13000

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

APR 18 1995

011499

#434A

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: BUTYLATE:---Tox Data Submitted Under MRID 434522-01.

ID # 041405

PC Code
[redacted] 041405 (434A)
RD Record: S477473
DP Barcode : D209675

FROM: Irving Mauer, PH.D., Geneticist
Toxicology Branch-I
Health Effects Division (7509C)

03-03-95

TO: Linda Probst/Judith Loranger, PM # 73
Special Review and Reregistration Division (7508W)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I
Health Effect Division (7509C)

4/7/95

Registrant: Zeneca Ag Products, Wilmington, DE

Request: Review and evaluate the following subchronic neurotoxicity assay:

(82-7) Butylate: Subchronic Neurotoxicity Study in Rats, performed at Zeneca's Central Toxicology Laboratory (CTL), Cheshire (UK), Final Report No. CTL/P/4423 (of Study No. PR0970), dated September 02, 1994 (MRID No. 434522-01).

TB CONCLUSIONS/EVALUATION: This study is judged ACCEPTABLE in demonstrating no evidence of either structural or functional neurotoxicity in rats fed test article for 13 weeks at doses up to the HDT, 5000 ppm in the diet (providing compound intakes up to 336/mg/kg/da in males, 382 mg/kg/da in females), which caused 10% to 15% reductions in bodyweight and food consumption.

ATTACHMENT: DER

14

DISK 4: SIZE 3.5:209675:MAUER:MB

BUTYLATE (82-7) SUBCHRONIC NEUROTOXICITY

EPA Reviewer: Irving Mauer, PH.D. Date: 03-01-95
Immediate Office, Toxicology Branch-I (7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D. Date: 4/7/95
Immediate Office, Toxicology Branch-I (7509C)

DATA EVALUATION RECORD

MRID No.: 434522-01
PC No.: 041405
RD Record No.: S477473
EPA ID No.: 041405
Tox Chem. No.: 434A
Project No.: D209675

I. SUMMARY

STUDY TYPE: (82-7) Subchronic neurotoxicity screening battery - rat

CHEMICAL: Butylate

SPONSOR: Zeneca Ag Products, Wilmington, DE

TESTING FACILITY: Zeneca Central Toxicology Laboratory (CTL), Cheshire (UK)

TITLE OF REPORT: Butylate: Subchronic Neurotoxicity in Rats

AUTHOR: A. Brammer

STUDY NUMBER: PRO970 (Report No. CTL/P/4423)

DATE ISSUED: September 02, 1994

EXECUTIVE SUMMARY: Rats were fed test article for 13 weeks at dietary levels of 0, 250, 1000 and 5000 ppm, and assessed for neurological function (FOB, LA) monthly (Study Weeks 5, 9, 14). At termination, neural tissues were processed for gross and histopathological examination.

No evidence of either structural (organic) or functional (FOB/LA) impairment was found up to the HDT, which caused 10-15% reductions in bodyweight and food consumptions (actual mean intakes up to 366 mg/kg/day in males, and 382 mg/kg/day in females).

TB-I EVALUATION: ACCEPTABLE. This study satisfies data requirements for GDLN 82-7 (Subchronic Neurotoxicity)

BUTYLATE (82-7) SUBCHRONIC NEUROTOXICITY

II. DETAILED REVIEW

011499

A. TEST MATERIAL: Butylate

Description:	Amber-colored liquid
Batches (Lots):	Y06370/007
Purity (%):	95.7%
Solvent/carrier/diluent:	Incorporated in feed (diet)

B. TEST ORGANISM: Rodent

Species:	Rat
Strain:	Alpk: APfSD
Age:	28 days
Weights - males:	209-211 g
females:	164-168 g
Source:	Zeneca's (SPF) Animal Breeding Unit, Cheshire (UK)

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the neurotoxic potential of the test article when administered in the diet to male and female rats for 90 days, and recording any functional changes in a battery of tests (FOB), and/or histoneuropathological lesions, according to established (published) procedures and FIFRA Test Guidelines.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice (GLP) was provided.

D. PROCEDURES/METHODS OF ANALYSIS: Following two weeks' acclimatization, groups of animals (12/sex/dose) were fed diets containing 0, 250, 1000 and 5000 ppm butylate[1] for 13 weeks. Animals were observed daily, weighed weekly, and quantitative assessments of neurological function (functional observation battery, FOB[2] and locomotor

---

[1]NB: The dose levels for this study were stated to have been selected on the basis of a "preliminary dietary study conducted in this laboratory", but neither bibliographic reference to which, nor results, were included in this Final Report.

[2]FOB:

Landing foot splay	Piloerection	Ptosis
Tail-flick test	Exophthalmus	Convulsions/tremors
Fore-/hind-limb grip	Urination	Abnormal reactivity to stimuli
Lachrymation	Defecation	Level of arousal
Salivation	Pupillary function	Alterations in sensimotor responses/respiration

activity (LA)[3] were monitored during the week prior to start of feeding test article, as well as during Study Weeks 5, 9 and 14.

At study termination, one-half of the animals from each group, i.e., six so "designated" per sex per dose group, were anesthetized, and perfusion-fixed with Karnovsky's Fluid; the remainder were exsanguinated, their brains removed, and kept on ice for biochemical analysis (cholinesterase, etc.). From perfusion-fixed animals, neuromuscular tissues (brain; spinal cord and roots; dorsal root ganglia; Gasserian ganglia; sciatic, sural and tibial nerves; gastrocnemius muscle) were removed, and prepared for histological examination as follows: (1) Right-hand portions were dehydrated in alcohol, cleared in toluene, embedded in paraffin wax, sectioned at one micron, and stained with alum hematoxylin/eosin; (2) the remaining (left-hand) portions were post-fixed in osmium tetroxide, dehydrated and cleared in acetone, embedded in resin, and "semi-thin" sections stained with toluidine blue. Only control (Group 1) and high-dose (5000 ppm) perfusion-fixed animals were actually examined microscopically.

At termination, plasma and erythrocyte cholinesterase activities were determined in exsanguinated blood of six rats/sex/dose group; as well, the left half of the (above) ice-cooled brains were submitted for cholinesterase determination, while the right halves were analyzed for "neuropathy" (neurotoxic) target esterase (NTE).

Resultant data were analyzed statistically, separately for males and females, as follows:

Analysis of Covariance: For body weights; brain weight, length and width.

Analysis of Variance (ANOVA): For food consumption and utilization
-motor activity measurements
-time-to-tail flick
-landing foot splay
-grip strength
- neuropathy target esterase and cholinesterase activities

[3] LA: By means of an automated activity recording apparatus; each observation period divided into ten scans of five minute duration each.

Differences from control values were tested by Student's "t"-test (two-sided).

E. RESULTS: Determination of test article in diets used in this study were considered satisfactory in terms of concentration (mean within 4% of nominal/range, 1% to 7% - Report Tables 2, 3); homogenicity (3% - Report Table 4), and stability (within the period of repeat preparation - Report Table 5).

