FXR-3070

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

JUL 6 1983

003070

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Memorandum

TO: Janet Remmers, Butylate Project Manager
Fungicide/Herbicide Branch
Registration Division (TS-767)

THRU: Christine F. Chaisson, Ph.D.
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Toxicology Branch (TS-769C)
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Chief Concurrence for CFC 7/6/83

SUBJECT: Toxicology Registration Standard for Butylate (Sutan)

Attached is the Registration Standard on Butylate. The §158.135, the generic toxicological requirements, is attached following the Introduction and Tolerance Reassessment Sections. After §158.135, the toxicology "one liners" are attached which summarize the results of the document is the data evaluation records (DER) of each toxicology study.

Butylate is conditionally recommended for continued registration. There are, however, a number of data gaps which should be filled as soon as possible. The recommendation for continued registration is dependent on the immediate initiation of the studies listed as data gaps. Especially important is the immediate fullfillment of the inhalation toxicity data gap.

A two-year rat study was submitted late and still is under Toxicology Branch review. I will communicate the results of this study, and any changes the study makes in the present toxicology position, which can be appended to the present Standard as an addendum.

J. W. Holder 7/6/83

James W. Holder, Ph.D.
Toxicology Branch
Hazard Evaluation Division
(TS-769C)

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REGISTRATION STANDARD

for

BUTYLATE (SUTAN)

James W. Holder, Ph.D.
Section IV
Toxicology Branch
Hazard Evaluation Division, OPP
TS-769C

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*** ATTACHMENTS ***

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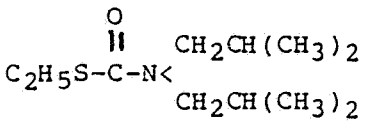
Introduction

Butylate

BUTYLATE, SUTAN, R-1910

Caswell Number: 434A



S-Ethyl diisobutylthiocarbamate

Butylate is a selective herbicide registered solely for use on corn. Approximately 43 to 59 million pounds of the active ingredient are used annually in the United States. Of the total domestic butylate use, 29% is applied as the single active ingredient, and the remaining 71% usage consists of combinations with atrazine and/or cyanazine. Application rates of the single active ingredient range from 3 to 6.14 lb ai/A. U.S. tolerance on corn was set on negligible residues, 0.1ppm.

Single active ingredient formulations of butylate consist of 1.33-10% G, 4 lb/gal Mcap, 6-7 lb/gal EC, and 85.1% EC. These formulations are applied with ground equipment and are generally soil incorporated immediately after application. Butylate may be injected into center pivot irrigation systems or butylate may be injected into soil before or after planting. Applicators need not be certified or under the direct supervision of applicators certified to apply butylate.

Tolerance Reassessment for Butylate

The LEL for butylate is based on a number of effects observed in the 2-year CD-1 mice study (Attachment F).

LEL: At 80 mg/kg/day feeding effects in the mouse kidney were amyloidosis, chronic nephritis and lymphocytic foci and in the mouse liver were cellular infiltrates and focal necroses. Also lower food consumption at 80 mg/kg/day was observed in female mice. It is concluded at this time that 80 mg/kg/day is the LEL for butylate.

No other effects from any other study reviewed to date

have more important toxicological effects that are as low or lower than the 80 mg/kg/day dose in two-year mouse study in Attachment F.

NOEL: The next lowest dose in the two-year mouse study, 20 mg/kg/day, for which these nonneoplastic lesions were not observed, is the NOEL for butylate. NOEL = 20 mg/kg/day.

At a NOEL = 20 mg/kg/day (=133 ppm) the ADI is calculated to be 0.20 mg/kg/day (1.33 ppm) with a 100-fold safety factor.

In order to compare present exposure to this established ADI, reference is made to 40 CFR Section 180.232:

> § 180.232 S-Ethyl diisobutylthiocarbamate; tolerances for residues.
> Tolerances are established for negligible residues of the herbicides S-ethyl diisobutylthiocarbamate in or on the raw agricultural commodities corn grain (including popcorn), fresh corn including sweet corn (kernels plus cob with husk removed), and corn forage and fodder (including sweet corn, field corn, and popcorn) at 0.1 part per million.

If butylate residues are assumed to be at tolerance levels for all corn products in the U.S. (food factor = 2.52%) and the percent of corn treated with the herbicide butylate is assumed to 100%, then the dietary burden (DB) can be calculated for an average U.S. citizen weighing 60 kg and eating 1500 g of food total per day:

DB = TMRC in average U.S. diet from corn products per unit body weight

$$DB = \frac{(0.1 \times 10^{-6}) \times (1.5 \times 10^{6} \text{ mg/day}) \times (.0252)}{60 \text{ kg}}$$

DB = 0.000063 mg/kg/day

The above calculation assumes that 100% of U.S. corn is treated with Butylate, and that this corn is consumed throughout the U.S. by individuals represented by an "average person" who eats 1.5 kg per day and weighs 60 kg, and that all corn products constitute 2.52% of the total dietary intake on the average throughout the the year. The calculation further explicitly assumes that there is no loss in Butylate residues in commercial processing of corn

products or in domestic preparation.

This DB calculation of 0.000063 mg/kg/day (= 3.8 micrograms/person/day) shows no more than .032% of the ADI (= 0.2 mg/kg/day) is used up by this Butylate use as a herbicide in U.S. corn production.

CONCLUSIONS

It is concluded that the present tolerance 0.1 ppm in or on corn adequately covers, at present, all toxicology concerns at this time.

There are no special pathological effects, acute or chronic, of Butylate exposure which would preclude the continued registration of Butylate in the U.S. at this time. It is understood that results from any of the many data gaps (below and pages 4 & 5) could change this statement.

In the 1968 reviewed acute studies by J.E. Schulz, congestion of liver and kidney was observed in those rats which died. The acute inhalation study showed all dose groups had similar types of congestions in the lungs. Further, a subchronic 3-week dermal study showed congestion in the kidneys and lungs at autopsy although no problems were observed histopathologically. No congestions were noted in either a subchronic rat or a subchronic dog study.

In light of the congestions (in highly blood-perfused organs) observed in the acute oral, acute inhalation, and subchronic dermal studies, Toxicology Branch requires that this pathologic effect be investigated thoroughly in the studies yet to be done on butylate (listed below) and that specific comments be addressed to this issue upon reporting the data of these studies.

Further, since congestion was observed in the acute inhalation study even at the lowest does group (8.7 mg/l, still in Category III range), it has been concluded that a no effect level has not been established for inhalation. Since the inhalation data is a data gap, and is insufficient at this time, Toxicology Branch recommends that a label restriction be placed on the label stating insufficient inhalation data on butylate exists and that a respirator is recommended when handling butylate formulations.

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Accordingly, Toxicology Branch recommends for the continued registration of Butylate, but only on the condition that the outstanding studies (below) be initiated as soon as possible and the results be submitted to the Agency for review and placement in the Butylate file as part of the butylate Standard.

DATA GAPS - STUDIES TO BE DONE ON BUTYLATE

1. Non-rodent 90-day feeding study of technical butylate.
2. Non-rodent chronic toxicity study of technical butylate.
3. A rabbit teratology study of technical butylate.
4. A rodent teratology study of technical butylate.
5. A two-generation reproduction study on technical butylate.
6. Appropriate mutagenicity studies.
7. Metabolism study to determine the fate of butylate labeled in the isolbutyl moiety.
8. Acute inhalation study with LD_{50} and observation for 2-weeks.
9. A two-year rat oncology study has been submitted and is still under review.

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* This data requirement is required by Toxicology Branch as soon as possible and should be accorded top priority by the registrant.

