: Signs of toxicity at 5129 mg/kg were slight depression, ruffled and stained fur, yellow ano-genital stains and some loss of fur. No animals died in this dose group, which is rather surprising in view of results in both sexes at 5000 mg/kg. No explanation is given for this surprising result. Signs of toxicity at lower doses included the above signs as well as tremors, salivation, weakness, and diarrhea in a few animals. One rat at 5000 mg/kg had convulsions. Several rats showed shallow respiration prior to death.

Necropsy showed no abnormalities at the high dose. At 5000 mg/kg, necropsy revealed bloated, fluid-filled intestines, dark-edged liver, and bright-to-dark-red lungs. These symptoms were distributed among 4 animals which died during the test. The remaining 6 who died during the test and the 20 who were sacrificed on day 14 showed no abnormalities except for three who had black-tipped spleens. At lower doses the above abnormalities were seen at necropsy we well as pale kidneys, pale liver and reddened intestines. Incidence

and frequency of the symptoms decreased at lower doses. At the four lowest doses the only abnormality at necropsy was dark red lungs and one animal also had reddened intestines. Survivors showed no lesions at necropsy.

Controls showed some hair loss (in 5 rats) but otherwise showed no signs of toxicity and at necropsy revealed no abnormalities

Conclusions:

Sutan Technical (98.0% pure) produces definite toxic effects in Sprague-Dawley albino rats. Males and females showed similar susceptibility to the effects of Sutan Technical.

This acute oral toxicity test was performed over 14 days in male and female Sprague-Dawley albino rats. The LD_{50} value in males was 4850 mg/kg (Toxicity Category III), (95% Confidence Interval = 4657-5050) and in females was 4786 mg/kg (Toxicity Category III), (95% C.I.= 4051-5655).

Toxicity Category: III

Core Classification: Minimum

1. Males were dosed at 2780 mg/kg and 2208 mg/kg, however, no results were reported.
2. Mortality in females at the highest dose tested, 5129 mg/kg, was 0/10 and at the next highest dose tested, 5000 mg/kg, was 10/30. This appears odd and further explanation is necessary to clarify this result.
3. The time for symptoms to appear after administration of the test substance did not appear in the report.

Table I

Acute Oral Toxicity of Sutan Technical (98.0% pure, Butylate)/Report No. T-6460

Mortality in Male and Female Rats

Dose (mg/kg)	Males			Females		
	Mortality	Time to death	Return to normal	Mortality	Time to death	Return to normal
normal[1]						
5129	Dose not tested			0/10	----	day 9
5000	14/30	days 1-3	day 5	10/30	days 1-2	day 6
4786	11/30	days 2-6	day 8, day 14[1]	6/20	days 1-2	day 9
4613	3/10	days 1-2	day 5	1/10	day 2	day 5
4571	8/20	days 1-4	day 7, day 14[1]	Dose not tested		
4406	3/10	days 1-3	day 6, day 14[1]	4/10	days 1-2	day 5
3972	0/10	-----	day 5	0/10	----	day 5
3802	1/10	day 1	day 4	Dose not tested		
3664	0/10	----	day 5	2/10	day 2	day 5
3500	1/20	day 2	day 6 [2]	Dose not tested		
3155	0/10	----	day 5	1/10	day 1	day 5
2780	2/10	no information	no info.	1/10	day 1	day 3
2208	0/10	no information	no info.	0/10	----	day 3

1. Several rats showed signs of depression and some showed remaining toxic signs at day 14.
2. Slight fur loss was still evident at day 14 in several animals.

Study Type: Acute Dermal Toxicity in Rabbits

Accession Number: 254690, Vol. 2 of 3; Butylate Registration Standard Toxicology Data; Stauffer Chemical Co., Sept. 10, 1984; Appendix I, Sec. B. Toxicology Laboratory Report T- 6376.

Contracting Lab: Stauffer Chemical Co.

Sponsor: Same

Date: April 4, 1979

Test Chemical: Sutan Technical, 97.7% pure (Butylate)

Experimental Protocol:

Sutan Technical, 97.7% pure, was applied to four New Zealand albino rabbits from each sex ranging in weight from 1.7 to 2.1 kg. 5000 mg/kg of the test substance were applied to clipped abdominal skin and covered with a protective binder. Prior to application of the test substance half the skin in the test area was abraded and half left intact. Dosing occurred for a 24 hour period during which the test area was covered by a protective binder and wrapped in gauze. After 24 hours the binder was removed and the test area rewrapped in gauze for 3 more days when the gauze was completely removed. Observation continued for a total of 14 days from the start of the test.

Results:

Signs of toxicity were slight depression lasting several hours and localized mild erythema in all animals.

Necropsy showed no gross abnormalities.

Conclusions:

The test report is sketchy, notably with regard to doses tested and numbers of animals at the start and at the end of the test. A discussion of these discrepancies follows.

Toxicity Category: Not determined

Classification: Supplementary.

1. Discrepancies in the study concerning the number of animals tested need to be resolved. The protocol states that 4 animals of each sex were selected for testing, for a total of eight animals, at the start. One animal was sacrificed during the test due to a broken leg. The results section shows 0/6 deaths, and the report states one animal died during the test. The sex of the dead animals is not mentioned. If both were of the same sex, the test group could possibly be diminished to two animals. Further explanation is necessary to explain the discrepancies in numbers. The report is unclear concerning the selection of controls. It is not clear whether control animals were selected from the original group of eight or were selected in addition to the test group. The 1982 Pesticides Guidelines specify 10 animals, including 5 from each sex, should be chosen for the test.

2. Sex differences in susceptibility to test substance are not discussed.

3. Test animals weighed from 1.7 to 2.1 kg, which is slightly underweight compared to Guideline specifications of 2 to 3 kg.

4. Only one dose level was tested. In light of the questions about the number of animals tested, it is impossible to conclude whether a sufficient number of animals or dose levels was tested.

Study Type: Acute Dermal Toxicity in Rabbits

Accession Number: 254690; Vol 2 of 3; Butylate Registration Toxicology Data; Stauffer Chemical Co.; Sept. 10, 1984; Appendix 2; Sec B; Toxicology Laboratory Report No. T-6460.

Contracting Lab: Stauffer Chemical Co.

Sponsor: Same

Date: December 15, 1981

Test Chemical: Sutan Technical, 98.0% pure (Butylate), a pale yellow liquid. The vehicle for the test substance was "Tween 80 and Water".

