Caswell

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

006467

SEP 2 1987

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Propoxur studies

TO: Mr. Dennis Edwards
Product Manager 12

FROM: Byron T. Backus, Toxicologist
Toxicology Branch, HED (TS-769C)

THROUGH: Marcia van Gemert, Ph.D.
Section Head, Review Section III
Toxicology Branch, HED (TS-769C)

and

Theodore M. Farber, Ph.D., D.A.B.T.
Branch Chief
Toxicology Branch, HED (TS-769C)

Project No. 7-0675

Tox. Chem. No. 508

Action Requested:

Review of 4 studies (urinary metabolism - rat, mutagenic with S. cerevisiae, mutagenic - SCE in human lymphocytes, 13-week subchronic in rhesus monkey) on Propoxur.

Conclusions and Recommendations:

1. The urinary metabolism study has been classified as core supplementary data. However, the data does provide important qualitative and quantitative information regarding urinary metabolites of Propoxur during the first 24 hours after dosage. Additionally, the complexity of the metabolic pathways involving Propoxur was illustrated by the statement that although 11 urinary metabolites were identified, about 14-20% of the radioactivity was distributed among a group of other (unidentified) "metabolites of very low concentration."

2. The S. cerevisiae mutagenic study is acceptable. There was no evidence of an increased incidence of mutagenic effects (point mutations, mitotic crossing-over or mitotic gene conversion) as a result of exposure of the test organism to concentrations of up to 10,000 mcg/ml Propoxur with or without concomitant S-9 exposure. The study was conducted in replicate and the positive controls elicited the appropriate responses.

006467

12519

3. The sister chromatid exchange study utilizing human lymphocyte cultures has been classified as acceptable without S-9 activation, but not acceptable with S-9 activation. No evidence was found of an increased incidence in SCE as a result of exposure of up to 500 ug/ml Propoxur (without S-9) or at up to 1000 ug/ml Propoxur (with S-9). However, a sufficient level of cytotoxicity at the high dose level was demonstrated only in the absence of S-9.

4. The subchronic dosing study utilizing rhesus monkeys has been classified as core supplementary data. The value of this study is somewhat limited, in part because of the lack of simultaneous controls, administration of the test material at only one dose level, and considerable variation in the test animals (weights: 3.37-10.03 kg; ages: 2.5-6 yrs; origins: two from China, one from Charles River Labs, U.S.A., two having been bred in the laboratory, and one from a zoo).

   There was no evidence of any bladder changes similar to those observed in a previously conducted rat chronic feeding/oncogenicity study. However, the test material was given as part of the diet in that study (at levels of 200, 1000 and 5000 ppm) and that study had a duration of 2+ years.

   A noteworthy finding in this subchronic rhesus dosing study was the occurrence of considerable (50% or more) plasma ChE inhibition at one hour after administration of the test material. Whether this was the maximum level of plasma ChE inhibition is unknown. It is also noteworthy that there were possible (but not definite) indications that RBC ChE inhibition may also have occurred, and that transient symptoms of ChE inhibition occurred in some monkeys. From these considerations, 40 mg/kg is a maximally tolerated dose (MTD) level in the rhesus monkey when administered on a daily single dose basis.

2

006467

Reviewed by: Byron T. Backus, Toxicologist Byron T. Backus 8/21/87
Section III, Tox. Branch (TS-769C)

Secondary Reviewer: Marcia Van Gemert, Ph.D., Section Head
Section III, Tox. Branch (TS-769C) M. Van Gemert 8/24/87

DATA EVALUATION REPORT I

STUDY TYPE: Metabolism - rat TOX. CHEM. NO.: 508

ACCESSION NUMBER: 266038 MRID NO.: not given

TEST MATERIAL: Baygon, Propoxur

STUDY NUMBER: Report No. 90441

SPONSOR: Mobay Chemical Corp.
Kansas City, MO

TESTING FACILITY: Bayer AG

TITLE OF REPORT: Biotransformation of Propoxur - Quantitative Determination of Metabolite Spectrum in Rats Dosed Once with [^{14}C] Propoxur After Being Fed Compound at Three Subchronic Dietary Levels

AUTHOR(S): Karl, W.