TOXICOLOGY GENERIC DATA REQUIREMENTS FOR BUTYLATE

Data Requirement	Composition and Use Pattern 1/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?
§158.135 Toxicology				
ACUTE TESTING				
81-1 - Oral LD_{50} - Rat	tech./A	yes	00063486	no
81-2 - Dermal LD_{50}	tech./A	yes	00063486	no
81-3 - Inhalation LC_{50} - Rat	tech./A	partially	00063488/	yes --> note 13
81-4 - Primary Eye Irritation - Rabbit	tech./A	yes	00063487	no
81-5 - Primary Dermal Irritation	tech./A	yes	00063487	no
81-6 - Dermal Sensitization	Not required for the use pattern ----------> Note 8			no
81-7 - Acute Delayed Neurotoxicity - Hen	Not required for this type of chemical--> Note 9			no
SUBCHRONIC TESTING:				
82-1 - 90-Day Feeding - Rodent,	tech./A	yes	00035843	no
nonrodent	none	no	00021846	yes --> note 1
82-2 - 21-Day Dermal	tech./A	yes	00026312	no
82-3 - 90-Day Dermal	Not required for this use pattern ----------> Note 10			no
82-4 - 90-Day Inhalation - Rat	Not required for this use pattern ----------> Note 11			no
82-5 - 90-Day Neurotoxicity - Hen and mammal	Not required for this type of chemica ---> Note 12			no
CHRONIC TESTING:				
83-1 - Chronic Toxicity - 2 spp. Rodent and Non-rodent (missing)	tech./A	partially	00035844	yes --> note 2
83-2 - Oncogenicity Study - 2 spp. Rat(missing) and mouse	tech./A	patially	00035844	yes --> note 3
83-3 - Teratogenicity - 2 species	_	partially	00026311	yes --> note 4
83-4 - Reproduction, 2-Generation	_	no	_	yes --> note 5
MUTAGENICITY TESTING:				
84-2 - Gene Mutation	_	no	_	yes --> note 6
84-2 - Chromosomal Aberration	_	no	_	yes --> note 6
84-2 - Other Mechanisms of Mutagenicity	_	no	_	yes --> note 6
SPECIAL TESTING:				
85-1 - General Metabolism	tech./A	partially	000436600 000436681 000436682	yes --> note 7

1/ Composition of the material to be tested is technical grade unless otherwise specified in footnotes. The use patterns are coded as follows: A=Terrestrial, Food Crop; B=Terrestrial, Non-Food; C=Aquatic, Food Crop; D=Aquatic, Non-Food; E=Greenhouse, Food Crop; F=Greenhouse, Non-Food; G=Forestry; H=Domestic Outdoor; I=Indoor.

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NOTES TO GENERIC REQUIREMENTS TABLE

Note 1 A non-rodent 90-day study is missing and should be done and submitted to the Agency. There is no chronic non-rodent study which could substitute for this requirement.

Note 2 A chronic mouse study statisfies the rodent requirement, but a non-rodent chronic toxicity study has not been submitted and is a data gap.

Note 3 The Agency has already reviewed a two-year mouse chronic feeding and oncology study (combined) and is currently reviewing a two-year rat study.

Note 4 The Agency has a rabbit teratology study which was categorized as supplementary data mainly because the dose did not go high enough. This rabbit study should be repeated using higher Butylate doses. Furthermore, another species other than the rabit should be tested for teratogenic effects.

Note 5 No reproduction studies on Butylate have been submitted to the Agency.

Note 6 No studies submitted. Refer to Pesticide Assessment Guidelines, Subdivision F, Series 84 (1982).

Note 7 This requirement is almost completed (see review in Butylate Standard, Attachment G). Completion of this requirement entails submission of ^{14}C-tracer studies on Butylate labeled at the C-1 position in the isobutyl moiety. Both kinetic and organ location with metabolite identification should be done.

Note 8 Dermal sensitization is required only if repeated contact with human skin is expected to occur. Use of Butylate is on corn, once a year, and by soil injection. Thus, the exposure is not expected to be repeated on human skin.

Note 9 Only organophosphate compounds are tested for acute delayed neurotoxicity.

Note 10 This 90-day dermal test is required only if pesticide is purposely applied to the skin or it is known that the pesticide is, in fact, metabolized differently in the skin than the oral route. Neither is the case here so 90-day dermal test is not required for Butylate.

Note 11 Use is not repeated inhalation since application of Butylate is only once/year.

Note 12 A 90-day neurotoxicity study is required if neuropathy is observed in the acute oral, dermal, and/or inhalation studies, or if the acute delayed neurotoxicity is positive (or a structurally related compound is positive for delayed neurotoxicity). Neither is the case for Butylate, so this test is not required.

Note 13 Neither the 1968 study nor the 1980 study were adequate to state the LC_{50} is greater than 5.0 mg/l. A new study must be initiated immediately to insure handler safety; a study of innalation to rats should done. The study should include an acute exposure at several doses up to 5 mg/l, observed letnalities, and pathology of all rats both gross and general and histological pathology.

ONE-LINERS OF BUTYLATE ACUTE STUDIES

EPA Chem. No. 434A

Study/Lab/Study #/Date	Material *	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.	MRID No.
Acute Oral LD_{50} - Rat Raltech #744849 09/28/79	S-Ethyl diisobutylthio-carbamate 85.71% (748-EGR)	244788	LD_{50} = 3.34 g/kg (M) 95% conf. = 3.04-3.62 g/kg LD_{50} = 3.0 g/kg (F) 95% conf. = 2.69-3.28 g/kg	III	Guideline 001201	00063486
Acute Dermal LD_{50} - Rabbit Raltech #733422 9/12/79	S-Ethyl diisobutylthio-carbamate 85.71% (748-EGR)	244788	LD_{50} >2 g/kg slight to severe erythema edema	III	Guideline 001201	00063486
Acute Inhalation LC_{50} - Rat Cosmopolitan Labs #3456-0334, 05/02/80	S-Ethyl diisobutylthio-carbamate 85.71% (748-EGR)	244788	LC_{50} >5 mg/liter (nominal concentration) & LC_{50} = 0.15 mg/l (analytical conc.)	not determined	Supplementary 001201	00063488
Primary Eye Irritation - Rabbit Raltech #733422 09/12/79	S-Ethyl diisobutylthio-carbamate 85.71% (748-EGR)	244788	Corneal opacity in 1/6 animals; iris irritation, redness, blanching chemosis. Corneal opacity still present by day 21 (unwashed eyes). No corneal opacity in washed eyes.	II	Guideline 001201	00063487
Primary Dermal Irritation - Rabbit Raltech #733422 09/12/79	S-Ethyl diisobutylthio-carbamate 85.71% (748-EGR)	244788	PIS was 1.9/8.0 slight to well defined erythema very slight edema.	III	Guideline 001201	00063487

* technical grade

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Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
21 Day Dermal - Rabbit Woodard Research Corp. 8/17/67	Technical	007101608 MRID 00026312	21 daily dermal application to normal or abraded skin: no systemic effects at 20 and 40 mg a.i./kg Local effects on skin - mild erythemia, some dryness and fissuring with sloughing		Minimum
21 Day Subchronic Feeding Study - Woodard Research Corp. - Rat 8/17/67	Technical	007101602 MRID Number 00026307	No hemotological or gross organ effects at doses 8, 16, 32 mg a.i./kg		Minimum
13 Week Subchronic Feeding - Woodard Research Corp. Dog 8/17/67	Technical	007101610 MRID Number 00026314	No effects in behavior, body weights neurological , opthalmological, hemotological, blood chemistries brain A ChE, or gross organ appearances or weight. No mortalities		Minimum
Teratology Study - Mouse Woodard Research Corp. 1967	Technical 97.6%	MRID Number 00026311	No maternal effects No fetal effects in natural or caesarian births at doses of 4, 8, 24 mg/kg No malformations observed related to dose		Supplementary
56 Week Feeding - Rat J.A. Trutter and F.E. Reno of Hazelton Research Labs. - Sprague-Dawley Rats 9/22/78	Technical	MRID Number 00035843 (Interim) 00021846 (Final)	Doses of 10 and 30 mg/kg produced no major effects; but at HDT 180 mg/kg liver pericholagitis, uterine and testicular changes with focal hemorrhage were all observed. Blood clotting parameters affected at lowest dose, 10 mg/kg/day		

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
24 Month Feeding Study Mouse - International Research and Development Corporation 1979	Technical	MRID # 00035844	No dose related oncological effects at any dose - HDT = 320 mg/kg/day LEL set on basis of Non-neoplastic effects: Liver, cellular infiltrates focal necrosis Kidney, amyloidosis, chronic nephritis, lymphocytic foci LEL = 80 mg/kg/day NOEL = 20 mg/kg/day		Minimum
Metabolism of ^{14}C - Butylate labelled at C-1 position in the ethyl or the isobutyl moeity Rat 1980	^{14}C-Sutan spiked Technical	MRID Number 00043680 00043681 00043682	Ethyl label - 66% in CO_2 in 24 hr. 12% urine, 2.7% feces Ehtyl methyl sulfoxide, ethyl methyl sulfone, ethane sultonic acid, ethane sulfinic acids found in the urine Conclusion: ethyl moeity of butylate rapidly enters the cullular oxidative process Isobutyl label: Incomplete data		Invalid
Mutagenicity - Genetics 83 54 1976	Not reported	MRID Number 05017208	No data given Butylate reported to have inncrease the number of sex linked recessives in Drosophila Melanguster.		Invalid

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Attachment A

Acute-oral, -dermal, and -inhalation lethality studies have been submitted to the Agency as well as acute eye and skin irritation studies. The first and older set of studies have been reviewed in 1968 by J.E. Schulz and appear in the Caswell file under file #434. These studies also appear as Attachment B. These studies generally show a low degree of acute toxicity except for acute inhalation. The acute inhalation studies did not indicate an accurate assessment of the dosing and did not establish a NOEL. Dr. Schulz requested the inhalation studies be repeated.