Experimental Protocol:

Sutan Technical, 98.0% pure, was applied to New Zealand albino rabbits. Four animals of each sex, which ranged in weight from 1.548 to 2.050 kg. were selected for the test. A patch of abdominal skin on each animal was closely clipped and half of the patch was then abraded. The test substance was applied to this prepared area beneath a protective binder. After 24 hours the binder was replaced with gauze wrapping, which was removed after three days. The animals were inspected for irritation at the first changing and observation continued for a total of 14 days from the time of initial application of the test substance.

Necropsies were performed on all animals which died during the test and survivors at 14 days.

Results:

One application of 5000 mg/kg was given. No deaths resulted in the six animals dosed during the test. Mild depression was the only observed toxic sign and all animals returned to normal on day one, showing no further toxic signs during the 14 day period. The test patch showed mild erythema in three rabbits and no effect in three others.

Necropsy showed no gross abnormalities.

Conclusions:

Toxicity Category: Not determined

Core Classification: Supplementary.

1. Number of animals tested: The protocol states 8 animals were selected but the results discuss only 6 animals and no mention is made of controls or fate of the 2 missing animals.
2. Erythema was observed in half of the dosed animals but the sex of these animals is not stated. The report should clearly indicate whether there were any gender differences in response to the test chemical.

Study Type: Primary Dermal Irritation in Rabbits

Citation: 254690; Vol 2 of 3; Butylate Registration Standard Toxicology Data; Stauffer Chemical Co.; Sept., 10, 1984; Appendix 1, Sec C; Toxicology Laboratory Report T-6376.

Contracting Lab: Stauffer Chemical Co.

Sponsor: Same

Date: April 4, 1979

Test Chemical: Sutan Technical, 97.7% pure (Butylate), a pale yellow liquid.

Experimental Protocol:

Six New Zealand albino rabbits received .5 ml of Sutan Technical on a patch applied to areas of both abraded and intact skin. The test substance was applied under a one-inch square of gauze which was secured with rubberized damming and adhesive. The patch was undisturbed for 24 hours, when the first set of scores was taken (0 hrs.). Two scores were assigned to each animal at successive readings, one for erythema and scar formation and one for edema. Further readings were made at 48 and at 72 hours after application of the test substance.

Results:

Table I[a] shows the Draize scores from all three readings. Upon patch removal, half of the animals showed well-defined erythema and half showed slight erythema. Twenty four hours after patch removal, erythema disappeared in two from the latter category and all others showed slight erythema. At 48 hours after patch removal, five animals appeared normal and one showed slight erythema. No difference between intact and abraded skin was seen with regard to erythema formation. No observations were made at 72 hours after patch removal.

Conclusions: The primary dermal irritation score, calculated as the average of the scores from six separate animals, was 0.86 for six New Zealand albino rabbits. The substance can be classified as a mild irritant at 48 hours. Most symptoms of erythema and edema disappeared at 48 hours after removal of the test patch and animals appeared generally asympomatic.

Toxicity Category: IV

Core Classification: Minimum

1. Animals were not followed to 72 hours or disappearance of toxic signs.

a. Note that times given in the table start with patch removal.

Table I

Primary Dermal Irritation in New Zealand Albino Rabbits/Report T-6376

Sutan Technical (97.7% Pure, Butylate)

	Erythema/eschar formation			Edema Observation			Total	Score[1]
Rabbit No./skin	0 hr.[2]	24 hr.	48 hr.	0 hr.	24 hr.	48 hr.		
1/ Intact	2	1	1	0	0	0	8	1.33
Abraded	2	1	1	0	0	0		
2/ Intact	1	1	0	1	0	0	6	1.00
Abraded	1	1	0	1	0	0		
3/ Intact	2	1	0	0	0	0	7	1.17
Abraded	2	1	0	1	0	0		
4/ Intact	1	0	0	0	0	0	2	.33
Abraded	1	0	0	0	0	0		
5/ Intact	2	1	0	0	0	0	6	1.00
Abraded	2	1	0	0	0	0		
6/ Intact	1	0	0	0	0	0	2	.33
Abraded	1	0	0	0	0	0		

Primary Irritation Score..... 0.86

1. Score is defined as the sum of individual values for each rabbit divided by six.
2. Time is measured starting with patch removal, after leaving the patch on for 24 hours.

004275

Study Type: Primary Dermal Irritation in Rabbits

Citation: Accession No.: 254690; Vol. 2 of 3; Butylate Registration Standard Toxicology Data; Stauffer Chemical Co.; Sept. 10, 1984; Appendix 2; Sec.C; Toxicology Laboratory Report T-6460.

Contracting Laboratory: Stauffer Chemical Co.

Sponsor: Same

Date: December 15, 1981

Test Chemical: Sutan Technical 98.0% pure (Butylate), a pale yellow liquid.

Experimental Protocol: Six New Zealand albino rabbits received .5 ml of Sutan Technical on a patch applied to areas of both abraded and intact skin. The test substance was applied under a one-inch square of gauze which was secured with rubberized damming and adhesive. The patch was undisturbed for 24 hours, when the first set of scores was taken (0 hr.). Two scores were assigned to each animal at successive readings, one for erythema and scar formation and one for edema. Further readings were made at 24 and at 48 hours after patch removal.

Results: Table I[a] shows the Draize scores for dermal irritation. Upon patch removal, four of the animals showed well-defined erythema and two showed slight erythema. Two of the animals showed edema. Twenty four hours after patch removal, erythema was still well-defined in two animals and four showed slight erythema. Edema subsided completely at 24 hours after patch removal. At 48 hours after patch removal, five of six animals showed sustained effects. The animals were not followed to 72 hours after patch removal. Control dose sites were scored as zero throughout the test period.

Conclusions: The primary dermal irritation score, calculated as the average of the scores from six separate animals, was 1.44. The substance is therefore a mild dermal irritant.

The study indicates readings at 24 hours and at 48 hours after patch removal. Since five animals still showed sustained effects at 48 hours after patch removal, observation should have continued until at least 72 hours after patch removal or until disappearance of any effects. Apparently, control dose sites showed no erythema or edema although no description of these areas is found in the test report.

Toxicity Category: IV

Core Classification: Minimum

1. There was a failure to follow animals to 72 hours after patch removal or disappearance of symptoms.

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a. Note that the times given in the table start at patch removal.