There were no treatment-related deaths, and no apparent clinically significant adverse effects in butylate-treated animals (Report Table 9). One mid-dose (1000 ppm) female (F83) was sacrificed during Week 13 "due to the presence of an abscess." Both high-dose (5000 ppm) males and females lost weight throughout the study, maximally 9% and 12%, as did 1000 ppm females (up to 7%), but not mid-dose males (Report Table 6; Figs. 1-4). Comparable reductions in food consumption (10-20%) were noted in both high-dose groups as well as mid-dose females, greatest (15-25%) during Study-Week 1 (Report Table 7), suggesting to the investigator, a "palatability effect." A smaller (non-significant) reduction was also noted during Study Week 1 in mid-dose males. Reductions in food utilization were statistically significant in affected female groups only (Report Table 8).

The actual intakes of test article were calculated in terms of mg butylate/kg body weights/day, and revealed a rapid decline during the study, due to "period of rapid growth to week 5" (Report APPENDIX I; Fig. 5). Mean doses of butylate received were 18.7, 76.0 and 366.1 mg/kg/day for the low, mid and high-dose male groups; 21.5, 80.6 and 382.5 mg/kg/day for the comparable female groups.

There were no observable treatment related effects in any dose group in any of the FOB tests (Report Tables 10/landing-foot splay; 11/time-to-tail flick; 12/grip strength), nor in LA measurements (Report Table 13/Fig. 6-9). Additionally, there were no compound-related changes recorded in brain, plasma or erythrocyte cholinesterase activities (Report Table 14[4]), nor in neuropathy target esterase activity (Report Table 15). Brain measurements (weight, length, width) in treated groups were no different from controls (Report Table 16), and no macroscopic (Report Table 17) or microscopic (Report Table 18) changes from controls were discernible in treatment groups.

Since the HDT, 5000 ppm, was fairly close to, if not at an MTD, as indicated by the significant weight loss at that level, the

[4]The singular statistically significant increase in the female high-dose (5000 ppm) mean erythrocyte cholinesterase activity was discounted by the investigator as "of no biological significance".

investigator concluded there was no evidence of neurological or structural impairment of the nervous system, and the assay providing a NOEL for neurotoxicity > 5000 ppm.

F. TB EVALUATION: ACCEPTABLE.

ATTACHMENTS: Report Tables

BUTYLATE (82-7) SUBCHRONIC NEUROTOXICITY

Disk 4:Size 3.5: 434522.01:MAUER:MB

BUTYLATE : SUBCHRONIC NEUROTOXICITY STUDY IN RATS

TABLE 2

ACHIEVED DIETARY CONCENTRATION SUMMARY

Group No.	Nominal Concn. (ppm)	Number of batches analysed	Mean Concn. (ppm)	% of Nominal Concn.	Concn. range (ppm)
1	Control	4	ND		
2	250	4	258	103.2	246 - 267
3	1000	4	967	96.7	879 - 1014
4	5000	4	5070	101.4	4710 - 5318

Limit of detection 15ppm ND - not detected

BUTYLATE : SUBCHRONIC NEUROTOXICITY STUDY IN RATS

TABLE 3

ACHIEVED DIETARY CONCENTRATION

Preparation Date	Group No.	Nominal Concn. (ppm)	Analysed Concn. (ppm)			Mean. Concn. (ppm)	% of Nominal Concn.
10/11/93	1	Control	ND				
	2	250	265	254	267	262	104.8
	3	1000	1011	1010		1011	101.1
	4	5000	5460	5270	5223	5318	106.4
22/11/93	1	Control	ND				
	2	250	256	253		255	102.0
	3	1000	1009	1018		1014	101.4
	4	5000	5305	5322		5314	106.3
15/12/93	1	Control	ND				
	2	250	246	246		246	98.4
	3	1000	944	985		965	96.5
	4	5000	4850	5027		4939	98.8

Limit of detection 15ppm ND - not detected.

BUTYLATE : SUBCHRONIC NEUROTOXICITY STUDY IN RATS

TABLE 3

ACHIEVED DIETARY CONCENTRATION

Preparation Date	Group No.	Nominal Concn. (ppm)	Analysed Concn. (ppm)		Mean. Concn. (ppm)	% of Nominal Concn.
4/ 2/94	1	Control	ND			
	2	250	249	285	267	106.8
	3	1000	851	906	879	87.9
	4	5000	4698	4722	4710	94.2

Limit of detection 15ppm ND - not detected.

BUTYLATE : SUBCHRONIC NEUROTOXICITY STUDY IN RATS

TABLE 4

HOMOGENEITY OF BUTYLATE IN DIET

Preparation Date: 10/11/93 30kg batch size

Nominal Concn. (ppm)	Sampling Point	Analysed Concn. (ppm)			Mean Concn. (ppm)	Overall Mean Concn. (ppm)	% Deviation
250	BOTTOM	287	287	283	286		-11.7
	MIDDLE	351	343	237	310		-4.3
	TOP	373	381	377	377	324	+16.4
5000	BOTTOM	7021	6889	7194	7035		+28.0
	MIDDLE	5399	5895	5168	5487		-0.2
	TOP	4084	3835	3996	3972	5498	-27.8

% Deviation = Deviation of mean concentration from overall mean concentration.

BUTYLATE : SUBCHRONIC NEUROTOXICITY STUDY IN RATS

TABLE 4

HOMOGENEITY OF BUTYLATE IN DIET

Preparation Date: 22/11/93 30kg batch size

Nominal Concn. (ppm)	Sampling Point	Analysed Concn. (ppm)		Mean Concn. (ppm)	Overall Mean Concn. (ppm)	% Deviation
250	BOTTOM	229	248	239		-1.6
	MIDDLE	248	239	244		+0.4
	TOP	249	244	247	243	+1.6
5000	BOTTOM	5083	4994	5039		-2.2
	MIDDLE	5122	5176	5149		+0.0
	TOP	5262	5265	5264	5151	+2.2

% Deviation = Deviation of mean concentration from overall mean concertration.

BUTYLATE : SUBCHRONIC NEUROTOXICITY STUDY IN RATS

TABLE 4

HOMOGENEITY OF BUTYLATE IN DIET

Preparation Date: 15/12/93

15kg batch size

Nominal Concn. (ppm)	Sampling Point	Analysed Concn. (ppm)		Mean Concn. (ppm)	Overall Mean Concn. (ppm)	% Deviation
250	BOTTOM	255	249	252		+0.4
	MIDDLE	259	255	257		+2.4
	TOP	243	246	245	251	-2.4
5000	BOTTOM	5005	4981	4993		+0.8
	MIDDLE	4904	4927	4916		-0.8
	TOP	4977	4935	4956	4955	+0.0

% Deviation = Deviation of mean concentration from overall mean concentration.