The summary of more recent studies (1979 & 1980) have been reviewed, Caswell file # 434, and are reproduced below. These studies also indicate a low degree of toxicity by the oral, dermal, eye routes; these toxicities were rated as Category III. The inhalation study did not rule out Category II toxicity insofar as the maximum analytical concentration attained was 0.15 mg/l (the Agency does not consider the nominal concentration a usefull parameter here). Therefore, the acute inhalation study is a data gap.

Because pulmonary congestion was observed in the earlier inhalation studies, the Agency recommends that verified doses up to at least 5 mg. a.i./liter of respirable particles of Butylate be administered by the inhalation route. Observations should be made for 14 days with all dying animals or animals sacrificed in extremis being autopsied with general histology, including lung, being done also. The same procedure should be followed on animals surviving the 14 day period.

ONE-LINERS OF BUTYLATE ACUTE STUDIES

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Primary Eye Irritation - Rabbit Raltech #733422 09/12/79	S-Ethyl diisobutylthiocarbamate 85.71% (748-EGR)	244788	Corneal opacity in 1/6 animal iris irritation, redne blanching chemosis. Cornea opacity still present by day 21 (unwashed eyes). No corneal city in washed eyes.	II	Guideline 001201	00063487
Primary Dermal Irritation - Rabbit Raltech #733422 09/12/79	S-Ethyl diisobutylthiocarbamate 85.71% (748-EGR)	244788	PII s 1.9/8.0 s : to well defined er, nema very slight edema.	III	Guideline 001201	00063487

Attachment B

JESholz:mw
March 1, 1968

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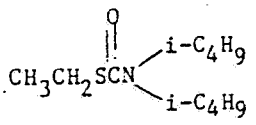
Product Name:	Sutan
Company Number:	R-1910
Chemical Name:	S-Ethyl Diisobutylthiocarbamate
Chemical Formula:	
Empirical Formula:	$C_{11}H_{23}OSN$
Molecular Weight:	217
State:	Liquid
Company	Stauffer Chemical Company
Tolerance:	Petition for a 0.1ppm negligible residue for corn (for seed use)
Use:	Herbicide

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Acute Oral (Rat)	:	LD_{50} = 4659(2864-7570) mg/kg for LD_{50} = 5431(3999-7384) mg/kg for Symptoms include depression, lacrimation and blood in urine. Pathology findings include congestion of liver, kidneys and adrenals with hemorrhage in lungs.
Acute Oral (Guinea Pig)	:	LD_{50} = 1659 mg/kg (1222-2260 mg/kg) for guinea pigs. Symptoms included depression and "prostration" prior to death. Gross pathology included congestion of liver and "lung erythema".
Acute Oral of Formulation (Rat)	:	LD_{50} = 4659 mg/kg (2864-7570 mg/kg) for rats fed 75% formulation LD_{50} - 5431 mg/kg (3999-7384 mg/kg) for rats. Symptoms included depression and weakness preceding death. Pathology included congestion of liver, kidneys and adrenals with hemorrhage in lungs.
Acute Dermal (Rabbit)	:	Mildly irritating at all levels (200, 632 and 2000 mg/kg) but reaction subsided after four days. One death at 632 mg/kg "unrelated to compound administration".
Acute Dermal of Formulation (Rabbit)	:	Severe dermal irritation caused by 4640 mg/kg application. Persistent at 14 days. No mortality pathologic finding.
Acute Inhalation (Rat)	:	Two deaths at the 19.0 mg/l (2 hours exposure) with "reddening and consolidation" in the lungs at all test levels. No effect level not established (lowest level in 8.7 mg/l). No LC_{50} determined.
Acute Irritation (Rabbit)	:	Technical product is not irritating to eye.

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Acute Eye Irritation of Formulation (Rabbit)	:	Mildly irritating with no evidence of damage after seven days.
Subacute Dermal (Rabbit)	:	No effect seen when 20 and 40 mgm/kg of active ingredient were applied 5 days/week for three weeks.
Subacute Oral (Rat)	:	No effect seen in rats on 8, 16 and 32 mgm/kg per day levels for 13 weeks.
Subacute Oral (Dogs)	:	No effect seen in dogs at 450, 900 and 1800 ppm dietary levels for 15 weeks.

Recommendations and Summary:

This material has a relatively low degree of toxicity. The compound appears to present no problem from either acute oral or dermal exposure.

The inhalation data presented did not show a no effect level and did not establish an LC_{50}. Even at the lowest level of exposure (8.7 mgm/l) there was evidence of significant lung pathology 14 days after the exposure. A crude estimate of the concentration applicators would be exposed to reveals a concentration of approximately .5-1 mgm/l. It is impossible to say from the data submitted whether this concentration will cause significant lung damage in humans. A repeat inhalation study should be done showing a no-effect level for this lung pathology. If this is not done, the label should warn against inhalation of the test material and recommend the use of a mask.

SUTAN

Acute Oral (Rat)

The technical product (S-ethyl diisobutyldithiocarbamate) was administered to the test animals via a stomach tube. The technical material was 97.4% pure and was fed to the animals at the dosage levels of 1,000, 2,150, 4,640, and 10,000 µl 1/kg. Five (5) male (weighing between 203 to 246 gms) and five (5) females (weighing between 192 to 209 gms) rats were used at each dosage level and were fasted overnight prior to the administration of the test material. The animals were observed for 14 days after the exposure to the compound.

Results:

At the lowest dosage level (1,000 µl/kg) there was no mortality and no toxic symptoms seen in the test animals. At the 2,150 µ/l test level there was no mortality observed. The female animals appeared normal throughout the observation period. The male animals appeared "to be slightly depressed" the day after the administration of the test material. At the 4,640 µ kg dosage level 3 of 5 male animals died, and 2 of 5 female animals died. These deaths occurred two days after administration of the compound. At the 10,000 µl/kg dosage level 4 of 5 female and 4 of 5 male rats died approximately 2 days after the administration of the compound. At both the higher dosage levels the animals exhibited depression, weakness, soft feces, lacrimation, and one female rat exhibited blood in the urine. The toxic symptoms started approximately 4 to 24 hours after dosage, and the 2 surviving animals appeared normal 6 days after dosage. The animals that

exhibited congested livers, kidneys and adrenals. There was also hemorrhage present in the lungs of the animals that died. Gross findings on the survivors were normal. No microscopic pathology or necropsy was done. The LD_{50} was concluded to be 4,659 mg/kg (with 95% confidence limits between 2,864 to 7,570 mg/kg). For the female rat the LD_{50} was determined to be 5,431 mg/kg with the 95% confidence limits between 3,999 to 7,384 mg/kg.

Acute Oral (Guinea Pig)

Male guinea pigs weighing between 231 to 350 gms were given single oral doses of the test material at the following levels: 464 µl/kg, 1,000 µl/kg, 2,150 µl/kg, 4,640 µl/kg. The animals were observed for 14 days after the administration of the test material.

Results:

At the lower two dosage levels (464 and 1,000 µl/kg) there was no mortality observed and all the test animals appeared to be normal throughout the observation period. At the 2,150 µl/kg dosage level four animals died during the 14 day period with one death at 2 days, 2 deaths at 3 days, and the fourth death on the 7th day. At the 4,640 µl/kg dosage level 4 of the 5 animals died on the second day and the 5th animal died on the third day after the administration of the compound. At the highest dosage levels the animals exhibited severe depression with gradual increasing weakness and prostration prior to death. After 4 days, the lone survivor at the 2,150 µl/kg dosage level appeared normal. The autopsy findings (gross autopsies only) included congested livers and "lung erythema". The surviving animals showed no gross pathogic pathology. The LD_{50} for male

guinea pigs was determined to be 1,659 mg/kg with the 95% confidence limits of 1,222 to 2,260 u/kg.

Acute Oral of Formulation (Rat)

Single oral doses of the formulation were given to male (206 to 230 gms) and female (185 to 218 gms) Sprague-Dawley rats and the animals were observed for 14 days for toxic effects. The formulation consisted of 78.25% active ingredient and 21.75%"inert ingredients".