Table I

Primary Dermal Irritation in New Zealand Albino Rabbits/Report T-6460

Sutan Technical (98.0% Pure, Butylate)

	Erythema/Eschar Formation			Edema Observation			Total	Score[1]
Rabbit No./skin	0 hr.[2]	24 hr.	48 hr.	0 hr.	24 hr.	48 hr.		
1/ Intact	1	1	0	0	0	0		
Abraded	1	1	0	0	0	0	4.0	0.67
2/ Intact	2	1	1	0	0	0		
Abraded	2	1	1	0	0	0	8.0	1.33
3/ Intact	2	2	1	1	0	0		
Abraded	2	2	1	1	0	0	12.0	2.00
4/ Intact	2	2	2	1	0	0		
Abraded	2	2	2	1	0	0	14.0	2.33
5/ Intact	1	1	1	0	0	0		
Abraded	1	1	1	0	0	0	6.0	1.00
6/ Intact	2	1	1	0	0	0		
Abraded	2	1	1	0	0	0	8.0	1.33

Primary Irritation Score.....1.44

1. Score is defined as the sum of individual values for each rabbit divided by six.
2. Time is measured from the time of patch removal, which is after 24 hours.

Study Type: Primary Occular Irritation in Rabbits 004275

Citation: 254690; Vol 2 of 3; Butylate Registration Standard Toxicology Data; Stauffer Chemical Co.; Sept., 10. 1984; Appendix 1 Sec. D; Toxicology Laboratory Report T-6376.

Contracting Lab: Stauffer Chemical Co.

Sponsor: Same

Date: April 4, 1979

Test Chemical: Sutan Technical, 97.7% pure, (Butylate), a pale yellow liquid.

Experimental Protocol: Nine New Zealand albino rabbits received .1 milliliter of the test chemical applied to one eye. The untreated eye served as control. The treated eye was washed with water at 2 seconds in three animals and at 4 seconds in three other animals. The treated eyes of one group of three remained unwashed.

Results: No readings were taken at 1 hour after application of the test chemical. Observations were recorded at 24, 48, and 72 hours post-application and at 7 days post-application. All Draize scores were zero.

Conclusions: The test report shows no positive effects of administration of the test chemical.

Toxicity Category: Not determined

Core Classification: Supplementary

1. The 1982 Pesticides Guidelines specify testing at least six animals and that treated eyes should not be washed for 24 hours after treatment. In this study, 9 animals were dosed originally but 3 had eyes washed at 2 seconds and 3 had eyes washed at 4 seconds after exposure. Three remainded unwashed. The rationale for proceeding as reported should be stated in the test report, since the procedure may have reduced the test group to an insufficient number for determining significant results.

2. No observations were made at 1 hour. It is therefore unknown whether any effects occurred in the period immediately following administration of the test chemical. The onset as well as the occurrence of any toxic effects should be reported.

Study Type: Primary Occular Irritation in Rabbits

Citation: 254690; Vol 2 of 3; Butylate Registration Standard Toxicology Data; Stauffer Chemical Co.; Sept., 10, 1984; Appendix 2; Sec. D; Toxicology Laboratory Report T-6460.

Contracting Lab: Stauffer Chemical Co.

Sponsor: Same

Date: December 20, 1978

Test Chemical: Sutan Technical, 98.0% pure, Butylate, a pale yellow liquid.

Experimental Protocol: Nine New Zealand albino rabbits received .1 milliliter of the test chemical applied to tone eye. The untreated eye served as control. The treated eye was washed with water at 2 seconds in three animals and at 4 seconds in 3 other animals. The treated eyes of one group of three remained unwashed.

Results: No readings were taken at 1 hour after application of the test chemical. Observations were recorded at 24, 48, and 72 hours post-application and at 7 days post-application. All Draize eye scores were zero.

Conclusions: The test report shows no positive effects of administration of the test chemical.

Toxicity Category: Not determined

Core Classification: Supplementary

1. The 1982 Pesticide Guidelines specify testing at least six animals and that treated eyes should not be washed for 24 hours after treatment. In this study, 9 animals were dosed originally but 3 had eyes washed at 2 seconds and 3 had eyes washed at 4 seconds after exposure. Three remained unwashed. The rationale for proceeding as reported should be stated in the test report, since the procedure may have reduced the test group to an insufficient number for statistically significant results.

2. No observations were made at 1 hour. It is therefore unknown whether any effects occurred in the period immediately following administration of the test chemical.

004275

Study Type: Acute Inhalation Toxicity in Rats

Citation: 254690; Vol 2 of 3; Butylate Registration Standard Toxicology Data; Stauffer Chemical Co.; Sept. 10, 1984; Appendix 3; Toxicology Laboratory Report T-6376.

Contracting Lab: Stauffer Chemical Co.

Sponsor: Same

Date: April 4, 1979

Test Chemical: Technical Sutan, 97.7% pure, an amber liquid.

Introduction: The acute inhalation LC_{50} for the test chemical was greater than 5.2 mg/l (mean actual aerosol chamber concentration) for 4 hours. Exposure at this level demonstrated no mortality and therefore qualifies as a limit test for inhalation toxicity as described in the 1982 Pesticides Guidelines.

Experimental Protocol: Ten male and ten female Sprague Dawley rats purchased from Charles River Laboratories, Portage, Michigan were acclimated for ten days prior to testing at 21°±2° C. The male rats ranged in weight from 207 to 240 grams and the female rats ranged in weight from 181 to 216 grams at the start of the test.

Chamber, Delivery Apparatus, and Measurement of Concentrations and Particle Size:

Chambers supplied by Young and Bertke, Co. were made of stainless steel and glass forming a volume of 447 l. Chamber air was maintained at 21°± 2°C and 60 ± 10 % humidity. Total air flow through the chamber was 110 l/min which effected 15 air changes per hour.

Aerosol was produced by a Solo-Sphere generator. For a mean exposure level of 5.2 mg/l, the generator pressure was 50 psi, the air flow was 6.5 l/min. and the auxiliary air flow was 5-25 l/min. The aerosol was delivered through stainless tubing and conditioned by an aerosol discharger (Model 3054, Thermo Systems, Inc.) which removed excess static charges on the droplets.

B-5

Actual aerosol concentration in the chamber was measured every 60 ± 15 min. during the 3.8 hour exposure. Samples were taken at the breathing zone of the animals approximately every hour. Particle size was measured analytically using samples collected in filters placed in the sampling apparatus. Nominal concentration was calculated as the ratio of amount of material aerosolized from the generator to the total chamber air flow. Aerosol particle size was analyzed twice during the test. Particle size was reported in units of mass mean aerodynamic resistance diameters ($MMAD_{ar}$). A recovery study demonstrated that virtually quantitative recovery of test substance from the aerosol filter samples could be achieved.