BUTYLATE : SUBCHRONIC NEUROTOXICITY STUDY IN RATS

TABLE 5A

CHEMICAL STABILITY OF BUTYLATE IN DIET AT ROOM TEMPERATURE

Nominal Concn. (ppm)	Preparation Date	Analysis Date	Analysis Interval (Days)	Analysed Concn. (ppm)		Mean Concn. (ppm)	% of Initial Concn.
250	22/11/93	23/11/93	0	256	253	255	100.0
		30/11/93	7	215	217	216	84.7
		7/12/93	14	219	215	217	85.1
		9/12/93	16	230	225	228	89.4
	15/12/93	15/12/93	0	246	246	246	100.0
		18/12/93	3	245	245	245	99.6
		21/12/93	6	248	249	249	101.2
		9/ 2/94	56	248	248	248	100.8
5000	22/11/93	23/11/93	0	5305	5322	5314	100.0
		30/11/93	7	4782	4754	4768	89.7
		7/12/93	14	4992	4819	4906	92.3
		9/12/93	16	4877	4670	4774	89.8
	15/12/93	15/12/93	0	4850	5027	4939	100.0
		18/12/93	3	5059	4962	5011	101.5
		21/12/93	6	4742	4916	4829	97.8
		9/ 2/94	56	4297	4459	4378	88.6

BUTYLATE : SUBCHRONIC NEUROTOXICITY STUDY IN RATS

TABLE 5B

CHEMICAL STABILITY OF BUTYLATE IN DIET AT -20°C

Nominal Concn. (ppm)	Preparation Date	Analysis Date	Analysis Interval (Days)	Analysed Concn. (ppm)		Mean Concn. (ppm)	% of Initial Concn.
250	22/11/93	23/11/93	0	256	253	255	100.0
		30/11/93	7	214	214	214	83.9
		7/12/93	14	222	235	229	89.8
		9/12/93	16	229	228	229	89.8
		21/12/93	28	231	241	236	92.5
	15/12/93	15/12/93	0	246	246	246	100.0
		9/ 2/94	56	263	249	256	104.1
5000	22/11/93	23/11/93	0	5305	5322	5314	100.0
		30/11/93	7	4991	4744	4868	91.6
		7/12/93	14	4782	4897	4840	91.1
		9/12/93	16	4913	4679	4796	90.3
		21/12/93	28	4440	4471	4456	83.9
	15/12/93	15/12/93	0	4850	5027	4939	100.0
		9/ 2/94	56	4663	4637	4650	94.1

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS

GLOSSARY FOR STATISTICAL TABLES

Key to results of statistical tests:

** Statistically significant difference from the control group mean at the 1% level (Student's t-test, two-sided).

* Statistically significant difference from the control group mean at the 5% level (Student's t-test, two-sided).

The following results were excluded from statistical analyses :

Table 6	Bodyweights	Male 15 , Week 3.
Table 16	Brain Parameters	Female 61 , brain weight.

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 6
INTERGROUP COMPARISON OF BODYWEIGHTS (g) - MALES

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Week 1	MEAN	211.4	211.7	209.1	211.1
	S.D.	9.7	13.4	10.2	14.7
	N	12	12	12	12
Week 2	MEAN	263.3	263.3	257.7	248.1**
	S.D.	13.4	17.8	10.9	18.0
	N	12	12	12	12
Week 3	MEAN	308.6	312.8	304.1	288.8**
	S.D.	18.0	17.3	12.4	18.5
	N	12	11	12	12
Week 4	MEAN	343.9	348.3	341.8	321.3**
	S.D.	19.5	20.6	15.6	18.5
	N	12	12	12	12
Week 5	MEAN	376.6	380.9	373.8	350.6**
	S.D.	20.6	22.0	19.8	22.5
	N	12	12	12	12
Week 6	MEAN	399.7	407.9	400.6	374.0**
	S.D.	23.9	27.3	23.7	23.1
	N	12	12	12	12
Week 7	MEAN	427.3	430.8	426.7	396.9**
	S.D.	28.6	28.3	24.9	27.9
	N	12	12	12	12
Week 8	MEAN	446.8	449.0	445.0	408.9**
	S.D.	28.4	27.7	28.7	27.3
	N	12	12	12	12
Week 9	MEAN	464.8	469.6	463.3	424.7**
	S.D.	33.0	29.0	32.5	29.5
	N	12	12	12	12
Week 10	MEAN	484.3	485.2	483.8	443.8**
	S.D.	36.5	30.8	34.9	30.6
	N	12	12	12	12
Week 11	MEAN	497.3	499.3	496.7	454.6**
	S.D.	38.8	33.8	39.0	32.8
	N	12	12	12	12
Week 12	MEAN	512.0	519.1	512.8	468.3**
	S.D.	40.8	38.4	42.3	38.1
	N	12	12	12	12
Week 13	MEAN	517.9	527.3	523.0	474.0**
	S.D.	38.1	38.7	42.7	41.4
	N	12	12	12	12
Week 14	MEAN	523.4	536.4	529.9	479.6**
	S.D.	37.9	39.7	45.1	42.0
	N	12	12	12	12

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 6
INTERGROUP COMPARISON OF BODYWEIGHTS (g) - FEMALES

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Week 1	MEAN	167.9	164.3	164.8	167.6
	S.D.	5.8	9.7	7.9	12.4
	N	12	12	12	12
Week 2	MEAN	192.0	186.8	183.0*	178.5**
	S.D.	10.9	10.1	8.9	11.0
	N	12	12	12	12
Week 3	MEAN	209.4	202.8	199.2*	194.8**
	S.D.	13.4	9.6	7.8	13.6
	N	12	12	12	12
Week 4	MEAN	224.1	217.8	207.6**	204.4**
	S.D.	12.0	14.4	8.6	15.3
	N	12	12	12	12
Week 5	MEAN	234.3	228.5	215.3**	214.2**
	S.D.	11.8	13.0	8.0	14.8
	N	12	12	12	12
Week 6	MEAN	247.0	241.5	228.9**	222.4**
	S.D.	13.9	13.5	8.0	17.6
	N	12	12	12	12
Week 7	MEAN	260.3	250.7	238.7**	229.2**
	S.D.	15.7	11.1	9.9	16.8
	N	12	12	12	12
Week 8	MEAN	259.8	255.0	241.6**	234.1**
	S.D.	13.6	13.2	11.4	19.7
	N	12	12	12	12
Week 9	MEAN	264.8	257.8	244.9**	235.9**
	S.D.	13.1	12.5	11.7	17.2
	N	12	12	12	12
Week 10	MEAN	273.1	267.3	253.3**	242.7**
	S.D.	15.6	11.4	9.2	19.7
	N	12	12	12	12
Week 11	MEAN	273.7	269.6	257.0**	244.2**
	S.D.	13.4	12.1	12.0	19.7
	N	12	12	12	12
Week 12	MEAN	276.7	279.4	259.3**	248.5**
	S.D.	12.2	13.9	13.7	19.6
	N	12	12	12	12
Week 13	MEAN	282.7	277.2	261.9**	252.5**
	S.D.	15.8	12.3	11.9	18.8
	N	12	12	12	12
Week 14	MEAN	285.4	277.8	263.9**	251.3**
	S.D.	17.5	12.3	11.2	19.3
	N	12	12	11	12