REPORT ISSUED: April 18, 1985

STUDY CLASSIFICATION: Core supplementary data

CONCLUSIONS:

1. The complexity of the metabolic pathways involving Propoxur is illustrated by the fact that although 11 urinary metabolites are identified, approximately 14-20% of the radiocativity was "distributed among a large number of metabolites of very low concentration."

2. While only one sex was used, and although the dosage protocol for the study was somewhat different from that specified by the section F Guidelines, the study does provide important qualitative and quantitative information regarding urinary metabolites of Propoxur during the first 24 hours after dosage.

006467 3

A. MATERIALS:

1. Test compound: Ring-UL-^{14}C-Propoxur (specific radioactivity: 112 iCi/mg = 4.1 MBq/mg), with chemical purity determinations of 98% (TLC) and 98.8% (HPLC).

2. Test animals: Female Wistar rats: BOR: WISW (SPF, Cpb); from Winkelmann, Borchen, Kreis Paderborn, average body weight of 200 g.

B. STUDY DESIGN:

1. Groups of 5 rats received 50, 250 or 5000 ppm unlabeled propoxur in their diet for approximately 5 months. They then received a single oral dose of 1 mg/kg labeled propoxur, administered in physiological saline. Rats were then placed in metabolism cages for separate collection of urine and feces (intervals of 0-24 and 24-48 hr).

2. Samples were analyzed using both thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC). Additionally, some urine samples were incubated for 60 hr in B-glucuronidase/arylsulfatase and arylsulfatase.

3. Quality Assurance: There is no quality assurance statement.

C. RESULTS:

1. Urinalysis

According to the text (p. 8) the 0-24 hr urine samples contained between 87.8 and 99.8% of the radioactivity, while the 24-48 hr samples contained less than 1%. However, one female (#348) in the 50 ppm diet group, as well as one (#354) in the 250 ppm group, had considerably lower values (less than 50% of the radioactivity was excreted), and data from these rats were not used in calculating group means. Since most of the radioactivity was in the 0-24 hr samples, determinations of the metabolites were made from these (presumably from pooled group samples).

Using thin-layer chromatography it was demonstrated that 97-98% of the urinary activity remained at the origin, and were due to either conjugated metabolites or to extremely polar metabolites of unknown structure.

Subsequently, several steps utilizing enzymatic cleavage were used, resulting in identification of 80-86% of the radioactivity as specific metabolites. The remaining 14-20% was "shown to be distributed among a large number of unknown metabolites of very low concentration."

The appended sheets show the structures of the identified metabolites as well as the relative amounts of radioactivity associated with each.

D. DISCUSSION:

The complexity of the metabolic pathways involving Propoxur is illustrated by the fact that although 11 urinary metabolites are identified, approximately 14-20% of the radiocativity was "distributed among a large number of metabolites of very low concentration."

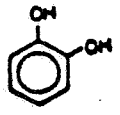
While only one sex was used, and although the dosage protocol for the study was somewhat different from that specified by the section F Guidelines, the study does provide important qualitative, as well as quantitative information regarding urinary metabolites of Propoxur during the first 24 hours after dosage. The study is classified as core supplementary data.

5

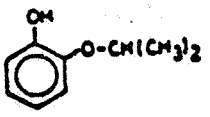
ylcarbam

d metabolit
e of rats f
hronic expe
tes, their
ort No. 128
structures

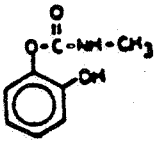
objective
rum differs
for approx.
of 50, 250
iolabelled
to the high
for more t
24 h, quant
24-h urine.