Exposure and Observations: Rats were caged individually in two compartmentalized stainless steel wire cages with ten compartments each inside the exposure chamber. The cages were stacked on wire shelves with a vertical clearance of 6 inches. Exposure lasted for four hours and the rats remained in the cages for one more hour at an increased flow rate of 40 air changes/hr. to facilitate evaporation of the test substance from their pelts. No deaths occurred during or after the exposure period.

Onset of toxic signs could not be noted during the 4 hour test period because the aerosol density reduced visibility into the cages. Rats were observed twice daily and weighed on the day of exposure and on days 3, 7, and 14. Upon necropsy at 14 days, the following tissues were placed in 10% neutral buffered formalin:

- trachea
- larynx
- nasal passages
- lung
- liver
- kidney
- heart
- any abnormal tissue

Results: The mean actual concentration of aerosol was 5.2 mg/l (4.8 - 5.6) for the 3.8 hours of exposure. Variability in chamber concentration (mean/range X 100) was 15% which can be considered an essentially constant concentration of aerosol. Particle size ranged from 3.3-3.5 microns with 6% variability. These particles were therefore of respirable size.

Toxic Signs: Males showed mild lethargy immediately after exposure until day 2 when they returned to normal. Mean body weight was 6% less than controls which was a significant difference according to Student's t-test. Weights continued to be less than controls but with a smaller difference for

the remainder of the test.

Females also showed mild lethargy immediately after exposure until day three. In addition, they showed mild to moderate incidence of blood-like flecks about the face and yellow anogenital stains. Appearance returned to normal by day 3. No significant difference in body weight between controls and the test group was observed.

Necropsy Results: Four of ten males showed small foci on the lung surface. Individual rats showed various red-brown foci on different lobes of the lung. A single instance of dilatation of a renal pelvis occurred with similar frequency to the control group.

One female showed a circular brownish focus on the diaphragmatic lobe of one lung. Another showed moderate dilatation of the right renal pelvis but, as in males, this symptom also occurred in controls with similar frequency.

Conclusions: The test results show the LC_{50} is greater than 5 mg/l for a 4 hour exposure. These particles measure 3.3 to 3.5 microns, which is well within the size limits considered respirable (10 - 15 microns). Therefore, the animals showed no notable toxicity resulting from a four hour exposure to the test substance at the limit level of exposure (5 mg/l).

Most details of the testing procedure appear complete as presented in the test report. The test report shows several areas of weakness which are noted following the core classification.

Toxicity Category: III

Core Classification: Minimum

1. The arrangement of animals in the exposure chamber is not clearly described. The cages were stacked with 6 inches of clearance between layers. This could result in uneven exposure to the animals. Also, there was no information about the distribution of males and females within the exposure chamber.

2. Due to the description of the exposure chamber, the animals appear to be able to move around within the cage rather than being restrained during the test period. Since the substance was rather wet, the fur was most likely saturated and the rats could have increased their exposure by swallowing some of the test substance during preening. Therefore, the

level measured at the breathing zone probably underestimated total exposure which included the amount inhaled and the undetermined amount ingested.

3. No discussion of control animals is in the test report. The table of weights gives mean weight of the control animals and it seems that equal numbers of test animals and controls were used. However, this is not clear from the test report. Further, no information is given about the treatment of controls, their diet, or whether they were given any vehicle. The mean weight of the controls, by itself, is not helpful in deciding on the toxicity of this chemical.

Study Type: Acute Inhalation Toxicity in Rats

Citation: 254690; Vol 2 of 3; Butylate Registration Standard Toxicology Data; Stauffer Chemical Co.; Sept. 10, 1984; Appendix 4; Toxicology Laboratory Report T-6460.

Contracting Lab: Stauffer Chemical Co.

Sponsor: Same

Date: December 20, 1978

Test Chemical: Technical Sutan, 98.2% pure, Lot #GGC-0301.

Introduction: The acute inhalation LC_{50} for the test chemical was greater than 4.7 mg/l (mean actual aerosol chamber concentration) for 4 hours. Exposure at this level demonstrated no mortality and therefore qualifies as a limit test for inhalation toxicity as described in the 1982 Pesticides Guidelines.

Experimental Protocol: Ten male and ten female Sprague Dawley rats purchased from Charles River Laboratories, Portage, Michigan were acclimated for ten days prior to testing at 21°±2°C. The male rats ranged in weight from 195 to 230 grams and the female rats ranged in weight from 172 to 199 grams at the start of the test.

Chamber, Delivery Apparatus, and Measurement of Concentrations and Particle Size:

Chambers supplied by Young and Bertke, Co. of Cincinnati, Ohio were made of stainless steel and glass forming a volume of 447 l. Chamber air was maintained at 21°±2°C and 60±10% humidity. Total air flow through the chamber was 110 l/min which effected 15 air changes per hour.

Aerosol was produced by a Solo-Sphere generator. For a mean exposure level of 4.7 mg/l, the generator pressure was 50 psi, the air flow was 4 l/min. and the auxiliary air flow was 22-23 l/min. The aerosol was delivered through stainless tubing and conditioned by an aerosol discharger (Model 3054, Thermo Systems, Inc.) which removed excess static charges on the droplets.

Actual aerosol concentration in the chamber was measured every 60 ± 15 min. during the 4 hour exposure. Samples were taken at the breathing zone of the animals approximately every hour. Particle size was measured analytically using samples collected in filters placed in the sampling apparatus. Nominal concentration was calculated as the ratio of amount of material aerosolized from the generator to the total chamber

air flow. Aerosol particle size was analyzed twice during the test. Particle size was reported in units of mass mean aerodynamic resistance diameters ($MMAD_{ar}$). A recovery study demonstrated that virtually quantitative recovery of test substance from the aerosol filter samples could be achieved.

Exposure and Observations: Rats were caged individually in two compartments each inside the exposure chamber. The cages were stacked on wire shelves with a vertical clearance of 6 inches. Exposure lasted for four hours and the rats remained in the cages for one more hour at an increased flow rate of 40 air changes per hour to facilitate evaporation of the test substance from their pelts. No deaths occurred during of after the exposure period.

Onset of toxic signs could not be noted during the 4 hour test period because the aerosol density reduced visibility into the cages. Rats were observed twice daily and weighed on the day of exposure and on days 3, 7 and 14. Upon necropsy at 14 days, the following tissues were placed in 10% neutral buffered formalin:

trachea
larynx
nasal passages
lung
liver
kidney
heart
any abnormal tissue

Results: The mean actual concentration of aerosol was 4.7 mg/l (4.5-4.9) for the 4 hours of exposure. Variability in chamber concentration (mean/range X 100) was 9% which can be considered an essentially constant concentration of aerosol. Particle size ranged from 4.0 to 4.2 microns with 5% variability. These particles are therefore of respirable size.