Results:

At the 1,000 and 2,150 µl/kg dosage level there were no toxic effects seen in either the male or female rats and there was no mortality observed. At the 4,640 µl/kg dosage level one of 5 male animals died and 4 of 5 female animals died. These deaths occurred on the 2nd, 3rd and 7th days after the oral ingestion of the compound. For the animals at the 10,000 µl/kg dosage level there were 5 deaths in 5 male rats tested and 4 deaths in the 5 female rats tested. For the male rats all the animals died 2 days after the administration of the compound, and for the female rats the deaths occurred on the 2nd and 5th days after the ingestion of the compound. As was the case in the previous experiments, the animals exhibited depression and generalized weakness preceding death at the higher dosage levels. Gross autopsy findings included congested livers, kidneys and adrenals and also hemorraghic lungs. Gross pathology on the surviving animals was norma.. The acute LD_{50} for the formulation for male rats was determined to be 5,366 mg/kg (95% confidence limits between 3,951 through 7,296 mg/kg). For the female rats the LD_{50} was determined to be

3,874 mg/kg with confidence limits between 2,325 to 6,451 mg/kg.

Acute Dermal (Rabbit)

Two albino rabbits (age and sex not given) weighing between 2 to 3 kgs had the hair clipped from their trunk, and the test material was applied at the dosage levels of 200, 632, and 2,000 mg/kg in the liquid form. The area of application was wrapped with rubber and the test material was left in place for 24 hours after which time the area was washed and gently wiped. The reactions were observed for a total of 15 days and graded on a 1 to 4+ scale for erythema, edema, sloughing and necrosis. Gross autopsies were performed at the end of the observation period.

Results:

The dermal reactions consisted of erythema and edema and were classified as 2 to 3+ reactions which disappeared after 4 days. One rabbit died on the 13th day of the study that had received 632 mg/kg but this death was "apparently from causes unrelated to compound administration". The autopsy findings on this rabbit were not given in the report. The autopsies on the other rabbit that survived the 14 day observation period were negative. It was concluded the LD_{50} for the dermal application of the compound was greater than 2,000 mg/kg.

Acute Dermal of Formulation (Rabbit)

Two male and two female albino rabbits weighing between 2.2 to 2.5 kgs were given single dermal applications of 4,640 mg/kg of the test material applied to the closely clipped abdominal skin. The material was left in place for 24 hours after which time it was washed from the skin, and the

animals were observed for 14 days for dermal effects.

Results:

The individual reactions were not given in the report, but in general all animals exhibited severe erythema and edema with necrosis seen in all exposure areas. There was also desquamation of the surrounding tissue at the time of autopsy. There was no gross pathology present at the time of autopsy.

Acute Inhalation (Rat)

Five male and five female Charles River rats weighing between 194 and 382 gms were exposed to vapors of the test material (the formulation) at the following concentrations: 19.0 mg/l for 2 hours, 15.8 mg/l for 1 hour and 8.7 mg/l for one hour. The test material was vaporized using a "vaponephrine" nebulizer which made droplets ranging in size from one to five microns. The exposure chamber was 30 liters. The animals were observed for 14 days after the exposure, during which time they had food and water available ad lib. All animals that died and all animals that survived the observation period were examined grossly for pathology in the heart, liver, kidneys, lungs, gonads, and brain. The method of calculating the chamber concentrations was not given in the report.

Results:

At the highest dosage level (19.0 mg/l for 2 hours) there were 2 deaths observed from a total of 10 animals. One animal died 24 hours after exposure and the second animal died 2 days after exposure. Autopsy findings

on the 2 animals showed "congested lungs" in both animals. The surviving animals had difficulty breathing throughout the 14 day observation period. At the lower dosage levels (8.7 mg/l and 15.8 mg/l for one hour exposure) there were no deaths observed and the animals did not exhibit the difficulty in breathing seen in the rats at the higher dosage levels. The test animals at all dosage levels exhibited decreased body wieght gain for the 14 day observation period after the exposure to the test material. Absolute and relative organ to body weight ratios were calculated and there appeared to be no difference in the 3 test levels, however no control group was used for comparison. At autopsy animals at all dosage levels exhibited "reddening and consolidations in the lungs". This was present in 7 of 8 animals autopsied at the highest dosage level, 5 of 10 animals autopsied at the 15.8 mg/l dosage level and 3 of 5 male rats at the 8.7 mg/l level. It is thus obvious that a no effect level was not established for the lung damage done by the compound. Although an LC_{50} was not determined for the formulation, it could be assumed that the LC_{50} would be considerably below the level of 200 mg/l necessary to be classified as a Class II compound. The LC_{50} of the compound could be determined, and a no effect level should be established for the lung pathology (consolidation). If neither of these values are determined the label should be worded in such a way as to warn against dangers from inhalation, and possibly recommend the use of a mask when applying this compound.

Acute Eye Irritation (Rabbit)

Six albino rabbits had 0.1 milliliters of the test material placed in the

conjunctival sac of one of their eyes. The other eye was used as a control. The eyes were examined at 1, 24, 48 and 72 hours and at 5 and 7 days. The irritation was scored by the method of Draize and involved a numberical scale of 0 to 4 for irritation seen in the iris, cornea and conjunctiva. The maximum possible score using this scale would be 110.

Results:

There was minimal corneal irritation seen in 2 of the 6 test rabbits. There was no evidence in any of the rabbits of irritation to the iris. The conjunctiviae showed erythema in all the test animals and this was given a score of 1 or 2 on a 4 point system. There was also evidence of conjunctival edema in 4 of the 6 animals tested. All these reactions had subsided within 24 hours and the eyes remained normal from that time on. The maximum score achieved by any one rabbit was 11, and this had returned to 0 after 24 hours. The test material is therefore not irritating to the eye.

Acute Eye Irritation of the Formulation (Rabbit)

0.1 milliliters of the formulation (75% active ingredient) was instilled into the left conjunctival sac of 3 male and 3 female albino rabbits. The animals were observed for a total of 7 days for evidence of eye irritation.

Results:

As was the case in the previous study, the evidence of eye irritation was scored by the method of Draize. There was no evidence of irritation to cornea or iris in any of the test animals at any time during the 7 day

observation period. The conjunctivae showed erythema, edema, and discharge in all the test animals, and this persisted for a maximum of 4 days in one animal. All the animals were classified as having no evidence of eye irritation after 7 days. It was concluded that the test material was mildly irritating to the eye.

Subacute Dermal (Rabbit) (MRID No. 00026312)

Sixty New Zealand albino rabbits were divided into 3 groups of 10 male and 10 female rabbits per group, and acclimated for 3 weeks prior to administration of the test material. An area of 8 x 10 cm was clipped from the trunk of each rabbit, and one half the animals had small superficial abrasions made over the clipped area. 2.0 ml/kg was applied to each rabbit for 5 days a week for a total of three weeks. The 3 separate groups were a control group, a test group exposed to a 10% solution of the active ingredient, and a test group exposed to a 20% solution of the active ingredient. The test material was held in place by a gauze pad over which was placed a fiber glass screen that was taped in place. This was left in contact with the animal's skin for 24 hours after which the area was examined for evidence of dermal irritation. At the end of the study serum, erythrocyte, and brain cholinesterase activities were determined, heomgrams were taken, and gross autopsies were performed. Histologic examinations were made of the liver, kidneys, adrenal, thyroid, and the gonads.

Results:

There was no mortality observed in any of the test groups or in the con-

trol animals. Both the test group and the control animals gained a significant amount of weight during the three week test period. The food intake between the groups were comparable. There was no significant difference in the primary dermal irritation seen when the test group was compared with the control animals. The hematological values were similar for the test and control animals. The compound appeared to have no effect on erythrocyte, plasma, and brain cholinesterase activity. The gross necropsy findings showed a significant amount of congestion present in the kidneys and lungs in the test animals which was not present in the control group. Histopathological examination did not reveal any significant differences between the test and control group. Assuming the information given in the report is correct, applying 2 milliliters of a 10 and a 20% solution of the active ingredient would be equivalent to the daily application of 20 and 40 mgms/kg of the active ingredient.

Subacute Oral (Rat)

130 weanling albino rats (Charles River strain) were divided into 4 groups of 20 male and 20 female rats which were used as controls, 15 male and 15 female rats which were used as test animals at the following levels: 8, 16, 32 mgms/kg per day. The test material was mixed with acetone and incorporated into the animal's daily diet. This was adjusted 3 times during the 13 week study so that the mg/kg/a was maintained at a constant level. Periodic examinations were made of the animals and any changes in general appearance, behavior, survival, body weight gained, or food intake was recorded.

Hematological examinations were done and included hemoglobin, micro-hematocrit, coagulation time, and leucocyte counts on 5 male and 5 females at each of the test levels. Other blood chemistrys done terminally included blood glucose, prothrombin time, serum glutamic pyruvic transaminase, red and red blood cell and plasma cholinesterase determinations Gross autopsies were performed at the end of the study, and histopatholog examination was made of the heart, liver, kidneys, spleen, lungs, brain, gonads, adrenal, thyroid, pituitray, prostate, uterus, duodenum,urinary bladder, pancreas, mesenteric lymph node, bone marrow, stomach, skin, and peripheral nerve in 5 male and 5 female rats at each test level.