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 7
INTERGROUP COMPARISON OF FOOD CONSUMPTION (g/RAT/DAY) - MALES

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Week 1	MEAN	28.8	28.2	27.6*	24.5**
	S.D.	1.2	1.8	1.1	1.0
	N	3	3	3	3
Week 2	MEAN	30.9	30.7	29.9	27.6**
	S.D.	1.8	1.3	1.1	0.2
	N	3	3	3	3
Week 3	MEAN	30.6	30.9	29.8	27.3**
	S.D.	1.0	1.6	0.8	0.4
	N	3	3	3	3
Week 4	MEAN	31.9	31.5	30.8	28.3**
	S.D.	1.1	2.6	1.6	0.7
	N	3	3	3	3
Week 5	MEAN	29.5	29.8	29.6	26.9
	S.D.	0.3	2.6	1.1	0.8
	N	3	3	3	3
Week 6	MEAN	30.2	29.7	30.4	27.8
	S.D.	0.7	3.0	1.4	1.3
	N	3	3	3	3
Week 7	MEAN	29.7	29.0	29.6	26.5*
	S.D.	0.4	1.9	1.3	1.5
	N	3	3	3	3
Week 8	MEAN	29.2	28.7	29.1	25.9*
	S.D.	0.9	2.4	1.1	1.5
	N	3	3	3	3
Week 9	MEAN	29.7	28.3	29.5	26.3*
	S.D.	1.5	2.4	1.2	1.1
	N	3	3	3	3
Week 10	MEAN	30.0	29.7	30.3	27.0*
	S.D.	1.3	1.8	1.4	1.5
	N	3	3	3	3
Week 11	MEAN	30.4	30.4	30.6	27.4*
	S.D.	0.8	2.3	1.4	1.6
	N	3	3	3	3
Week 12	MEAN	29.8	30.0	31.1	26.9*
	S.D.	0.4	2.0	1.5	1.4
	N	3	3	3	3
Week 13	MEAN	29.9	30.1	31.1	26.9*
	S.D.	0.4	2.2	1.6	1.1
	N	3	3	3	3

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 7
INTERGROUP COMPARISON OF FOOD CONSUMPTION (g/RAT/DAY) - FEMALES

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Week 1	MEAN	21.5	21.4	19.0**	16.6**
	S.D.	0.5	1.1	1.2	0.6
	N	3	3	3	3
Week 2	MEAN	21.6	20.7	19.3*	19.0**
	S.D.	0.7	1.5	0.3	0.8
	N	3	3	3	3
Week 3	MEAN	21.2	21.0	18.1**	17.4**
	S.D.	0.9	2.0	0.7	0.9
	N	3	3	3	3
Week 4	MEAN	21.9	21.2	18.8**	17.5**
	S.D.	1.4	1.2	1.1	0.7
	N	3	3	3	3
Week 5	MEAN	20.6	20.2	18.0**	16.5**
	S.D.	1.7	1.9	0.9	1.2
	N	3	3	3	3
Week 6	MEAN	21.9	21.0	19.0**	17.2**
	S.D.	1.2	1.9	1.1	1.0
	N	3	3	3	3
Week 7	MEAN	20.9	20.6	18.2**	16.6**
	S.D.	0.3	2.2	0.8	0.7
	N	3	3	3	3
Week 8	MEAN	19.9	19.2	17.6**	15.7**
	S.D.	0.4	1.4	0.5	0.6
	N	3	3	3	3
Week 9	MEAN	20.1	19.6	17.6*	15.9**
	S.D.	1.4	1.2	0.1	1.2
	N	3	3	3	3
Week 10	MEAN	20.7	20.6	18.3**	17.0**
	S.D.	1.6	1.8	0.8	1.5
	N	3	3	3	3
Week 11	MEAN	19.8	21.1	17.6**	16.3**
	S.D.	0.8	2.0	0.6	1.3
	N	3	3	3	3
Week 12	MEAN	20.6	19.7	18.1**	16.5**
	S.D.	1.0	1.4	0.5	1.4
	N	3	3	3	3
Week 13	MEAN	20.8	19.5	17.9**	16.6**
	S.D.	1.1	1.7	0.6	1.7
	N	3	3	3	3

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 8
INTERGROUP COMPARISON OF FOOD UTILISATION (g GROWTH / 100g FOOD)

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Males					
Weeks 1-4	MEAN	19.3	20.0	19.9	18.5
	S.D.	1.8	1.1	0.5	2.1
	N	3	3	3	3
Weeks 5-8	MEAN	10.6	10.8	10.8	9.9
	S.D.	1.4	1.3	1.0	0.8
	N	3	3	3	3
Weeks 9-13	MEAN	5.6	6.4	6.2	5.8
	S.D.	0.7	0.3	0.6	1.0
	N	3	3	3	3
Overall (Weeks 1-13)	MEAN	11.4	12.0	11.8	11.0
	S.D.	1.1	0.7	0.5	1.1
	N	3	3	3	3
Females					
Weeks 1-4	MEAN	11.0	10.9	9.6**	9.4**
	S.D.	1.1	0.5	0.5	1.3
	N	3	3	3	3
Weeks 5-8	MEAN	5.2	5.2	5.8	4.7
	S.D.	0.6	0.7	1.4	0.1
	N	3	3	3	3
Weeks 9-13	MEAN	2.9	2.9	2.7	2.6
	S.D.	0.5	0.1	0.5	1.0
	N	3	3	3	3
Overall (Weeks 1-13)	MEAN	6.2	6.1	5.9	5.5*
	S.D.	0.3	0.4	0.6	0.5
	N	3	3	3	3

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS

GLOSSARY FOR TABLE 9

CLINICAL OBSERVATIONS

[11499

Key:

NO.	-	number
OBS.	-	observations
INCR/D	-	increased
INCONTIN	-	incontinence
AREA 20	-	underside of tail
SEE FREE TEXT	-	Female 83 (1000ppm) - Head leaning to left hand side.

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 9
CLINICAL OBSERVATIONS

SEX: MALE	0 PPM		250 PPM		1000 PPM		5000 PPM	
KILLED TERMINATION								
NO. OF OBS.	12		12		12		12	
NO. OF ANIMALS	12		12		12		12	
WEEKS FROM - TO	14	14	14	14	14	14	14	14
SCABS (CODED BY AREA 20)								
NO. OF OBS.	1		7		4			
NO. OF ANIMALS	1		1		1			
WEEKS FROM - TO	5	5	6	12	5	8		
INCR/SD RESPONSE TO TOUCH								
NO. OF OBS.					1			
NO. OF ANIMALS					1			
WEEKS FROM - TO					14	14		
REDUCED SPLAY REFLEX								
NO. OF OBS.			2				1	
NO. OF ANIMALS			1				1	
WEEKS FROM - TO			5	6			14	14
TAIL BLEEDING								
NO. OF OBS.	1							
NO. OF ANIMALS	1							
WEEKS FROM - TO	6	6						
TAIL DAMAGED								
NO. OF OBS.	28				11		9	
NO. OF ANIMALS	4				2		2	
WEEKS FROM - TO	5	14			5	14	8	14
TEETH TRIMMED								
NO. OF OBS.	1							
NO. OF ANIMALS	1							
WEEKS FROM - TO	14	14						

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 9
CLINICAL OBSERVATIONS

	0 PPM	250 PPM	1000 PPM	5000 PPM
SEX: FEMALE				
EXOPHTHALMUS				
NO. OF OBS.			1	
NO. OF ANIMALS			1	
WEEKS FROM - TO			13	
HAIR LOSS (GENERAL)				
NO. OF OBS.	12			
NO. OF ANIMALS	2			
WEEKS FROM - TO	14			
HUNCHED				
NO. OF OBS.		1	2	
NO. OF ANIMALS		1	2	
WEEKS FROM - TO		14	5	9
KILLED FOR HUMANE REASONS				
NO. OF OBS.			1	
NO. OF ANIMALS			1	
WEEKS FROM - TO			13	
KILLED TERMINATION				
NO. OF OBS.	12	12	11	12
NO. OF ANIMALS	12	12	11	12
WEEKS FROM - TO	14	14	14	14
PALE				
NO. OF OBS.			1	
NO. OF ANIMALS			1	
WEEKS FROM - TO			5	
REDUCED SPLAY REFLEX				
NO. OF OBS.	2	9		7
NO. OF ANIMALS	2	1		3
WEEKS FROM - TO	14	5 14	13	9 14