Toxic Signs:

Males exhibited lethargy following the exposure period. Blood-like flecks were observed about the faces. All rats appeared normal by day 2. No statistically significant changes in body weight between control and experimental groups was noted.

Females appeared similar to males, being lethargic immediately following exposure and with blood-like flecks about the face. Likewise, they returned to normal by day 2. Body weight was significantly less than the weight of controls on day 14, showing a 5% decrease.

Necropsy:

Males and females showed no compound-related abnormalities at necropsy.

Conclusions: No mortality occurred after exposing the test species to an aerosol of the compound at a mean concentration of the test chemical of 4.7 mg/l for 4 hours. The LC_{50} is therefore above the level used in this limit test for acute inhalation toxicity.

Toxicity Category: III

Core Classification: Minimum

1. The animals were caged individually, however, since they were not restrained within the cage, nothing prevented them from moving about inside the cage which allowed the animals to preen and to consequently ingest some of the test chemical via the oral route. This would affect the total exposure level but may not have changed the amount inhaled. One must consider that the total exposure was probably greater than the actual aerosol concentration over the time period of the test.

2. The cages were stacked and there is no description of the distribution of animals by sex within the exposure chamber in the test report. The animals on the upper rows could have received a different amount of the test chemical than those in the lower rows. No information in the test report allows conclusions regarding this issue. It is therefore possible that the animals and the two sexes did not receive identical treatment during the test period. If any differences in handling occurred, these may have affected the exposure of the animals and the test results.

Study Type: Acute Inhalation Toxicity in Rats

Citation: 254690; Vol 2 of 3; Butylate Registration Standard Toxicology Data; Stauffer Chemical Co.; Sept. 10, 1984; Appendix 5; Toxicology Laboratory Report T-6134.

Contracting Lab: Stauffer-Chemical-Co. International Research and Developme... Corp.

Sponsor: Same. Stauffer Chem. Co.

Date: December 19, 1977

Test Chemical: Sutan + 6.7E, Lot No. ZGC 0801, a yellow brown liquid, of unspecified purity.

Experimental Protocol: Thirty male and thirty female Charles River CD rats were selected for the test. Males ranged in weight from 206 to 300 grams and the females ranged in weight from 200 to 237 grams. The rats were kept in temperature- and humidity-controlled cages before and after exposure.

During exposure, three rats were placed, by sex, in each wire-mesh cage and then placed in a 160 liter cubical stainless steel-glass chamber during exposure. Constant air flow was maintained during each exposure and varied with each concentration. The exhaust was filtered before discharge to the outside.

Production of aerosol was achieved by using a precision dual syringe pump to introduce the test liquid into a positive pressure atomizer located near the chamber air inlet at the top of the chamber. Air pressure of 10 psig (units) was used to aerosolize the liquid.

To achieve the desired concentration, the aerosols were diluted by the incoming chamber air. Nominal concentration was calculated as the ratio of rate of liquid dissemination to total air flow (volume of air ejected from the atomizer + volume of make-up air).

Table I shows the five exposure groups and their respective levels

Table I

Group	Chamber Air Flow l/min.	Calculated Chamber Concentration mg/l
1	50	30.20
2	23	15.19
3	25	13.97
4	35	12.59
5	25	10.28

The animals were observed for signs of toxicity and mortality during and immediately following exposure. Daily observations were made from the end of the exposure period until day 14. Body weights were recorded "periodically", although the length of this term is not defined in the test report. All rats were necropsied at 14 days.

Results:

Table II. Mortality in Rats Admininstered Butylate[a]

Exposure Concentration mg/l	Number of Deaths Hrs. 0-4		Days 1		2		3		4		5		6		7-14		Total Mortalities		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	Male	Female	Tot.
30.20	3		3	2		4											6/6	6/6	12/12
15.19					4	6					1						5/6	6/6	11/12
13.97																	0/6	0/6	0/12
12.59																	0/6	0/6	0/12
10.27																	0/6	0/6	0/12

Signs of Toxicity:

Onset of toxic signs occurred immediately in the highest doses: 30.20 mg/l, and 15.19 mg/l. There was an immediate increase in activity. After 30 minutes of exposure at these doses, some animals exhibited eye squint, salivation, nasal discharge, and some hypoactivity. At 60 minutes, all animals exhibited most of the above symptoms and some also showed dyspnea and lacrimation. After two hours of exposure, the animals showed dyspnea and ataxia in addition to the other symptoms. All deaths occurred at these doses. Mortality is summarized in the Table II.
In the 30.20 mg/l group, three males died after 5 hours, and the remaining three died within the first day. Two females died in the first day and the remaining four exhibited ataxia, salivation, nasal discharge, and gasping. These four females died on day two. In the 15.19 mg/l group, four males and all the females died on day two. One male died on day 5.

The three remaining dose groups, 13.97 mg/l, 12.59 mg/l, and 10.29 mg/l showed similar toxic signs. The animals demonstrated eye squint, dyspnea, salivation, lacrimation, and nasal discharge during the exposure period. In the 13.97 mg/l group these signs persisted for several hours post-exposure. By day one post-exposure, most animals appeared normal with some loss of body weight. By day five, all animals appeared normal. In the 12.59 mg/l group, these signs persisted for one day. By day two, all were normal except for slight loss of body weight. By day four all had gained weight. In the 10.29 mg/l group, some dyspnea was observed at three hours in addition to the above symptoms. On day one post-

a. Table II is found in the report for Acute Inhalation Toxicity, T-6134, page 5.

exposure the animals showed sightly lowered respiratory rate and the other signs of toxicity persisted. After day one, all appeared normal, however body weight was lower than before exposure for one week. Table III shows mean body weights for these three groups at the start and finish of the test.

Table III. Comparative Mean Body Weights of Survivors

Dose/Sex (mg/l)	Initial Body Weight (g)	Final Body Weight (g)
13.97/		
Females	209	222
Males	257	294
12.59/		
Females	219	254
Males	232	312
10.29/		
Females	219	246
Males	281	316

Necropsy:

Necropsy was performed on animals who died during the test and on all survivors at 14 days.

In the highest dose groups: 30.20 mg/l and 15.19 mg/l the majority of animals showed congestion and focal hemorrhages of the lungs. Further, ulceration of the glandular mucosa of the stomach was seen in many at the highest dose and in one animal at the next-highest dose. In the 15.19 mg/l dose group, three animals showed liver congestion. The one survivor demonstrated lung congestion at sacrifice.