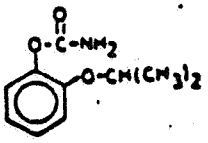
M 1



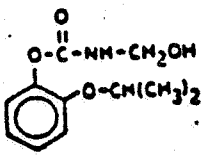
M 2



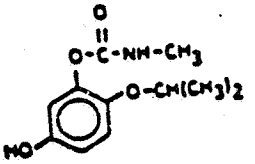
M 3



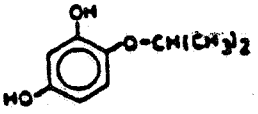
M 4



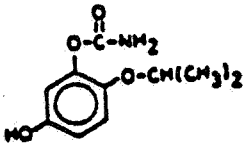
M 5



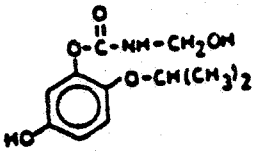
M 6



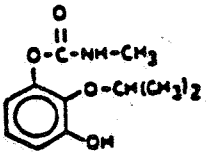
M 7



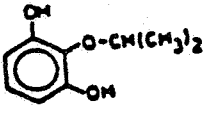
M 6CII



M S3



M 7A



M 8

Abb. 1: Renale Metaboliten von Propoxur (nach Enzymspaltung)

Figure 1: Renal metabolites of propoxur (after enzymatic cleavage)

006467

Reviewed by: Byron T. Backus, Toxicologist Byron T. Backus
Section III, Tox. Branch (TS-769C) 08/21/87

Secondary Reviewer: Marcia Van Gemert, Ph.D., Section Head
Section III, Tox. Branch (TS-769C) M. Van Gemert 8/24/87

DATA EVALUATION REPORT II

STUDY TYPE: Mutagenic - S. cerevisiae TOX. CHEM. NO.: 508

ACCESSION NUMBER: 266038 MRID NO.: not given

TEST MATERIAL: Baygon, Propoxur

STUDY NUMBER: Report No. 13966

SPONSOR: Mobay Chemical Corp.
Kansas City, MO

TESTING FACILITY: Bayer AG Institute of Toxicology

TITLE OF REPORT: BOQ 5812315 c.n. Propoxur - Test on S. cerevisiae to evaluate for point mutagenic effect.

AUTHOR(S): Herbold, B.

REPORT ISSUED: October 30, 1985

STUDY CLASSIFICATION: Acceptable

CONCLUSIONS:

1. No increased incidence of mutagenic effects (point mutations, mitotic crossing-over or mitotic gene conversion) occurred in S. cerevesiae as a result of exposures of up to 10,000 mcg/ml Propoxur with or without concomitant S-9 exposure. The study was conducted in replicate, and the positive controls elicited the appropriate responses.

2. The study is acceptable.

A. MATERIALS:

1. Test compound: BOQ 5812315 [redacted] batch no. 234401878, 99.8% pure. DMSO was used as solvent.

2. Positive controls: a) Methyl methane sulphonate (MMS), batch 1171879, used without S-9 mix; and b) Cyclophosphamide, batch 044438, used only with S-9 mix.

3. Test organism: Diploid strain Saccharomyces cerevisiae D7 developed by Zimmermann et al. (1975) with the following genotype:

$$\frac{\text{ade 2-40, trp 5-12, ilvI-92}}{\text{ade 2-119, trp 5-27, ilvI-92}}$$

"With the alleles ade 2-40 and ade 2-119 it permits detection of induced mitotic crossing-over, and with alleles trp 5-12 and trp 5-27 detection of induced mitotic gene conversion. The market ilvI-92 permits testing for induced point mutations."

"Detection of a point mutagenic effect is by means of the marker ilvI-92, which is located and homoallele on both homologous chromosomes. The ilvI locus is the structure gene for the threonine dehydratase. This enzyme is sensitive to inhibition of feedback of L-isoleucine. Due to this mutation S. cerevisiae is isoleucine-dependent. Reversion...of this mutation usually takes place by allele-specific or locus-specific suppressor mutations... Consequently both base pair substitutions and frameshift mutations can be detected at this locus..."

4. S-9 Mix: obtained from the livers of at least 6 adult male Sprague-Dawley rats, wts approx. 200-300 g, which had been given IP injection of aroclor 1254 at 500 mg/kg five days before preparation

B. STUDY DESIGN:

1. "1 ml yeast suspension was...incubated for five hours at 28° C and 150 rpm. The culture is then set at a cell density of approx. 100 million cells per ml with medium, and used immediately for the test."

0.5 ml of the set yeast suspension was incubated in tubes for 16 hrs together with the appropriate medium components and buffers, along with 0.05 ml test substance solution. Suspensions were then centrifuged and washed.

0.1 ml suspension per tube was then applied to 10 isoleucine-free nutrient broth plates, which were incubated for 6-8 days and then evaluated.