LAC904-09/02

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 9
CLINICAL OBSERVATIONS

	0 PPM	250 PPM	1000 PPM	5000 PPM
SEX: FEMALE				
SIGNS OF URINARY INCONTIN				4
NO. OF OBS.				1
NO. OF ANIMALS				6
WEEKS FROM - TO				14
TAIL BLEEDING			1	
NO. OF OBS.			1	
NO. OF ANIMALS			5	
WEEKS FROM - TO			5	
TAIL DAMAGED		6		
NO. OF OBS.		1		
NO. OF ANIMALS		9		
WEEKS FROM - TO		14		
SCALY TAIL			11	
NO. OF OBS.			1	
NO. OF ANIMALS			4	
WEEKS FROM - TO			14	
SEE FREE TEXT				1
NO. OF OBS.				1
NO. OF ANIMALS				14
WEEKS FROM - TO				14
TIP TOE GAIT			5	
NO. OF OBS.			1	
NO. OF ANIMALS			9	
WEEKS FROM - TO			13	
		1		
		1		
		9		

26

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 10
INTERGROUP COMPARISON OF LANDING FOOT SPLAY (mm)

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Males					
Week -1	MEAN	58.6	60.3	56.7	52.0
	S.D.	17.1	13.8	12.8	9.1
	N	12	12	12	12
Week 5	MEAN	71.5	76.2	71.5	72.7
	S.D.	12.3	14.8	15.6	15.0
	N	12	12	12	12
Week 9	MEAN	65.5	74.1	68.7	63.8
	S.D.	16.2	19.6	20.8	14.1
	N	12	12	12	12
Week 14	MEAN	68.6	64.6	69.2	59.6
	S.D.	15.5	11.6	14.2	14.8
	N	12	12	12	12
Females					
Week -1	MEAN	43.3	43.3	47.0	47.1
	S.D.	9.0	7.8	9.7	8.3
	N	12	12	12	12
Week 5	MEAN	61.2	57.3	66.3	64.0
	S.D.	12.8	12.1	20.9	12.1
	N	12	12	12	12
Week 9	MEAN	60.6	59.2	54.0	56.9
	S.D.	8.6	12.5	10.0	10.1
	N	12	12	12	12
Week 14	MEAN	56.6	50.6	52.4	53.6
	S.D.	15.3	3.0	10.9	11.1
	N	12	12	11	12

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 11
INTERGROUP COMPARISON OF TIME TO TAIL FLICK (s)

		Dietary Concentration of Butylate (ppm)			
		0(Control)	250	1000	5000
Males					
Week -1	MEAN	6.4	9.5	7.6	6.4
	S.D.	4.1	6.7	4.6	4.3
	N	12	12	12	12
Week 5	MEAN	8.7	9.2	5.8	6.6
	S.D.	4.8	6.8	2.3	6.3
	N	12	12	12	12
Week 9	MEAN	10.2	14.0	8.5	8.8
	S.D.	6.1	6.7	5.2	5.5
	N	12	12	12	12
Week 14	MEAN	10.9	8.9	6.8	9.0
	S.D.	6.8	6.8	3.7	7.1
	N	12	12	12	12
Females					
Week -1	MEAN	5.9	8.3	5.1	5.3
	S.D.	3.1	5.0	2.5	3.1
	N	12	12	12	12
Week 5	MEAN	3.0	3.5	6.3**	3.7
	S.D.	2.2	1.9	4.1	2.1
	N	12	12	12	12
Week 9	MEAN	4.6	6.2	5.2	4.8
	S.D.	1.5	3.4	3.0	2.2
	N	12	12	12	12
Week 14	MEAN	6.3	4.5	5.1	4.3
	S.D.	4.0	1.4	2.6	1.9
	N	12	12	11	12

28

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 12A
INTERGROUP COMPARISON OF GRIP STRENGTH (g) - FORELIMB

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Males					
Week -1	MEAN	646	696	671	660
	S.D.	102	67	107	76
	N	12	12	12	12
Week 5	MEAN	1306	1369	1392	1383
	S.D.	133	277	209	182
	N	12	12	12	12
Week 9	MEAN	1575	1563	1581	1644
	S.D.	164	192	187	187
	N	12	12	12	12
Week 14	MEAN	1929	1890	1867	1790
	S.D.	193	188	455	206
	N	12	12	12	12
Females					
Week -1	MEAN	635	640	638	640
	S.D.	81	91	78	97
	N	12	12	12	12
Week 5	MEAN	1215	1185	1090*	1146
	S.D.	93	158	140	162
	N	12	12	12	12
Week 9	MEAN	1310	1313	1275	1279
	S.D.	183	162	112	200
	N	12	12	12	12
Week 14	MEAN	1548	1517	1455	1471
	S.D.	170	193	102	171
	N	12	12	11	12

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 12B
INTERGROUP COMPARISON OF GRIP STRENGTH (g) - HINDLIMB

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Males					
Week -1	MEAN	319	279	377	269
	S.D.	118	58	76	67
	N	12	12	12	12
Week 5	MEAN	602	627	544	594
	S.D.	160	85	132	123
	N	12	12	12	12
Week 9	MEAN	840	863	854	813
	S.D.	96	203	174	124
	N	12	12	12	12
Week 14	MEAN	879	983	960	850
	S.D.	154	291	261	167
	N	12	12	12	12
Females					
Week -1	MEAN	304	294	306	296
	S.D.	66	73	82	84
	N	12	12	12	12
Week 5	MEAN	506	423	410	444
	S.D.	109	129	134	132
	N	12	12	12	12
Week 9	MEAN	546	565	550	596
	S.D.	141	127	131	120
	N	12	12	12	12
Week 14	MEAN	623	515	559	556
	S.D.	193	186	175	156
	N	12	12	11	12

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 13
INTERGROUP COMPARISON OF MOTOR ACTIVITY - WEEK -1 MALES

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Minutes 1-5	MEAN	40.8	42.1	46.3	36.8
	S.D.	20.2	16.4	20.6	18.0
	N	12	12	12	12
Minutes 6-10	MEAN	40.0	46.0	48.1	38.8
	S.D.	21.4	15.4	11.9	18.1
	N	12	12	12	12
Minutes 11-15	MEAN	30.6	36.8	42.0	34.1
	S.D.	20.4	22.8	17.0	18.0
	N	12	12	12	12
Minutes 16-20	MEAN	19.1	17.1	20.8	15.2
	S.D.	20.5	23.7	21.6	16.4
	N	12	12	12	12
Minutes 21-25	MEAN	5.3	11.9	11.8	5.1
	S.D.	7.8	21.2	24.2	7.4
	N	12	12	12	12
Minutes 26-30	MEAN	3.6	5.2	3.2	4.5
	S.D.	7.6	12.2	9.4	12.8
	N	12	12	12	12
Minutes 31-35	MEAN	1.8	2.3	2.8	0.8
	S.D.	4.7	6.3	6.2	1.6
	N	12	12	12	12
Minutes 36-40	MEAN	5.4	3.3	1.3	4.5
	S.D.	13.4	9.4	4.3	9.4
	N	12	12	12	12
Minutes 41-45	MEAN	2.1	2.8	6.4	1.0
	S.D.	3.9	3.6	12.7	3.2
	N	12	12	12	12
Minutes 46-50	MEAN	1.8	1.6	4.1	2.0
	S.D.	5.2	3.4	10.0	4.5
	N	12	12	12	12
Overall (1-50)	MEAN	150.3	169.0	186.8	142.8
	S.D.	76.4	63.2	75.9	54.2
	N	12	12	12	12