No abnormalities were observed at necropsy of the 13.97 mg/l dose group. Three animals in the 12.59 mg/l dose group showed pulmonary congestion. The remaining animals in this group as well and those in the 10.29 mg/l dose group showed no lesions at necropsy.

Toxicity Category: Not determined

Core Classification: Supplementary

1. The purity of the test substance is not given in the test report.

2. Air changes per hour or minute were not discussed. The report states only that "air flow was constant" through the chamber and gives no data supporting this statement.

3. Actual concentration was not determined, only nominal concentration was given. The report gives no indication that samples were taken from the breathing zone of the animals.

4. Control animals were not discussed in the test report. Although weights of surviving animals were taken at the start and at the end of the test period, it is not possible to use these in a meaningful way, since control information is not available for comparison purposes.

5. The death table shows that all animals died at approximately 30 and 15 mg/l, but none died at the next lower dose (14 mg/l) or at any lower doses. There appears to be a discrepancy in these numbers and possibly a problem with the exposure chamber.

6. No mention of particle size or the details of any measurements to determine particle size appear in the test report.

Study Type: Acute Intraperitoneal Toxicity in Rats

Accession No.: 254690, Vol 2 of 3; Butylate Registration Standard Toxicology Data; Stauffer Chemical Co., Sept. 10, 1984; Appendix 6; Toxicology Laboratory Report T-6009.

Contracting Lab.: Stauffer Chemical Co.

Sponsor: Same

Date: November 8, 1977

Test Chemical: Sutan Technical, 97.8% pure (Butylate), Lot No. REJ1501, a dark tan liquid. The vehicle for the test substance was Tween 80 in 0.9% saline.

Experimental Protocol: About ten Sprague Dawley albino rats were used for each dose level tested. At the start of the test the animal weights ranged from 163 to 216 grams. The animals were dosed with a hypodermic injection administered directly into the peritoneal cavity. The five dose levels tested in males were 2500, 1750, 1000, 750, and 500 ml/kg. The seven doses tested in females were 5000, 4000, 2500, 1750, 1000, 750, and 500 mg/kg. The animals were observed for ten days for signs of toxicity and mortality. No discussion of control animals appears in the test report. The LD_{50} and 95% confidence intervals were calculated using the method of Litchfield and Wilcoxon (1949).

Results: Table I shows the mortality rates for each dose for males and females. Toxic signs at the high dose were convulsions, depression, salivation, exophthalmus and blood-like lacrimation for males and females. Several females showed the additional symptom of severe diarrhea. The onset of mortality and the times for survivors to return to normal, are included in Table I.

Necropsy showed irritated abdominal walls and adhesions of one or more organs to each other and/or to teh abdominal walls. Somme affected organs were enlarged and a few animals showed red fluid in the intestines.

The LD_{50} for both males and females was 1050 mg/kg. The 95% confidence interval for males was 780-1413 mg/kg and the 95% C.I. for females was 741-1488 mg/kg.

Comments:

1. The report does not discuss control animals in the experimental procedure.

Table I

Acute Intraperitoneal Toxicity of Sutan Technical (Butylate, 98.7 % pure)/Report No. T-6009

Mortality and Return to Normal in Male and Female Rats

Dose mg/kg	Males			Females		
	Mortality	Time to Death	Return to Normal	Mortality	Time to Death	Return to Normal
5000	Dose not tested			9/10	2 hours	day 2
4000	Dose not tested			16/19	2 hours	day 2
2500	10/10	days 1-2	----	18/20	day 1	day 3
1750	8/10	day 1	day 4	7/10	day 1	day 2
1000	9/20	days 1-3	day 4	7/10	day 1	day 2
750	6/15	days 1-2	day 3	7/15	days 1-3	day 2
500	0/10	No deaths	No info.	0/10	No deaths	day 2
	LD_{50} = 1050 mg/kg (95% Confidence Interval = 780-1413.			LD_{50} = 1050 mg/kg (95% Confidence Interval = 741-1488.		

DATA EVALUATION RECORD

004275

STUDY TYPE: Mutagenicity reverse mutation in Salmonella).

CITATION: Jagannath, D.R. and Brisick, D.J. Mutagenicity evaluation of Sutan Tech GGC-0301. (Unpublished Study No. T6310 prepared by Litton Bionetics, Inc., Kensington, MD for Stauffer Chemical Corporation, Western Research Centers, Richmond, CA; dated October 27, 1977.)

ACCESSION NUMBER: 254590.

LABORATORY: Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, MD 20795.

QUALITY ASSURANCE STATEMENT: Not present.

TEST MATERIAL: Sutan Tech GGC-0301, described as a light yellow liquid. No purity was given.

PROCEDURES:

Microbial Strains: Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 and the yeast strain Saccharomyces cerevisiae D4 were used.

Preparation of S9: The S9 homogenate was prepared from the liver of an adult male Sprague-Dawley rat induced by ip injection with Arochlor 1254. Components of the S9 mix were as follows:

Components of S9 Mix

TPN	4 µmoles/ml
Glucose-6-phosphate	5 µmoles/ml
Sodium phosphate (dibasic)	100 µmoles/ml
MgCl2	8 µmoles/ml
KCl	33 µmoles/ml
S9 fraction	0.1-0.15 ml/ml

Selective Media: The selective medium used in the Salmonella and Saccharomyces assays were not reported; however, it is assumed that Vogel Bonner media was used for Salmonella, since the author used the Ames[1] method. There were no reported methods and materials for Saccharomyces cerevisiae D4.

Preparation of Test Material: The test material was diluted with dimethylsulfoxide (DMSO) to final dose levels of 0.001, 0.01, 0.1, 1.0, and 5.0 µl/plate. As was stated in the report, the study protocol usually employs a dose range with "the highest of these doses being selected to show slight toxicity as determined by subjective criteria."

Controls: The negative (solvent) control was DMSO at 50 µl/plate. The positive controls were those listed in Table 1.

TABLE 1. Positive Controls

Strain	S9 Activation	Substance	Concentration (µg/plate)
TA1535	-	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	10
	+	2-Anthramine (ANTH)	100
TA1537	-	Quinacrine mustard (QM)	10
	+	8-Aminoquinoline (AMQ)	100
TA1538	-	2-Nitrofluorene (NF)	100
	+	2-Acetylaminofluorene (AAF)	100
TA98	-	NF	100
	+	AAF	100
TA100	-	MNNG	10
	+	ANTH	100
D4	-	MNNG	10
	+	DMNA*	100 µmoles

* This compound was not identified by the authors, but was presumed to be dimethylnitrosamine.