For determinations of cytotoxicity, dilutions were made from each tube, and 0.2 ml applied to each of 10 full medium plates. Dilutions were made in such a way that approximately 200 colonies would be expected to grow/plate if no cytotoxicity was present.

2. In the first run (readings made May 9, 1985?) Propoxur was tested at 0, 625, 1250, 2500, 5000 and 10,000 ug/ml; the positive controls were MMS (without S-9) at 30 nl/ml and cyclophosphamide (with S-9) at 60 mcg/ml. In the second run (readings made May 22, 1985) the same doses were used. In a third run (dated October 11, 1985) doses used were 0, 75, 150, 300, 600 and 1200 mcg/ml Propoxur and 60 mcg/ml Endoxan (this was done only with S-9).

C. RESULTS

Mean number of isoleucine revertants/plate:

		RUN 1		RUN 2	
Substance Tested	Dose mcg/ml	without S9	with S9	without S9	with S9
Negative control	-	5.5	3.9	2.1	1.2
Propoxur	625	3.5	5.6	1.3	5.6
"	1250	1.9	4.2	0.3	2.0
"	2500	2.7	2.9	0.8	1.1
"	5000	2.8	1.7	0.5	0.3
"	10000	0.5	1.7	0.4	0.7
MMS	0.03	31.3†	-	18.8†	-
Endoxan	60	-	24.4†	-	13.4†

†Reported as "mutagenic effect."

Plate counts for Propoxur at 10,000 mcg/ml in the absence (but not in the presence) of S-9 showed means of 27.7 and 36.7% viability (as compared to respective negative control values of 74 7 and 64.5%).

In the third run (utilizing S-9 only) there was no evidence of either cytotoxicity or a mutagenic effect at the highest dose level of Propoxur used (1200 mcg/ml). It is not clear why this third study, done after the first two, utilized such a considerably lower high dose level of Propoxur, particularly when it had been demonstrated that there was evidence for cytotoxicity at this level.

D. DISCUSSION:

The study is acceptable. There was no evidence of a mutagenic response with or without activation under the assay conditions with a highest dose level of 10,000 mcg/ml.

006467

Reviewed by: Byron T. Backus, Toxicologist Byron T. Backus 05/21/87
Section III, Tox. Branch (TS-769C)

Secondary Reviewer: Marcia Van Gemert, Ph.D., Section Head
Section III, Tox. Branch (TS-769C) [illegible] 5/24/87

DATA EVALUATION REPORT III

STUDY TYPE: Mutagenic - Human Lymphocyte culture. TOX. CHEM. NO.: 508

ACCESSION NUMBER: 266038 MRID NO.: not given

TEST MATERIAL: Baygon, Propoxur

STUDY NUMBER: Study No. T 2018788

SPONSOR: Mobay Chemical Corp.
Kansas City, MO

TESTING FACILITY: Bayer AG Institute of Toxicology

TITLE OF REPORT: BOQ 5812315 c.n. Propoxur - Sister Chromatid Exchange in Human Lymphocyte Cultures in vitro to test for DNA-modifying effects

AUTHOR(S): Herbold, B.

REPORT ISSUED: October 9, 1985

STUDY CLASSIFICATION: Without S-9 activation: Acceptable
With S-9 activation: Not Acceptable

CONCLUSIONS:

1. No evidence was found of an increased incidence in SCE in human lymphocytes as a result of exposure of up to 500 ug/ml Propoxur (without S-9 activation) or at up to 1000 ug/ml Propoxur (with rat S-9 activation). However, a sufficient level of cytotoxicity at highest dose was demonstrated only in the absence of S-9.

2. Without S-9 activation, the positive control elicited the appropriate response on 3/4 occasions. The one time it failed to result in a significantly increased incidence of SCE, the high-dose (500 ug/ml) exposure level to Propoxur was not evaluable. However, after considering the overall combined results (from both blood cultures) on that one occasion, it is concluded that there was sufficient evidence of a lack of induction of SCE in the absence of S-9.