Results:

There appeared to be no significant difference between the control group and the test animals when they were compared using the perameters mentioned previously.

Subacute Oral (Dog)

Twenty-six purebred Beagle dogs (4 to 8 months of age) were pre-treated against distemper, hepatitis, leptosprirosis, rabies, and worms. The dogs were then divided into four groups of 4 male and 4 female dogs which were used as a control group, 3 male and 3 female dogs which were placed on an 1800 ppm dietary level of the test material, 3 male and 3 female dogs which were placed on a dietary level of 900 ppm, and 3 male and 3 female dogs which were placed on a dietary level of [illegible] ppm. The test material was mixed with a dry dog meal and was fed to the animals at the level of 200 gms/day accompanied with 45 gms/day of canned beef.

Periodic examinations were made of the weight, behavior, urine, stool, heart rate, respiratory rate, activity, blood pressure, and heart rate (by doing EKG's). Neurological and ophthalmolgical examinations were don at the beginning of the study and the termination of the study. Hematological values including hemoglobin, hematocrit, sedementation rate, coagulation time, thrombocyte counts and leucocyte counts were done initi and 4, 8, and 13 weeks. Blood chemistries including BUN, serum, alkalin phosphatase, blood glucose, prothrombin time, SGPT, SGOT, plasma, cholinesterase, and red blood cell cholinesterase were determined on the animal initially and at 4, 8, and 13 weeks. Brain cholinesterase determinations were done at the termination of the study. Urinalysis was done on each o the dogs at the same intervals as the blood chemistry determinations. At the end of the study (13 weeks) the dogs were sacrificed using sodium pentobarbital and autopsied. Gross and histopathological examination wa performed on the heart, lungs, liver, kidneys, spleen, thyroid, adrenal, prostate, uterus, gonads, pituitary, brain peripheral nerve, esophagus, small intestine, large intestine, stomach, pancreas, parotic gland, thymus, trachea, gall bladder, skeletal muscle, urinary bladder, mesente lymph node, femoral bone marrow, spinal cord, abdominal skin, eyes, aort and mammary gland. Organ to body weight ratio were also calculated for each of the above organs.

Results:

Using the perimeters mentioned, there appeared to be no difference when the control group was compared to the test animals. There was no mortal observed in any of the animals, and the test animals were comparable to the control group throughout the 13 week test period. Both gross and

microscopic necropsy findings were comparable for the two groups. One dog at the 450 ppm dietary level exhibited several symptoms that were extremely suggestive of an infectious process. This seemed to be unrelated to the chronic ingestion of the compound.

Attachment C

These three subchronic studies were originally reviewed by J.E. Schulz (3/4/68) and were re-reviewed by J.W. Holder (1/17/83). All three studies were classified as core minimum.

Subchronic Studies on Butylate

Study #1 - Dermal (Rabbit)

MRID No. 00026312
Authors: Woodard, M., Woodard, G., Cronin, M.
Date: 1967

Twenty-one Day Dermal Applications (daily) of technical butylate to New Zealand Rabbits. Three groups were treated: Control, 10% solution (= 20 mg a.i./kg) and 20% solution (= 40 mg a.i./kg) were tested.

Results were: No skin toxicological effects were observed when normal or abraded rabbit skin were treated at doses of 20 and 40 mg a.i./kg at 5 days/week for 3 weeks. Treated areas showed mild erythemia, dryness, fissuring, and sloughing. Gross necropsy findings show congestion present in kidneys and lung of treated rabbits, but no histological findings supporting any particular pathology was found.

o Study Classification: Core Minimum.

Study #2 - Oral (Rat)

MRID No. 00026307
Authors: Woodard, N., Woodard G., Cronin, M.T.
Date: 1967

Fifteen Charles River Rats/sex/dose group where fed 0, 8, 16, and 32 mg a.i./kg b.w. via the diet. The dosage in the feed was adjusted three times during the 3-week test period so as to keep the dosage rate constant per unit of body weight.

Hematological examinations at three weeks that were done: hemoglobin, microhematocrit, coagulation time, and leucocyte counts on 5 male and 5 females at each of the test levels. Other blood chemistries done included blood glucose, prothrombin time, serum glutamic pyruvic transaminase, red and red blood cell and plasma cholinesterase determinations.

Gross autopsies were performed at the end of the study, and histopathological examinations were made of the heart, liver, kidneys spleen, lungs, brain, gonads, adrenal, thyroid, pituitary, prostate, uterus, duodenum, urinary bladder, pancreas, mesenteric lymph node, bone marrow, stomach, skin and peipheral nerves in 5 male and 5 female rats at the each test level, i.e., 8, 16, and 32 mg/kg.

Results: There appeared to be no significant differences in the above parameters between the control group and the test animals in this subchronic study. No congestion was reported in this experiment as in the higher dose, acute oral rat study.

o Study Classification: Core Minimum

o Study #3 - Oral (Dog)

MRID No. 00026314.
Authors: Woodard, M.W., Woodard, G., and Cronin, M.T.I.
Date: 1967

Twenty six Beagle dogs (4 to 8 months of age) were tested for 13 weeks by the oral route. The dogs were divided into four groups: 4 male and 4 female dogs wheich were used as a control group, 3 male and 3 female dogs per group were placed on an 1800 ppm, 900 ppm, and 450 ppm dietary level of the test material. These doses are equivalent to 45, 22.5, and 11.25 mg/kg b.w.

The test material was mixed with a dry dog meal and was fed to the animals at an approximate level of 200 gms/day accompanied with 45 gms/day of canned beef.

Periodic examinations were made of the behavior, weight, urine, stool, heart rate, respiratory rate, activity, blood pressure, and heart rate. Neurological and ophthalmological examination were done at the beginning of the study and at the termination of the sutdy. Hematological values of hemoglobin, hematocrit, sedemantation rate, coagulation time, thrombocyte counts and leucocyte coundts were done at the start of the 13 week study and then at 4, 8, and 13 weeks. Blood chemistries including BUN, serum, alkaline phosphatase, blood glucoase, prothrombin time, SGPT, SGOT, plasma cholinesterase, and red blood cell cholinesterase were determined on the animals initially and 4, 8, and 13 weeks.

Brain cholinesterase determinations were done at the termination of the study. At the end of the study (13 weeks) the dogs were sacrificed and autopsied. Gross and histopathological examinations were performed on the heart, lungs, liver, kidneys, spleen, thyroid, adrenal, prostate, uterus, gonads, pituitary, brain peripheral nerve, esophagus, small intestine, large intestine, stomach, pancreas, parotid gland, thymus, trachea, gall bladder, skeletal muscle, urinary bladder, mesenteric lymph node, femoral bone marrow; spinal cord, abdominal skin, eyes, aorta, and mammary gland, organ to body weight ratios were also calculated for each of the above organs.

Results: There appeared to be no significant difference when the control group was compared to the test animals. There was no congestion reported as in the acute rat oral and 3 week dermal rabbit studies.

There was no deaths observed in any of the animals. Both gross and microscopic necropsy findings were comparabel for the two groups. One dog at the 450 ppm dietary level exhibited several symptoms but were symptoms seemed to be unrelated to the chronic ingestion of the compound.

This study was originally reviewed by J.E. Schulz in 1968 before studies were core classified. This subchronic dog study was re-reviewed by J.W. Holder (TOX Branch) on 1/17/83.

o Study Classification: Core Minimum

Attachment D

Study Type: Teratology

Title: R-1910 [butylate] Safety Evaluation by Teratological Study in the Mouse

MRID No.: 00026311

Sponsor: Stauffer Chemical Company

Contracting Lab: Woodard Research Corp., 12310 Pinecrest Road, Herdon, Virginia 22070.

Date Reported to Sponsor: April 26, 1967

Reported By: Robt. P. Beliles, Ph.D. and Geoffrey Woodard, Ph.D.

Test Material: Yellow liquid labeled technical R-1910 97.6%; lot P50-39105-5 received by cotnract lab. 7/21/66.

Protocol:

Charles River mice (strain no specified) were bred (ratio M:F unspecified). Groups set up were:

Group	1	2	3	4
Dose (mg/kg/day)	0	4	8	24
Number of mice	40	20	40	20
No. of Pregnancies	32	17	34	15
Percent impreganated	80%	85%	85%	75%

Dosing of butylate was by the diet from day six of post-vaginal plug formation (day zero) until termination of gestation (days 17.5 to 19.5).

Body weights of gravid mice were measured on days 6, 11, 15 and 18. At weighing the behaviors of the mice were noted.