[1] Ames, et al. Mutat. Res. 31:347-364 (1975).

Mutagenicity Assay: Salmonella typhimurium: In the nonactivated system, the appropriate concentration of the test material and the control substances were mixed with 10^8 cells from overnight cultures of each tester strain in tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. In the activated system, 0.5 ml of the S9 mixture was added to the 2.0 ml of molten agar. The tubes were mixed then poured onto selective agar plates, one plate/dose/strain. The plates were allowed to solidify, then incubated at 37° C for 48 hr, after which the plates were scored for the number of revertant colonies. In the nonactivated Salmonella system, the appropriate concentration of the test material and the control substances were mixed with 10^8 cells from overnight cultures of each tester strain in tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. In the activated system, 0.5 ml of the S9 mixture was added to the 2.0 ml of molten agar. The tubes were mixed then poured onto selective agar plates one plate/dose/strain. The plates were allowed to solidify, then incubated at 37° C for 48 hours, after which the plates were scored for the number of revertant colonies.

Saccharomyces cerevisiae: No method nor a reference was reported for reverse mutation in the yeast strain D4.

Evaluation Criteria: If a chemical produced a positive dose response over three concentrations with the lowest increase equal to twice the solvent control in strains TA1535, TA1537, or TA1538, the chemical was considered mutagenic. If a chemical produced a positive dose response over three concentrations with the highest increase equal to twice the solvent control in strains TA98, TA100, or D4, it was considered mutagenic.

RESULTS:

The number of revertants reported for the assay of various doses of Sutan technical, with and without metabolic activation were essentially equal to those reported for the negative control. Results obtained for the negative and positive controls are presented in Table 2.

DISCUSSION:

The authors reported that all tester strains gave appropriate responses, with and without metabolic activation, for the solvent and positive controls. Each strain produced the expected numbers of revertant colonies. They stated that none of the plates treated with the test material produced a positive response but that the assay's sensitivity was adequate; therefore, it was concluded that Sutan technical GGC-0301 (Butylate) was not mutagenic.

Our assessment is that the data were insufficient to conclude that Sutan was not mutagenic. Although the authors stated that a toxicity test had been conducted, how toxicity was assessed in Salmonella or Saccharomyces was not reported. They reported that the low dose was nontoxic, but did not report what level was toxic, and no data were presented.. Thus, it

TABLE 2. Results for Solvent and Positive Controls Used in the Assay of Sutan Tech GGC-0301 (Butylate)

Strain	S9 Activation	Substance	Revertants[a] Per Plate
TA1535	-	DMSO	18
	+		28
	-	MNNG	>1000
	+	ANTH	234
TA1537	-	DMSO	22
	+		19
	-	QM	577
	+	AMQ	424
TA1538	-	DMSO	19
	+		34
	-	NF	>1000
	+	AAF	673
TA98	-	DMSO	31
	+		47
	-	NF	>1000
	+	AAF	>1000
TA100	-	DMSO	196
	+		198
	-	MNNG	>1000
	+	ANTH	>1000
D4*	-	DMSO	32
	+		23
	-	MNNG	573
	+	DMNA	48

[a] Results from a single plate.

* Try. convertants per single plate.

could not be confirmed that the highest dose tested was close to the limit of cytotoxicity. If there was no cytotoxicity, then the test material should have been assayed based on its limit of solubility or as according to Ames[1] at a maximum concentration of 50 µl.

The authors' protocol stated that in the dose range tested the highest dose should show slight toxicity as determined by "subjective criteria"; however, a definition of "subjective criteria" was not given.

There were no methods and materials reported for Saccharomyces cerevisiae D4, thus, it can only be assumed that it was performed according to the Ames[1] method, which is not the standard assay for Saccharomyces. In addition, no attempt was made to assure the reproducibility of the results, since one plate per dose per strain was assayed (CBI Table 1, page 4). According to the Ames[1] method, a minimum of duplicate plates per dose per strain should be performed in order to assess reproducibility of the assay.

CONCLUSIONS:

No conclusions can be drawn from these studies (S. typhimurium or S. cerevisiae) because: 1) it could not be confirmed that the dose range used was high enough; 2) what materials and methods were used to assay mutations in S. cerevisiae strain D4; and 3) whether the results obtained were reproducible.

CLASSIFICATION: Unacceptable.

00427

EPA: 68-01-6561
TASK: 20
January 10, 1985

DATA EVALUATION RECORD

BUTYLATE

Acute Delayed Neurotoxicity Study in Hens

STUDY IDENTIFICATION: Sprague, G.L. Acute delayed neurotoxicity study with technical Sutan in adult Hens. (Unpublished study No. T-6801 conducted by Richmond Toxicology Laboratory for Stauffer Chemical Co., Richmond, CA, dated October 1, 1980.) Accession No. 254690.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 1-10-85

00427S

1. CHEMICAL: Butylate, Sutan Technical.

2. TEST MATERIAL: Sutan, Lot #GHE-2501, 98.97% pure, density = 0.9317 g/ml; Practical tri-o-tolyl phosphate, Lot #A7C, (TOCP); and corn oil (Mazola).

3. STUDY/ACTION TYPE: Acute delayed neurotoxicity study in hens.

4. STUDY IDENTIFICATION: Sprague, G.L. Acute delayed neurotoxicity study with technical Sutan in adult Hens. (Unpublished study No. T-6801 conducted by Richmond Toxicology Laboratory for Stauffer Chemical Co., Richmond, CA, dated October 1, 1980.) Accession No. 254690.

5. REVIEWED BY:

Finis Cavender, Ph.D.
Project Scientist
Dynamac Corporation

Signature: Finis Cavender
Date: 1/11/85

Nicolas P. Hajjar, Ph.D.
Project Scientist
Dynamac Corporation

Signature: Nicolas P. Hajjar
Date: January 10, 1985

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Technical Quality Assurance
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 1-10-85

Chad Sandusky, Ph.D.
EPA Reviewer

Signature: Chad B. Sandusky
Date: Jan 23, 1985

Chris Chaisson, Ph.D.
Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. Butylate did not produce TOCP-like delayed neurotoxicity in adult hens treated with 9.3 g/kg.

B. This study was conducted and reported in an acceptable manner and is a core guideline study.

004275

8. RECOMMENDATIONS:

Not applicable.

9. BACKGROUND:

The acute toxicity of butylate in hens was tested with the highest dose being 9.3 g/kg. No hens died at this dose, thus, the oral LD_{50} in hens is greater than 9.3 g/kg.