006467

10

A. MATERIALS:

1. Test compound: BOQ 5812315, batch no. 234401740, 99.6% pure.

2. Positive controls: a) Mitomycin C, batch 1148865, an antibiotic which inhibits DNA synthesis, producing crosslinks in DNA, used without S-9 mix; and b) Cyclophosphamide, batch 044438, used only with S-9 mix.

3. "Lymphocytes from the blood of one male and one female healthy test person were used. There were four cultures per test group, two per donor." According to the text (p. 6) "blood was obtained fresh...on the test day." However, from the procedure reported actual exposure (of the blood culture) to the test substance did not occur until 48 hours later.

4. S-9 Mix: obtained from the livers of at least 6 adult male Sprague-Dawley rats, wts approx. 200-300 g, which had been given IP injection of aroclor 1254 at 500 mg/kg five days before preparation

B. STUDY DESIGN:

1. "Twenty-four hours after start of cultivation, 100 ug bromodeoxyuridine...was added per culture."

At 48 hrs: "the test substance, dissolved in DMSO at a volume of 0.1 ml, was added to the culture. For the negative control only the solvent was added. The positive controls cyclophosphamide and mitomycin C were added...dissolved in a volume of likewise 0.1 ml."

"0.1 ml S-9 mix was...added to...cultures with metabolic activation. To reduce toxic side-effects of the S-9 mix, these cultures were washed 2.5 hours after it had been added, and then again provided with bromodeoxyuridine." "0.1 ml Hank's saline solution was...added to the test group cultures without metabolic activation." "The total volume in all the cultures was therefore 10.1 ml...a volume of 10 ml was assumed for calculation of the concentrations."

"Colchicin was added to the cultures in a final concentration of 0.4 ug/ml 21 hours after the addition of the substance... Cultures were prepared (fixed?) three hours later. Two to three slides were produced per culture, which were stained by Schwarzacher and Wolf's method."

"The concentrations used were based on a pilot test, in which 5000 ug/ml, 500 ug/ml, 50 ug/ml, 5 ug/ml and 0.5 ug/ml were added to two cultures each. At 5000 ug/ml no mitoses were observed. The highest concentrations of 1000 ug/ml with S-9 mix and 500 ug/ml without S-9 mix were therefore chosen for the main test."

Two replicate studies were run.

2. Evaluation: "Twenty metaphases each in one male and one female culture were examined per concentration, and the incidence of sister chromatid exchanges per metaphase was found, the slides being scanned in a meandering pattern."

"The chi square test was used to statistically assess the mitosis index. A difference was considered significant if the error probability was below 1%. For the statistical assessment of the SCE rate the mean and 1s range were calculated for each culture of a treatment group. In addition, the Wilcoxon rank test was used to assess this group. Here a difference was considered significant if the error probability was below 1%, and/or if the mean was higher by a factor of 1.4 than the... negative control."

3. Quality assurance: The statement is made (p. 4) that the test complied with the OECD GLP Principles. While there is a quality assurance statement on p. 14 dated October 2, 1985, there is no signature.

C. RESULTS

Mitotic indices (4000 nuclei evaluated/dose):
First run:

Substance Tested	Dose mcg/ml	Number of Mitoses: without S9	As % neg. control	with S9	As % neg. control
Negative control	-	96	100	74	100
Propoxur	125	61	63.5	-	-
"	250	52†	54.2	58	78.4
"	500	30†	31.3	55	74.3
"	1000	-	-	41	55.4
Mitomycin C	0.01	91	94.8	-	-
Cyclophos-phamide	2.0	-	-	40	54.1

† P reported as $\leq$ 0.01 by chi^2 Test

Second run:

Substance Tested	Dose mcg/ml	Number of Mitoses: without S9	As % neg. control	with S9	As % neg. control
Negative control	-	58	100	42	100
Propoxur	125	35	60.3	-	-
"	250	26†	44.8	42	100
"	500	33	56.9	74	176.2
"	1000	-	-	29	69.1
Mitomycin C	0.01	79	136.2	-	-
Cyclophos-phamide	2.0	-	-	41	97.6

† P reported as $\leq$ 0.01 by chi^2 Test

First run:
Without S-9:

Substance Tested	Dose mcg/ml	"SCE rate" Male mean SCE/ mitosis	Male S.D.	Female mean SCE/ mitosis	Female S.D.
Negative control	-	14.95	4.87*	11.50	3.32
Propoxur	125	14.25	6.81	15.60	6.12
"	250	16.60	5.20	14.70	5.06
"	500	" not evaluable"		16.50	7.07
Mitomycin C	0.01	17.65	6.09	39.55¶	8.61

*Reported as 5.31 (table 4); value given above is calculated from data appearing in table 2.
†Reported as 5.30 (table 4); value given above is calculated from data appearing in table 2.
¶Significantly different from controls at $p \leq 0.01$ by the Wilcoxon rank test.