On day 18 one-half of the females were separated, chloroformed, and fetuses were delivered by Caesarian section. The other one-half of the females were allowed to go to term and deliver the pups naturally. The delivery date to the nearest one-half day was recorded as well as, number of fetuses per litter, number alive, number dead, fetal weight, and any gross fetal alterations. The anomalies of the dam's visceral organs were observed and recorded after parturition.

One-half of the fetuses in each litter were taken for visceral examination and preserved in Bouin's fluid. These fetuses were examined grossly. The limbs were then removed and examined. The head was sectioned and examined for changes. The body cavities open and the visceral examined.

The other one-half of the fetuses were examined for skeltoneal malformations by clearing, which renders the viscera translucent, and then staining bone tissue with Alizarin Red S.

Results:

There no deaths in the gravid female mice in any of the dose groups, Caesarian or natural deliveries. No behavior alterations were noted. The weights of the dams, that were pregnant, did not vary when control and treated dams were compared at 6 weeks and 18 weeks. It is concluded there were no maternal toxicity in this experiment. There were no organ changes due to butylate in the dams (examined after delivery).

In the Caesarian deliveries the fetal data were:

CAESARIAN BIRTHS

DOSE (mg/kg)	No RESORBSIONS/ No. Pregnant Mice	No VIABLE FETUSES/ No. Pregnant Mice	FETAL WEIGHT (g) (+ S.D.)
0	27/17 = 1.59	179/17 = 10.53	1.18 ± .21
4	14/10 = 1.40	101/10 = 10.10	1.22 ± .37
8	5/14 = 0.36	170/14 = 12.14	1.12 ± .21
24	12/9 = 1.33	95/9 = 10.56	1.09 ± .26

It can be seen from these data that no dose related effects occurred in the number of resorbitions, number of viable fetuses, or fetal weights. The same can be said for the fetuses obtained from the natural births (data following).

NATURAL BIRTHS

Dose (mg/kg)	No. Resorbsions/ No. Pregnant Mice	No. Viable Fetuses/ No. of Pregnant Mice	Fetal Weight (g) (± S.D.)
0	6/15 = 0.40	178/15 = 11.86	1.45 ± .23
4	5/7 = 0.71*	72/7 = 10.29	1.52 ± .35
8	14/20 = 0.70*	202/20 = 10.10	1.48 ± .15
24	5/6 = 0.83*	54/6 = 9.00	1.45 ± .24

Note: These values are greater than control but are not considered biologically significant: (1) No. viable >> No. resorptions, (2) small numbered ratios can give false results, (3) there is no difference in the No. of dams with resorptions (one or more) among the 4 groups (6/15, 4/7, 6/20, 2/6).

Additionally, the number of dead fetuses per litter (Casesarin, 6/17, 6/10, 4/14, 4/9 and natural, 0/15, 0/7, 14/20, 1/6) did not vary with butylate dosages. There were no significant differences between Caesarian and Natural birth fetal data except natural fetal weights were heavier which is to be expected.

The percentage of males occurring in the litters at doses 0, 4, 3, and 24 mg/kg were 49.7%, 44.0%, 46.5% and 44.0%. These data show by observation no change in sex frequency patterns with butylate.

In the skeletonal examinations certain developmental effects of butylate were observed at the highest dose, 24 mg/kg. The effect was non-fused supraoccipitals (NFO):

DOSE: (mg/kg)	0	4	8	24
No. observed: (Caesarian)	90	52	87	49
No. with NFO (Caesarian)	7	7	3	13*
No. observed (Natural)	90	39	107	26
No. with NFO (Natural)	0	0	0	0

* $p < .005$, level of significance

The effect is obviously cleared up if natural delivery is allowed to take place. This effect is considered by this reviewer to be a mild fetotoxic effects of butylate.

The malformations in the skeleton and viscera that were found were:

Dose (mg/kg)	No. Examined*	Malformations	
		Skeletal	Visceral
0	178	2 with sternebrae"off" 2 with sternebrae not aligned	None
4	86	None	(2 cleft palate (1 ureter (undulated
8	186	1 sternabrae not fused properly	(6 cleft palate (2 eye open (1 left testicle (dark
24	75	None	None

* The data was not broken out as to occurrence/litter, but only occurrance/dose group.

In the skeleton there does not appear to any butylate induced malformations, and the cleft palates in the low and mid dose groups occur at low frequencies (2.3 and 3.2%) and do not occur at all in the high dose group. For these reasons, it is concluded butylate did not induce visceral malformations.

Butylate did not cause effects at 24 mg/kg, but was mildly fetotoxic at the level in the mouse.

It was not certain GLF was attended to in this experiment. Further, since the highest dose here, 24 mg/kg for mice, is so much lower than rat LD_{50}, 3000-3340 mg/kg, it is expected that high enough doses, approaching the MTD, were not employed in this mouse teratology study, and thereby reduces this study to usefull, but supplementary information. Thus, higher doses should be administered in a repeat mouse study.

o Study Classification: Supplemental.

Note: A second species was not tested for the teratogenic effects. Teratogenesis is a butylate data gap. That is, the mouse teratology study that should be repeated, and another species should be tested for teratogenic effects.

Attachment E

Subchronic/Chronic Feeding Study (56-weeks) of Butylate to Sprague-Dawley Rats*

MRID No.: 00035843 (interim report) and 00021846 (final report)
Authors: J.A. Trutter and F.E. Reno (Hazelton Labs.)
Date: September 22, 1978

A 56-week feeding study was conducted to evaluate the toxicity of butylate (Sutan Technical) when administered orally to Sprague-Dawley albino rats. Four tests groups, 60 rats/sex/group, received 0, 10, 30, and 90 mg/kg/day. The control group received the vehicle corn oil at 1%, by weight of the diet. At 15 weeks, the dosing regimen for 10 animals from the high dose groups was changed to 180 mg/kg/day.

Parameters monitored during the in-life phase of the study included observations of overt toxicity, morbidity and mortality, body weights, food consumption, hematology, coagulatin parameters, blood chemistry including cholinesterase, and urinalysis. Gross necropsy observations, organ weights, organ to body weight ratios, and histologic evaluations were conducted on 10 animals/sex/group at 18 and 47 weeks. Histopathology was done on all animals found dead or sacrificed in extremis as well as the control and high dose group. No histopathology was done on the low- (10 mg/kg/day) and mid-dose (30 mg/kg/day) groups. No explanation was given. No NOEL and LELs for histopathology could therefore be established in this study because the low-and mid-dose groups were not done. This is a major reason this study was ultimately classified as a study to be useful for substitution for a "90-day rodent study" in section 158.135.

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* This study reviewed originally by Richard Herbert, M.S. and Jan Kurtz, M.S. of the Dynamac Corporation. Secondary review was by J.R. Strange, Ph.D., Dynamac Corporation. The review presented here is by J.W. Holder, Toxicology Branch.

Results show mortality was not a problem with all groups showing good survivability.

Body weights were affected by butylate in males at $\geq$ 30 mg/kg/day during early growth (-1 to 17 weeks) and then again toward the end of the test (44-55 weeks). Females were also affected but at higher doses, i.e., $\geq$ 90 mg/kg/day. Food consumption was unaffected by butylate.

Organ weight (absolute) changes were observed in kidney (increase), liver (increase), heart (increase), brain (increase), NOEL for organ weight changes is equal to the low dose (10 mg/kg/day) is this, approximately one year, feeding experiment (56 weeks).

Hemotology results were normal and comparable to negative control rats in hematocrit, RCB, WBC, lymphocytes, segmented granulocytes, monocytes, eosinophils, basophils, and banded leukocytes both for males and females. However, at the highest dose tested (HDT), the hemoglobin was suppressed in the females. The NOEL for hemotology is 90 mg/kg/day.

Individual platelet counts and coagulation times were reported for 10 animals of each group at weeks 13, 18, and 46-47 (see Table on the following page). Fibrinogen, prothrombin times, activated partial thromboplastin times (APTT) and Russell's viper venom times (RVV) were reported for weeks 18 and 46-47. At 13 weeks, no significant differences were seen in platelet counts and coagulation times (Table 5).

At week 18, platelet counts and fibrinogen levels in all treated groups (except for fibrinogen in low dose males) were higher than control. The differences were significant ($p<0.05$) for the high dose groups only. the mean RVV value for mid-dose females at 18 weeks was significantly higher than the control values, but this did not appear to be treatment-related. No other significant differences were seen at this time. At week 46, platelet counts of treated male groups were comparable to controls. Fibrinogen levels at week 46 for males where higher in all treated groups, relative to controls, but the differences were not significant at this time. At week 47, APTT values for all treated female groups were higher than control values. The differences were significant in the low, mid, and very high dose (180 mg/kg/day) groups. There were significantly higher coagulation times and RVV values in low dose females at week 47, but these values did not appear to be treatment-related.