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 13
INTERGROUP COMPARISON OF MOTOR ACTIVITY - WEEK -1 FEMALES

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Minutes 1-5	MEAN	47.8	49.1	44.5	51.6
	S.D.	8.2	18.6	16.9	8.3
	N	12	12	12	12
Minutes 6-10	MEAN	56.0	44.8	48.3	52.8
	S.D.	9.5	24.8	19.9	10.8
	N	12	12	12	12
Minutes 11-15	MEAN	40.8	32.0	47.5	35.3
	S.D.	18.2	24.4	17.6	17.1
	N	12	12	12	12
Minutes 16-20	MEAN	21.8	23.9	30.5	15.8
	S.D.	25.4	28.6	26.8	18.9
	N	12	12	12	12
Minutes 21-25	MEAN	11.8	8.1	17.9	7.6
	S.D.	18.3	15.6	21.7	14.4
	N	12	12	12	12
Minutes 26-30	MEAN	5.4	5.8	4.7	6.3
	S.D.	10.9	11.0	7.8	15.7
	N	12	12	12	12
Minutes 31-35	MEAN	0.4	1.5	8.3	5.5
	S.D.	1.2	3.3	16.5	17.2
	N	12	12	12	12
Minutes 36-40	MEAN	1.3	2.4	9.4	7.2
	S.D.	2.4	5.4	16.6	19.3
	N	12	12	12	12
Minutes 41-45	MEAN	1.3	6.2	12.8	4.5
	S.D.	4.0	15.6	19.0	9.7
	N	12	12	12	12
Minutes 46-50	MEAN	3.3	0.4	11.8	0.3
	S.D.	8.1	0.8	20.3	0.8
	N	12	12	12	12
Overall (1-50)	MEAN	189.8	174.2	235.8	186.8
	S.D.	72.6	109.6	75.3	84.2
	N	12	12	12	12

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 13
INTERGROUP COMPARISON OF MOTOR ACTIVITY - WEEK 5 MALES

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Minutes 1-5	MEAN	59.5	60.6	58.2	57.8
	S.D.	9.8	10.5	6.2	11.9
	N	12	12	12	12
Minutes 6-10	MEAN	57.8	60.3	58.3	55.8
	S.D.	10.6	10.9	16.2	6.9
	N	12	12	12	12
Minutes 11-15	MEAN	61.7	52.8	45.3*	49.8
	S.D.	14.1	14.9	20.6	14.1
	N	12	12	12	12
Minutes 16-20	MEAN	53.8	42.2	39.3	40.9
	S.D.	25.7	23.1	25.0	19.6
	N	12	12	12	12
Minutes 21-25	MEAN	48.5	39.7	48.4	41.3
	S.D.	17.4	22.2	23.3	23.2
	N	12	12	12	12
Minutes 26-30	MEAN	47.4	31.8	25.5*	44.1
	S.D.	28.2	23.0	22.0	22.9
	N	12	12	12	12
Minutes 31-35	MEAN	37.8	26.3	28.3	35.3
	S.D.	27.9	23.7	27.9	28.1
	N	12	12	12	12
Minutes 36-40	MEAN	30.0	18.3	23.8	31.6
	S.D.	26.3	26.0	28.4	24.4
	N	12	12	12	12
Minutes 41-45	MEAN	27.2	15.2	15.8	28.4
	S.D.	30.8	27.8	20.1	23.8
	N	12	12	12	12
Minutes 46-50	MEAN	17.7	11.4	8.7	25.4
	S.D.	26.5	21.5	14.5	25.2
	N	12	12	12	12
Overall (1-50)	MEAN	441.3	358.4	351.3	410.2
	S.D.	156.3	151.6	159.2	130.4
	N	12	12	12	12

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 13
INTERGROUP COMPARISON OF MOTOR ACTIVITY - WEEK 5 FEMALES

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Minutes 1-5	MEAN	51.9	54.2	54.6	57.6
	S.D.	6.2	8.0	7.3	9.0
	N	12	12	12	12
Minutes 6-10	MEAN	50.8	61.3**	58.0	53.8
	S.D.	6.9	8.7	8.2	12.2
	N	12	12	12	12
Minutes 11-15	MEAN	43.6	49.5	60.6*	53.1
	S.D.	25.0	19.3	7.4	10.4
	N	12	12	12	12
Minutes 16-20	MEAN	39.3	35.8	62.9**	46.2
	S.D.	22.2	24.6	7.9	19.8
	N	12	12	12	12
Minutes 21-25	MEAN	43.3	48.3	53.5	49.9
	S.D.	20.8	27.0	13.1	25.5
	N	12	12	12	12
Minutes 26-30	MEAN	41.3	49.5	54.5	48.5
	S.D.	25.4	14.0	17.7	18.1
	N	12	12	12	12
Minutes 31-35	MEAN	35.7	42.4	50.4	52.8
	S.D.	30.0	22.5	24.0	18.3
	N	12	12	12	12
Minutes 36-40	MEAN	35.1	38.0	45.9	53.3
	S.D.	28.8	25.8	27.8	22.9
	N	12	12	12	12
Minutes 41-45	MEAN	40.7	39.8	44.0	55.3
	S.D.	25.3	26.1	28.6	6.4
	N	12	12	12	12
Minutes 46-50	MEAN	49.3	46.0	38.8	56.6
	S.D.	26.7	24.4	29.8	16.6
	N	12	12	12	12
Overall (1-50)	MEAN	430.8	464.8	523.2	527.0
	S.D.	167.7	153.7	109.9	107.6
	N	12	12	12	12

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 13
INTERGROUP COMPARISON OF MOTOR ACTIVITY - WEEK 9 MALES

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Minutes 1-5	MEAN	61.2	60.7	55.4	64.0
	S.D.	10.1	8.4	10.5	8.2
	N	12	12	12	12
Minutes 6-10	MEAN	57.4	56.0	59.3	55.7
	S.D.	10.5	8.5	11.0	12.5
	N	12	12	12	12
Minutes 11-15	MEAN	47.0	43.3	53.7	54.4
	S.D.	21.1	20.8	8.0	14.8
	N	12	12	12	12
Minutes 16-20	MEAN	48.5	42.0	40.8	52.1
	S.D.	20.5	25.6	14.1	11.0
	N	12	12	12	12
Minutes 21-25	MEAN	38.3	30.7	41.9	40.7
	S.D.	22.7	24.8	20.7	17.1
	N	12	12	12	12
Minutes 26-30	MEAN	34.4	35.4	42.2	36.3
	S.D.	27.7	28.4	21.5	31.0
	N	12	12	12	12
Minutes 31-35	MEAN	44.1	38.9	32.4	26.5
	S.D.	27.6	23.2	27.4	27.2
	N	12	12	12	12
Minutes 36-40	MEAN	34.8	34.5	21.8	20.9
	S.D.	25.3	29.6	20.2	22.7
	N	12	12	12	12
Minutes 41-45	MEAN	30.3	17.2	18.0	20.0
	S.D.	29.9	20.4	22.3	24.5
	N	12	12	12	12
Minutes 46-50	MEAN	25.1	22.6	13.4	24.7
	S.D.	25.9	27.2	17.9	29.6
	N	12	12	12	12
Overall (1-50)	MEAN	421.1	381.3	378.9	395.2
	S.D.	154.5	141.2	108.0	159.6
	N	12	12	12	12