First run:
With S-9:

Substance Tested	Dose mcg/ml	"SCE rate" Male mean SCE/ mitosis	Male S.D.	Female mean SCE/ mitosis	Female S.D.
Negative control	-	17.00	6.00	14.20	4.82
Propoxur	250	16.10	4.51	14.40	4.62
"	500	15.35	4.38	13.90	5.15
"	1000	18.15	5.55	12.25	3.99
Cyclophos-phamide	2	30.65¶	9.98	15.10	5.33

¶Significantly different from controls at $p \leq 0.01$ by the Wilcoxon rank test.

Second run:
Without S-9:

Substance Tested	Dose mcg/ml	"SCE rate" Male mean SCE/ mitosis	Male S.D.	Female mean SCE/ mitosis	Female S.D.
Negative control	-	10.85	3.57	9.75	3.46
Propoxur	125	10.80	3.98	11.05	5.44
"	250	15.00	6.71	12.55	4.96
"	500	12.35	4.02	11.80	4.07
Mitomycin C	0.01	16.25¶	4.53	16.00¶	6.10

¶Reported as significantly different from controls at $p \leq 0.01$ by the Wilcoxon rank test.

Second run:
With S-9:

Substance Tested	Dose mcg/ml	"SCE rate" Male mean SCE/ mitosis	Male S.D.	Female mean SCE/ mitosis	Female S.D.
Negative control	-	10.80	3.37	10.90	5.10
Propoxur	250	12.20	3.38	11.00	3.26
"	500	12.65	5.05	10.30	3.05
"	1000	12.95	5.32	12.50	2.63
Cyclophos-phamide	2	26.05¶	8.41	23.80¶	6.90

¶Significantly different from controls at $p \leq 0.01$ by the Wilcoxon rank test.

D. DISCUSSION:

No evidence was found of an increased incidence in SCE in the human lymphocyte cultures used as a result of exposure of up to 500 ug/ml Propoxur (without S-9 activation) or at up to 1000 ug/ml Propoxur (with rat S-9 activation). However, a sufficient level of cytotoxicity at highest dose in concurrent assays was demonstrated only in the absence of S-9.

Without S-9 activation, the positive control elicited the appropriate response on 3/4 occasions. The one time it failed to result in a significantly increased incidence of SCE, the high-dose (500 ug/ml) exposure level to Propoxur was not evaluable. However, after considering the overall combined results (from both blood cultures) on that occasion, it is concluded that there was sufficient evidence of a lack of induction of SCE.

The study was acceptable (although only marginally so) without S-9 activation. That part of the study which involved S-9 activation is classified as not acceptable (because of lack of evidence for cytotoxicity at the highest dose level).

Reviewed by: Byron T. Backus 01/21/87
Section 3, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. 8/24/87
Section 3, Tox. Branch (TS-769C)

006467

DATA EVALUATION REPORT IV

STUDY TYPE: Subchronic (13-week) feeding rhesus monkey

TOX. CHEM. NO.: 508

ACCESSION NUMBER: 266038

MRID NO.: not given

TEST MATERIAL: Propoxur

SYNONYMS: Baygon, BOQ 58 12 315

STUDY NUMBER(S): Report no. 13779

SPONSOR: Mobay Corporation

TESTING FACILITY: Bayer AG Institute of Toxicology

TITLE OF REPORT: Subchronic study of toxicity to rhesus monkey after oral administration by stomach tube for 13 weeks to check for possible findings in the urinary bladder

AUTHOR(S): Hoffmann, K. and Ruehl C.