The changes in the hemostatic function, as evidenced by the various aberrations in the tests designed to measure potential disorders in bleeding or clotting times, do not describe a clearcut

Mean Coagulation Data for Rats Fed,
Via the Diet, Sutan Technical

These data were presented in MRID # 00021846, compiled by Dynamac, and reviewed by Dr. J. Strange of Dynamac

Time Interval (Weeks)	Sex	Dietary Dosage (mg/kg/day)	Platelets ($\times 10^3$)	Coagulation Time (sec.)	Prothrombin Time (sec.)	Activated Partial Thromboplastin Time (sec.)	Fibrinogen (mg/dl)	Russell's Viper Venom Time (sec.)
13	M	0	1066	50.4	--[a]	--	--	--
		10	1007	53.8	--	--	--	--
		30	893	59.1	--	--	--	--
		90	865	55.4	--	--	--	--
	F	0	931	65.8	--	--	--	--
		10	876	56.2	--	--	--	--
		30	891	65.2	--	--	--	--
		90	1026	66.1	--	--	--	--
	M	0	1072	74.9	15.19	19.57	90.0	20.73
		10	1241	81.1	15.69	20.15	80.0	17.33
		30	1166	66.1	16.21	20.55	115.0	20.56
		90	1369*	86.7	15.26	20.77	145.0*	16.98
	F	0	1040	63.1	13.41	20.31	50.0	14.09
		10	1102	64.3	13.82	20.34	75.0	15.77
		30	1200	69.5	13.36	20.03	83.3	16.67*
		90	1255*	64.0	13.16	20.27	170.0*	14.80
46	M	0	1356	131	18.58	22.06	155.0	5.91
		10	1346	114.2	17.67	20.05	200.0	6.07
		30	1277	104.5	17.57	23.27	260.0	6.05
		90	1184	108.4	19.83	24.47	250.0	6.16
		180	1187	96.5	20.72	23.20	275.0	6.22
47	F	0	1058	85.0	14.36	26.76	200.0	6.10
		10	1114	148.5*	11.99	20.19*	240.0	6.80*
		30	926	117.3	12.53	20.91*	210.0	6.33
		90	1087	87.23	13.63	21.60	165.0	6.17
		180	1344*	94.5	12.09	19.80*	125.0	6.21

[a] Not determined.

* Significantly different from control at $p < 0.05$.

hemopathy. For example, the transient elevations in platelets at the high dose in both sexes, the depression in all groups of the CF2 (prothrombin), as well as the scattered increases and decreases in clotting times are not descriptive of a specific hemopathy, i.e, no pattern is manifest.

The frequency and diversity of hemopathic responses suggest that the compound may either cause intermittent or transient changes. Therefore, considering all these factors, NOEL and LEL are not determinable from these data.

Clinical blood chemistries (SGPT, SGOT, BUN, Protein) were done at 13, 18, and 46-47 weeks of the feeding experiment. Some statistical differences were noted at $p = .05$, but none showed consistency with time or dose. This, the NOEL for blood chemistries is provisionally set at 180 mg/kg/day (HDT). An expanded set of chemistries should be done in the rat and thus is a butylate data gap for chronic oral exposure.

Cholinesterase determinations in erythrocytes and plasma were determined at 2, 4, and 13 weeks. Why the stidies were not done at mid and terminal dates was not explained by the Hazelton personnel. Nonetheless, at 2 weeks there was some depression in both males and fe male erythrocyte cholinesterase, but not plasma cholinesterase, at the mid (30 mg/kg/day) and high dose (90 mg/kg/day) levels. At 4 and 13 weeks, all values were comparable to controls, and it is believed the animals compensated at later times for earlier erythrocyte cholinesterase suppression. Thus, the NOEL is set at HDT, 180 mg/kg/day.

There were no compound related effects at 13 and 46 weeks in the urinalysis (pH, specific gravity, protein, bilirubin, ketones, and occult blood). All values appeared to be normal.

Only control and high dose groups were examined histopathologically. Tabulations and evaluation of histologic findings were reported for rats sacrificed at each of the interim sacrifices, the terminal sacrifice, and for unscheduled sacrifices and deaths.

Histologic findings of the lung, spleen, liver, and kidney were the most prevalent. Although higher in incidence, controls. Interstitial cell hyperplasia or tumors were not demonstrated in the testes of controls, while seven high dose males had these findings. Chronic interstitial prostatistis was present in 11 control males, the one mid-dose male examined, and 16 high dose males.

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There were no significant histological differences, which were toxicologically meaningfull and dose-related, in incidence between the controls and the treated dose groups for males or females.

Certain findings of interest, however, that were observed follow in discussion below.

Lung findings consisted of lymphoid hyperplasia, with a few observations of focal pneumonitis. Hemosiderosis of the spleen was commonly occurring, with a slightly higher incidence in females when compared to males.

Liver findings generally occurred with the same frequency in controls and high dose animals of both sexes; however, pericholang was more common in the high dose group for both sexes (control males-4, low dose males-1, high dose males-6; control females-4, high dose females-7). Other liver findings consisted of round cell aggregates in the hepatic parenchyma, necrosis, and a neoplastic nodule in one control female.

Examination of the kidneys revealed interstitial nephritis, interstitial lymphosytes, tubular changes, and chronic nephropathy with slightly higher incidence in males when compared to females. Females had a higher incidence of kidney related calcareous bodies. Papillary hyperplasia was reported for a high dose female and a renal tubular carcinoma was present in a low dose male.

Uterine findings were more common in high dose females than in controls. Uterine findings in the controls consisted of focal hemorrhage and idstended horns with one incidence of an endometria polyp, while three high dose females showed endometrial hyperplasi and two had endometrial polyps (control-1, high dose-5).

Males showed a higher incidence of change than females for the heart. Chronic myocarditis was much more common in males than females, and was elevated over control in both high dose groups (control males-12, high dose males-22, control females-5, high dose females-7). Interstitial hemorrhage of the heart was also more common in high dose males (control males-1, control females-5, high dose females-7). Interstitial hemorrhage of the heart was also more common in high dose males (control males-1, high dose males-3, females-0).

Interstitial changes in the testes and prostate were elevated in incidence for high dose males when compared to controls.

Since low and mid-dose group animals were not examined histopathologically, a NOEL or a LEL cannot be set. At the high dose (180 mg/kg/day) increases were demonstrated for the heart, liver (pericholangitis) and testes for males, and for the uterus and liver for females.

The provisional NOELs and LELs suggested by this 56-week study are:

56-WEEK STUDY

Parameter	Provisional NOEL (mg/kg/day) M	Provisional NOEL (mg/kg/day) F	Provisional LEL (mg/kg/day) M	Provisional LEL (mg/kg/day) F
Body Weights	10	30	30	90
Food Consumption	180	180	>180	>180
Hematology - Hemoglobin - Hematocrits WBC, RBC, Differential Leukocyte	180	90	>180	180
Clotting Parameters (overall)	ND	ND	ND	ND
Clinical Blood Chemistry	180	180	>180	>180
Cholinesterase*	180	180	>180	>180
Urinalysis	180	180	>180	>180
Organ Weights	10	10	30	30
Histopathology	Not determinable from data presented			

ND = note determined

* only 13 weeks, after which AChE was not done.

The inherent sensitive parameters in this 56-week feeding study are body and organ weights. This study was not conducted long enough to be considered a chronic study although it was conducted long enough to present usable information on the rat.

o Study Classification: Core-Supplementary.

Attachment F

Two-Year Combined Carcinogenicity & Feeding Study in CD-1 Mice*

(MRID No. 00025844)

A 24 month feeding study (submitted 1979) at International Research & Development Corp. was conducted for Stauffer Chemical Co. to evaluate the toxicity of butylate (Sutan Technical) when administered orally to CD-1 mice. Four test groups, 60 mice/sex group, received corn oil at 3 mg/kg of basal diet. Parameters monitored during the study included observations of overt toxicity, morbidity and mortality, body weight, food consumption, hematology, biochemistry, and urinalysis. Gross necropsy observations, organ weights, organ to body weight ratios, and histologic evaluation were conducted at 12 months (10 animals/sex/group) and at 24 months.

No recorded data were presented for individual superficial observations. Several incidental findings were reported in both the control and treated groups including tail lesions, corneal opacities, piloerection and eccentric pupils; while dark yellow urine was reported for animals in the high dose group.

Mortality data showed no significant differences between the control and treated groups for either sex; therefore, the NOEL for mortality was 320 mg/kg/day (HDT).