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 13
INTERGROUP COMPARISON OF MOTOR ACTIVITY - WEEK 9 FEMALES

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Minutes 1-5	MEAN	58.6	55.1	58.2	59.8
	S.D.	9.0	10.4	9.9	7.0
	N	12	12	12	12
Minutes 6-10	MEAN	60.3	51.8	57.3	54.3
	S.D.	15.4	12.0	13.7	13.8
	N	12	12	12	12
Minutes 11-15	MEAN	59.5	51.1*	57.8	58.1
	S.D.	9.5	16.4	8.8	9.7
	N	12	12	12	12
Minutes 16-20	MEAN	50.8	55.1	57.0	51.6
	S.D.	14.8	14.6	20.6	19.7
	N	12	12	12	12
Minutes 21-25	MEAN	44.5	35.8	43.4	51.8
	S.D.	26.8	22.5	23.4	21.7
	N	12	12	12	12
Minutes 26-30	MEAN	44.4	33.7	50.3	54.5
	S.D.	23.2	21.0	26.3	21.7
	N	12	12	12	12
Minutes 31-35	MEAN	52.8	54.4	41.6	52.3
	S.D.	15.1	14.2	24.4	16.4
	N	12	12	12	12
Minutes 36-40	MEAN	40.3	45.4	59.8*	52.2
	S.D.	28.3	17.2	21.5	19.6
	N	12	12	12	12
Minutes 41-45	MEAN	43.8	47.8	61.7*	58.1
	S.D.	25.7	25.5	10.4	19.3
	N	12	12	12	12
Minutes 46-50	MEAN	51.9	46.8	51.5	47.5
	S.D.	18.7	23.0	26.4	16.8
	N	12	12	12	12
Overall (1-50)	MEAN	506.9	476.9	538.6	540.0
	S.D.	117.6	82.8	134.0	121.5
	N	12	12	12	12

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 13
INTERGROUP COMPARISON OF MOTOR ACTIVITY - WEEK 14 MALES

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Minutes 1-5	MEAN	48.8	56.3	53.0	55.4
	S.D.	19.5	10.1	17.4	18.3
	N	12	12	12	12
Minutes 6-10	MEAN	37.3	45.0	49.5	52.4*
	S.D.	22.9	17.6	16.2	21.9
	N	12	12	12	12
Minutes 11-15	MEAN	31.4	35.2	38.4	37.8
	S.D.	26.7	19.2	32.6	23.6
	N	12	12	12	12
Minutes 16-20	MEAN	15.8	17.2	18.0	33.1*
	S.D.	19.3	18.1	19.7	27.9
	N	12	12	12	12
Minutes 21-25	MEAN	14.9	16.0	19.7	21.8
	S.D.	16.0	19.9	26.1	22.0
	N	12	12	12	12
Minutes 26-30	MEAN	11.9	13.3	9.8	12.3
	S.D.	16.9	12.7	12.8	17.6
	N	12	12	12	12
Minutes 31-35	MEAN	8.3	15.6	6.8	8.7
	S.D.	14.1	23.2	8.3	17.7
	N	12	12	12	12
Minutes 36-40	MEAN	5.8	11.3	2.7	5.7
	S.D.	7.8	12.4	3.7	10.6
	N	12	12	12	12
Minutes 41-45	MEAN	8.2	3.7	1.8	2.7
	S.D.	15.3	5.8	4.6	6.9
	N	12	12	12	12
Minutes 46-50	MEAN	8.9	2.2	3.3	1.0*
	S.D.	18.0	3.9	6.4	1.6
	N	12	12	12	12
Overall (1-50)	MEAN	191.3	215.6	202.8	230.8
	S.D.	108.0	65.4	86.2	128.0
	N	12	12	12	12

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 13
INTERGROUP COMPARISON OF MOTOR ACTIVITY - WEEK 14 FEMALES

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Minutes 1-5	MEAN	51.7	56.3	58.0*	52.6
	S.D.	7.3	9.5	7.9	7.5
	N	12	12	11	12
Minutes 6-10	MEAN	52.9	56.6	58.8	58.4
	S.D.	18.9	7.2	7.8	7.6
	N	12	12	11	12
Minutes 11-15	MEAN	53.8	49.0	62.8	50.3
	S.D.	19.6	20.3	13.3	19.0
	N	12	12	11	12
Minutes 16-20	MEAN	47.3	40.8	46.8	48.3
	S.D.	23.5	19.9	22.5	24.3
	N	12	12	11	12
Minutes 21-25	MEAN	36.8	35.4	51.7	47.8
	S.D.	27.2	29.0	27.5	20.5
	N	12	12	11	12
Minutes 26-30	MEAN	32.4	29.0	43.5	46.1
	S.D.	29.3	28.4	30.3	25.6
	N	12	12	11	12
Minutes 31-35	MEAN	35.5	35.0	51.8	53.5*
	S.D.	26.7	21.6	21.4	11.8
	N	12	12	11	12
Minutes 36-40	MEAN	42.8	31.1	56.5	51.6
	S.D.	29.6	24.7	19.7	9.6
	N	12	12	11	12
Minutes 41-45	MEAN	45.8	28.3	37.1	46.2
	S.D.	20.9	24.6	27.4	24.8
	N	12	12	11	12
Minutes 46-50	MEAN	41.3	21.7*	45.5	46.1
	S.D.	28.0	24.5	25.4	17.6
	N	12	12	11	12
Overall (1-50)	MEAN	440.2	383.1	532.6	500.8
	S.D.	164.6	133.2	143.6	103.7
	N	12	12	11	12

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 14
INTERGROUP COMPARISON OF CHOLINESTERASE ACTIVITY

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
Males					
Brain Cholinesterase (micromples/l/min/g)					
	MEAN	10.31	10.85	10.95	11.09
	S.D.	1.10	1.07	0.24	1.36
	N	6	6	6	6
Erythrocyte Cholinesterase (U/l)					
	MEAN	2128	2072	2483*	2168
	S.D.	300	178	324	246
	N	6	6	6	6
Plasma Cholinesterase (U/l)					
	MEAN	547	528	525	606
	S.D.	54	35	80	95
	N	6	6	6	6
Females					
Brain Cholinesterase (micromoles/l/min/g)					
	MEAN	11.34	10.99	11.77	11.90
	S.D.	0.33	0.61	0.39	0.70
	N	6	6	5	6
Erythrocyte Cholinesterase (U/l)					
	MEAN	2177	2338	2440	2498*
	S.D.	246	299	321	213
	N	6	6	5	6
Plasma Cholinesterase (U/l)					
	MEAN	1508	1672	1781	1654
	S.D.	243	252	352	232
	N	6	6	5	6