The maximum dose required for an acute delayed neurotoxicity study is 5 g/kg. Thus, a dose of 9.3 g/kg exceeds the dosing requirement for this test.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES:

Not applicable.

11. MATERIALS AND METHODS (PROTOCOLS): The complete Materials and Methods section of the report are given in Appendix A.

A. Materials and Methods:

1. Sutan technical (98.97%) pure); butylate.

2. Adult, white leghorn hens, 13 months old from Feather Hill Farms (Petaluma, CA).

3. Groups of hens were dosed twice with 9.3 g/kg butylate (15 hens); 500 mg/kg TOCP (12 hens); or 10 ml/kg corn oil (12 hens).

12. REPORTED RESULTS:

No hens given butylate or corn oil died on study while 3/12 hens receiving TOCP were sacrificed in extremis on day 37 of the study.

The incidence of selected pharmacotoxic signs observed during the study are summarized in Table 1. Transient motor incoordination was noted in 2 hens receiving butylate while all hens receiving TOCP exhibited motor incoordination.

Body weight effects were not noted for hens receiving butylate or corn oil, whereas hens receiving TOCP lost weight steadily over the 42-day study.

TABLE 1. Incidence of Selected Adverse Signs Observed in Hens Treated with Corn Oil, TOCP, or Butylate[a]

Adverse Sign	Incidence of Sign in Hens Treated Twice With Corn Oil (10 ml/kg)	TOCP (500 mg/kg)	Butylate (9317 mg/kg)
1. Appearance:			
Dry or atrophied comb	9/12[b]	9/12	1/15
Feather loss	12/12	10/12	12/15
Quiet or non-vocal behavior	0/12	0/12	15/15
2. Posture, Motor Coordination, Leg Strength:			
Wide stance	0/12	3/12	0/15
Leaning back	0/12	4/12	0/15
Transient motor incoordination	0/12	12/12	2/15
Progressive paralysis	0/12	11/12	0/15
Sitting on hocks	0/12	9/12	0/15
Unable to stand	0/12	6/12	0/15
3. Physiological			
Diarrhea	10/12	12/12	2/15
Soft-shelled egg	1/12	1/12	5/15

[a]This table was reproduced from the original text as submitted by the registrant.

[b]Number of hens showing the specified sign/number of hens in the group.

Walking behavior was affected on the days of dosing (days 1 and 22) in hens receiving butylate, whereas hens receiving TOCP exhibited progressive, impaired walking behavior starting on day 15 and continuing throughout the study. No evidence of impaired walking behavior was noted for hens receiving corn oil.

The incidence of selected histologic changes is summarized in Table 2. Hens receiving butylate while all hens treated with TOCP exhibited axonal degeneration in the brain and spinal cord, whereas none of these lesions were noted in tissues from hens receiving butylate.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Transient motor incoordination was noted in hens treated with butylate on the day of dosing. No other signs or histologic changes suggestive of delayed neurotoxicity were noted in these hens as compared with TOCP-treated hens.

B. This study was conducted in an acceptable manner and was inspected according to Good Laboratory Practice regulations. The quality assurance statement was present, signed, and dated October 2, 1980.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Hens were dosed with the maximum concentration of butylate possible in the dosing volume recommended for this type of study. No deaths occurred at this dose (9.3 mg/kg). Transient motor incoordination and impaired walking behavior was noted for butylate-treated hens only on the days of dosing. In contrast, TOCP-treated hens exhibited progressive motor incoordination and impaired walking behavior on day 15 of the study. There were no histologic lesions suggestive of acute delayed neurotoxicity, observed in butylate-treated hens as compared to TOCP-treated-hens. Therefore, butylate did not produce TOCP-like delayed neurotoxicity.

B. These conclusions agree with those of the author.

C. This study was conducted in an acceptable manner.

15. COMPLETION OF ONE-LINER FORM FOR STUDY:

Butylate does not produce TOCP-like delayed neurotoxicity in hens.

16. CBI APPENDIX:

Appendix A - Materials and Methods (reproduced from the original text as submitted by the registrant).

TABLE 2. Frequency of Occurrence of Histologic Changes in Nerves of Hens Treated with Corn Oil, TOCP, or Butylate[a]

Neurologic Tissue	Histopathologic Change	Frequency of occurrence (%) in hens treated twice with: Corn Oil (10 mg/kg)	TOCP (500 mg/kg)	Butylate (9317 mg/kg)
Brain	Axonal degeneration in cerebellar peduncles	0.0	100.0	0.0
	Focal gliosis	16.7	100.0	13.3
	Neuronal swelling and chromatolysis	0.0	8.3	6.7
Spinal Cord Cervical	Axonal degeneration in dorsal funiculi	0.0	100.0	0.0
	Random axonal degeneration	16.7	75.0	6.7
	Focal gliosis	66.7	100.0	60.0
	Neuronal swelling and chromatolysis	8.3	16.7	0.0
Thoracic	Axonal degeneration in ventral and lateral funiculi	8.3	100.0	0.0
	Random axonal degeneration	25.0	75.0	20.0
	Lymphocytic perivascular cuffing	16.7	66.7	53.3
	Focal gliosis	58.3	100.0	60.0
	Neuronal swelling and chromatolysis	58.3	41.7	40.0

[a]This table was reproduced from the original text as submitted by registrant.

TABLE 2. Frequency of Occurrence of Histologic Changes in Nerves of Hens Treated with Corn Oil, TOCP, or Butylate[a] (Continued).

Neurologic Tissue	Histopathologic Change	Frequency of occurrence (%) in hens treated twice with Corn Oil (10 mg/kg)	TOCP (500 mg/kg)	Butylate (9317 mg/kg)
Spinal Cord Sacro-lumbar				
	Axonal degeneration in ventro-medial funiculi	0.0	100.0	13.3
	Random axonal degeneration	0.0	50.0	6.7
	Focal gliosis	25.0	100.0	26.7
	Neuronal swelling and chromatolysis	75.0	41.7	66.7
Sciatic Nerve				
	Bilateral nerve fiber degeneration	16.7	91.7	0.0
	Unilateral nerve fiber degeneration	16.7	8.3	13.3
	Swelling of axis cylinders (R/L)[b]	8.3/8.3	66.7/66.7	0.0/0.0
	Schwann cell hyperplasia (R/L)	41.7/58.3	100.0/100.0	53.3/60.0

[a]This table was reproduced from the original text as submitted by registrant.
[b]R/L = right/left.

Butylate toxicology review

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