With respect to body weights, treated males showed no effect when compared to controls, while mid-dose female body weights were decreased when compared to controls. High dose females showed six incidences of statistically depressed body weights when compared to controls. Based on the significant body weight depression seen in females of the high dose group, the NOEL for body weight effects was 20 mg/kg/day (LEL = 80 mg/kg/day), while the body weight NOEL for males was 320 mg/kg/day (HDT).

Organ weight (absolute) and organ to body weight ratios (relative) showed an elevation of relative liver weights for all treated females at both intervals. A decrease was seen in both relative and absolute kidney weights for high dose males and females. Based on the statistical significance of liver and kidney effects in both sexes at the high dose, the NOEL for organ weights was 80 mg/kg/day (LEL 320 mg/kg/day).

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* This study was originally reviewed by Jan Kurtz, M.S., Dynamac Corporation (1/25/83). The review presented hereis by J.W. Holder, Toxicology Branch.

Food consumption data for males showed depression when compared to controls in the high dose group from weeks 27 to 86 but not at termination (104 weeks). Depressions in female food consumption occurred at the low dose from week 1-62, at the mid dose from week 14 to termination, and throughout the whole 104 week study for the high dose group. Based on the reversible effects on food consumption in high dose males, the NOEL was 80 mg/kg/day (LEL 320 mg/kg/day), while the NOEL for females was less than 20 mg/kg/day.

Hematology data at 12 or 24 months revealed no pattern of dose- or time related changes with either sex; therefore the NOEL for hematology was 320 mg/kg/day (HDT).

Clinical chemistry assays for treated female groups showed lower than control values for all parameters, excluding glucose, at the 24-month interval, while mid-and high-dose males showed values increased over controls for BUN, alkaline phosphatase, SGOT, and SGPT. None of the values, however, fell outside of predetermined ranges for the mouse. The NOEL for clinical chemistry is 320 mg/kg.

Urinalysis data revealed reduced urine volumes for treated male and female treated groups when compared to controls. High dose animals showed a slight evevation in specific gravity. High dose males showed a small reduction in pH when compared to controls. The effects seen are small, but fall into the normal ranges for mouse urine, and thus, the NOEL is set at 320 mg/kg/day (HDT).

Gross necropsy observations related to treatment were recorded for external, liver and kidney sites. External findings, primarily urine stained ventral surface, congestion and edema, and tail abnormalities, were higher in incidence for treated male and female groups when compared to controls. Liver findings, consisting of discolorations and nodules or masses, appear to be dose related in the males, with females showing an increased in incidence at the mid and high doses. Kidney observations, including cortical cysts, discoloration, and irregular shape and pitting, were increased above controls in all treated males as well as mid-dose females.

With respect to histologic evaluation, neoplastic lesions showed no difference in treated groups from controls for either sex. Therefore, Butylate does not appear to be carinogenic in the mouse.

Non-neoplastic lesions did demonstrate effects in the lung, liver, kidneys, and tail. Lung obserations included congestion and interstitial inflamatory infillrates at 12 months and atelecasis and aveolan cell hyperplasia, along with congestion at 24 months. Treated groups showed no greater incidence that controls, However.

Liver findings (cellular infiltrates and focal necrosis) where higher in incidence for the high dose groups of both sexes when compared to controls.

Kidney findings (amyloidosis, chronic nephritis and lymphocytic foci) were slightly higher in high dose males, and higher in both mid- and high-dose females when compared to controls. No kidney effects such as these were observed at the low dose (320 mg/kg/day).

Tail lesions, although not high in incidence, were more prevalent in high dose males and females when compared to controls.

Based on the dose-related effect seen in liver and kidney non-neoplastic lesions, the NOEL was 20 mg/kg/day for both sexes, while the LEL was 80 mg/kg/day. The latter non-neoplastic liver and kidney lesions with NOEL = 20 mg/kg/day represent the most sensitive (at lowest dose) pathologic effect observed in this 2-year study.

The 2-year mouse study was deficient in the following:

1. Compound storage or stability in feed was not tested (or reported).

2. The frequency at which Butylate was mixed with feed was not reported.

3. Ages of mice at study initiation was not reported.

4. Clinical chemistry values should have included Na+, K+, Ca#, and total protein values as well as other routine SMA analyses.

5. Organ weights for lung were not reported.

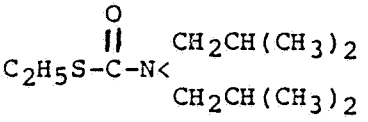
6. Complete histopathology (versus a partial list of tissues) should be been done for the low- and mid-dose groups.

o Study Classification: Core minimum

Attachment G

Metabolism of Butylate

The structure of butylate is:



S-Ethyl diisobutylthiocarbamate

Butylate is seen to be a thiocarbamate type of herbicide. Other names for this herbicide are Sutan™ and R-1910. Studies of butylate metabolism have used butylate labeled at the C-1 position in the isobutyl moiety, or at the C-1 position in the ethyl moiety. Both types of tracer studies will be described below.

[S-(1-^{14}C) ethyl]diisobutyl thiocarbamate

Bova, et al., (1978) followed the S-ethyl part of butylate in the rat [1]. Butylate (100 mg/kg) cleared rapidly from the rat; 81% was cleared in 24 hours distributed, approximately, 66% CO_2, 12.2% urine, and 2.7% feces. A total of 69% over 72 hours of the original ^{14}C dose was collected as CO_2; further, 61% of 69% CO_2 was expired in the first 6 hours after dosing. These later results suggest that the S-ethyl group is cleared from butylate and rapidly enters the cellular oxidative metabolic process.

Such is case as ^{14}C-urea and ^{14}C-hippuric acid were found in the urine [Thomas D.L.B., et al., (1980) Metabolism of (1-^{14}C-ethyl) Sutan in the Rat: Urinary Metabolite Identification, submitted by Stauffer Chemical Co., MRID No. 000436682]. Accordingly, ethyl methyl sulfoxide, ethyl methyl sulfone, ethane sulfonic acid, and ethane sulfinic acids were also found in urine.

All tissues assayed for (1-^{14}C-ethyl) Sutan did not show an excess of Sutan except for a slight excess in liver [Bova,

D.L. [1978] Metabolism of (1-^{14}C-ethyl) Sutan in Rat: Balance and Tissue Residues, submitted by Stauffer Chemical Company. MRID No. 000436682.

[^{14}C-1-isobutyl]-butylate Metabolism Study

When 100 mg/kg (as above) of butylate is administered where the ^{14}C-labeled is at the C-1 position in the isobutylate moiety, it was observed that the ^{14}-label was on the ethyl moiety [Thomas, D.B., et al., (1978) Metabolism of (1-^{14}C-Isobutyl) Sutan in the Rat. Balance and Tissue Residue Study. Submitted by Stauffer, MRID No. 00043680]. Thomas, et al., (1979) found that 99% was recovered in 24 hours. The distribution showed most of the label in the urine; 93.7% urine, 4.0% feces, 2% expired CO_2. After 72 hours less than 0.5% remained in the tissues with liver (as above) retaining only a slight excess of ^{14}C-label [3]. The chemical identity of meaolites in urine from this study were not reported but it was indicated Thomas that such a study was underway.

It is concluded from these metabolic studies that butylate (or Sutan) is absorbed quickly and the thiocarbamate structure is cleaved either to release ethyl mercaptan or [possibly] diisobutyl amine. In the first instance the ethyl mercaptan is oxidized via the Kreb cycle or the sulfur becomes progressively oxidized to sulfones, then to sulfinic acid, and then on to sulfonic acids. In the latter instance, identification and proof of the ^{14}C-metabolites from the [1-^{14}C-isobutyl]-butylate study is yet forthcomming and is identified as a data gap.

o Study Classification: Not valid

003070

Attachment H

Mutagenicity Study on Butylate

MRID No. 05017208
Genetics 83 54 (1976)

Author: Murnik, Mary Rengo, Western Illinois University, Macomb, Illinois.

The author has tested widely used herbicides for potential mutagenicity in Drosophila melanogaster. Herbicides tested included Butylate.

Butylate was reported, with no data presented, to have significantly increased the frequency of sex-linked recessive lethals in the fruitflies.

Tox views butylate mutagenciity to be a data gap. The present study abstract does not add to the toxicology profile for butylate since no data is presented.

o Study Classification: Unacceptable.

Butylate Standard Chronology:

1.) Study not presented in secondary review Feb 15, 1983 to Chad Sandusky & Wm. Burnam. Corrected in 2a
2.) Study corrected fo - secondary review 3/28/1987, Submitted to Christine Chaisson
3.) Standard returned from C.Chaisson secondary review 6/30/93 with request for 24-hour turn-around on