011499

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS

TABLE 15

INTERGROUP COMPARISON OF NEUROPATHY TARGET ESTERASE ACTIVITY

		Dietary Concentration of Butylate (ppm)				
		0(Control)	250	1000	5000	
Activity (moles/min/g wet weight)						
Males	MEAN	967	1032	1092*	1060	
	S.D.	126	85	144	63	
	N	3	3	3	3	
Females	MEAN	979	1060	1021	999	
	S.D.	103	101	34	144	
	N	3	3	3	3	

40

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 16
INTERGROUP COMPARISON OF BRAIN PARAMETERS

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
BRAIN					
MALES					
Terminal Bodyweight (g)	MEAN	524.4	535.8	529.8	481.5
	S.D.	35.0	41.1	44.9	41.4
	N	12	12	12	12
Organ Weight (g)	MEAN	2.13	2.17	2.18	2.15
	S.D.	0.07	0.08	0.08	0.07
	N	12	12	12	12
Organ to Bodyweight Ratio (%)	MEAN	0.41	0.41	0.41	0.45
	S.D.	0.03	0.02	0.04	0.03
	N	12	12	12	12
Organ Weight Adjusted For Bodyweight		2.13	2.15	2.17	2.18
FEMALES					
Terminal Bodyweight (g)	MEAN	285.0	275.3	260.4	249.7
	S.D.	17.7	12.8	12.4	20.0
	N	12	11	11	12
Organ Weight (g)	MEAN	1.98	1.97	1.97	1.95
	S.D.	0.06	0.09	0.08	0.05
	N	12	11	11	12
Organ to Bodyweight Ratio (%)	MEAN	0.70	0.72	0.76	0.79
	S.D.	0.03	0.04	0.04	0.07
	N	12	11	11	12
Organ Weight Adjusted For Bodyweight		1.97	1.97	1.97	1.96

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 16
INTERGROUP COMPARISON OF BRAIN PARAMETERS

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
BRAIN LENGTH					
MALES					
Terminal Bodyweight (g)	MEAN	524.4	535.8	529.8	481.5
	S.D.	35.0	41.1	44.9	41.4
	N	12	12	12	12
Brain Length (mm)	MEAN	28.1	28.6	28.3	28.6
	S.D.	2.1	2.1	1.9	2.1
	N	12	12	12	12
Brain Length to Bodyweight Ratio (%)	MEAN	5.4	5.4	5.4	6.0
	S.D.	0.5	0.6	0.6	0.6
	N	12	12	12	12
Brain Length Adjusted For Bodyweight		28.1	28.6	28.2	28.6
FEMALES					
Terminal Bodyweight (g)	MEAN	285.0	275.0	260.4	249.7
	S.D.	17.7	12.2	12.4	20.0
	N	12	12	11	12
Brain Length (mm)	MEAN	26.9	27.3	27.3	27.0
	S.D.	2.3	2.3	2.1	1.8
	N	12	12	11	12
Brain Length to Bodyweight Ratio (%)	MEAN	9.5	9.9	10.5	10.9
	S.D.	0.7	1.0	0.9	1.2
	N	12	12	11	12
Brain Length Adjusted For Bodyweight		27.0	27.3	27.2	27.0

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 16
INTERGROUP COMPARISON OF BRAIN PARAMETERS

		Dietary Concentration of Butylate (ppm) 0(Control)	250	1000	5000
BRAIN WIDTH					
MALES					
Terminal Bodyweight (g)	MEAN	524.4	535.8	529.8	481.5
	S.D.	35.0	41.1	44.9	41.4
	N	12	12	12	12
Brain Width (mm)	MEAN	15.7	15.5	15.5	15.4
	S.D.	0.5	0.5	0.7	0.8
	N	12	12	12	12
Brain Width to Bodyweight Ratio (%)	MEAN	3.0	2.9	2.9	3.2
	S.D.	0.2	0.2	0.2	0.3
	N	12	12	12	12
Brain Width Adjusted For Bodyweight		15.7	15.5	15.5	15.5
FEMALES					
Terminal Bodyweight (g)	MEAN	285.0	275.0	260.4	249.7
	S.D.	17.7	12.2	12.4	20.0
	N	12	12	11	12
Brain Width (mm)	MEAN	14.9	14.8	15.0	14.8
	S.D.	0.5	0.6	0.6	0.7
	N	12	12	11	12
Brain Width to Bodyweight Ratio (%)	MEAN	5.3	5.4	5.8	6.0
	S.D.	0.4	0.3	0.5	0.6
	N	12	12	11	12
Brain Width Adjusted For Bodyweight		14.9	14.8	15.0	14.8

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 17
INTERGROUP COMPARISON OF MACROSCOPIC FINDINGS

	MALES				FEMALES			
	0 PPM	250 PPM	1000 PPM	5000 PPM	0 PPM	250 PPM	1000 PPM	5000 PPM
ANIMALS ON STUDY	12	12	12	12	12	12	12	12
ANIMALS COMPLETED	12	12	12	12	12	12	12	12
KIDNEY								
NO.WITH FINDINGS BUT NOT SUBMITTED....	0	0	0	1	0	0	0	1
Pelvic dilatation.....	0	0	0	1	0	0	0	1
SKIN								
NO.WITH FINDINGS BUT NOT SUBMITTED....	0	0	0	0	2	0	0	0
Hair loss.....	0	0	0	0	2	0	0	0
TAIL								
SUBMITTED.....	1	0	1	1	0	0	0	1
NO.WITH FINDINGS.....	1	0	1	1	0	0	0	1
NO.WITH FINDINGS BUT NOT SUBMITTED....	1	0	1	1	0	1	1	0
Traumatised.....	0	0	0	1	0	0	0	0
Kinked.....	2	0	2	0	0	1	0	0
Scaly.....	0	0	0	0	0	0	1	1
Scab/s.....	0	0	0	1	0	0	0	0
URINARY BLADDER								
NO.WITH FINDINGS BUT NOT SUBMITTED....	0	0	0	0	0	0	0	1
Distended.....	0	0	0	0	0	0	0	1
Firm deposit/s.....	0	0	0	0	0	0	0	1

CTL/P/4423 - 71

011499

PAC921-10/04

BUTYLATE: SUBCHRONIC NEUROTOXICITY STUDY IN RATS
TABLE 18
INTERGROUP COMPARISON OF MICROSCOPIC FINDINGS

REMOVAL REASON: TERMINAL	MALES						FEMALES					
	0 PRM		250 PRM		1000 PRM	5000 PRM	0 PRM		250 PRM		1000 PRM	5000 PRM
ANIMALS ON STUDY	12	6	12	6	12 6	12 6	12	6	12	6	12 6	12 6
ANIMALS COMPLETED	12	6	12	6	12 6	12 6	12	6	12	6	12 6	12 6
BRAIN												
EXAMINED	6		6		6	6	6		6		6	6
NO ABNORMALITIES DETECTED	4		6		6	3	5		6		6	6
Neuronal cell necrosis (TOTAL)	2		0		0	3	1		0		0	0
minimal	2		0		0	3	1		0		0	0

45