REPORT ISSUED: August 27, 1985

STUDY CLASSIFICATION: Core Supplementary Data
Special Review Criteria (40 CFR 154.7)

CONCLUSIONS:

1. The value of this study is somewhat limited, in part because of the lack of simultaneous controls, administration of the test material at only one dose level, and considerable variation in the test animals (weights: 3.37-10.03 kg; ages: 2.5-6 yrs; origins: two from China, one from Charles River Labs, U.S.A., two having been bred in the laboratory).

2. There was no evidence of any bladder changes similar to those observed in a previously conducted rat chronic feeding/oncogenicity study. However, the test material was given as part of the diet in that study (at levels of 200, 1000 and 500 ppm) and the study duration was longer than 2 yrs.

3. A noteworthy finding in this study was the occurrence of considerable (50% or more) plasma ChE inhibition at one hour after administration of 40 mg/kg of the test material. Whether this was the maximum level of plasma ChE inhibition is unknown. It is also noteworthy that there were possible (but not definite) indications that RBC ChE inhibition may also have occurred, and that symptoms of ChE inhibition were also present in the monkeys. From these considerations, 40 mg/kg is a maximally tolerated dose (MTD) level in the rhesus monkey when administered on a daily single dose basis.

006467

15

A. MATERIALS:

1. Test compound: BOQ 58 12 315: Analysis by HPLC gave 99.6% active. 006467

2. Test animals: Species: rhesus monkey; three males and three females; Ages: 2.5-6 yrs, Weight: 3.37-10.03 kg, Source: four "...obtained at various times, and transferred after quarantine to the Institute's primate station, while two of them were bred by the Institute itself."

B. STUDY DESIGN:

1. Dosage level: All animals were dosed at 40 mg propoxur/kg body weight. "A special control group...was not used, since adequate experience (basic values) are available in respect to the examination criteria."

2. Test material preparation: The test material was suspended at a at a concentration of 0.5% in a 0.5% tylose solution. The suspension was given daily by stomach tube at an administered volume of 8 ml/kg body weight. The volume given was varied, on an individual basis, according to weekly body weight.

3. Animals received food (Altromin GmbH 6014 breeding diet for monkeys, up to 150 g/day), and fresh fruits and vegetables (apples, bananas, carrots, cabbage). Tap water was available ad libitum.

4. Statistics - Although a number of means (body weights, plasma and RBC ChE, absolute and relative organ weights) are presented (in addition to the individual values), the only comparison made was with mean bladder weights of monkeys from previous studies. This comparison utilized only means ± standard deviations.

5. Quality assurance: There is a quality assurance statement on p. 26, dated August 19, 1985. Although there is space designated for a signature, there is no signature.

C. METHODS AND RESULTS:

1. Observations:

Animals were inspected daily for signs of toxicity, appearance, and mortality.

Results:

All the animals survived until termination.

All of the animals coats are described as "smooth, glossy and groomed."

Four of the animals occasionally showed transient signs ("twitching in the head, limb and chest areas, salivation") typical of cholinesterase inhibition. These appeared shortly (approximately 15 minutes) after administration of Propoxur.

2. Body weights:

Animals were weighed twice before the initiation of the study and weekly tnereafter.

Results:

Two of the males had weight losses. In one of the animals (the heaviest one) there was a loss of 0.61 kg (or 6% from the week -1 value). All three females anu one male had slight weight gains.

3. Food consumption:

Results:

Apart from one animal in the second week of treatment, all the monkeys are reported as consistently consuming their feed rations.

4. Ophthalmalogical examinations

Ophthalmalogical examinations were not performed.

5. Blood was collected in the 12th and 13th weeks of the study, before treatment, and 1 and 24 hrs afterwards. Cholinesterase (plasma and RBC) activities were determined.

Results:

There was some depression (52% and 53.7%) in mean plasma ChE activities at 1 hour after dosage, with recovery by 24 hours. Mean RBC ChE activities were also somewhat depressed (25.5% and 6.7%) one hour after administration, but probably not significantly so.

6. Urinalysis*

Urine was collected from animals at 9 and 13 weeks. The CHECKED (X) parameters were examined.

	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*		Urobilinogen

* Required for chronic studies
* Not required for subchronic studies

Results: Findings were physiologically normal.

7. Sacrifice and Pathology -

All animals were sacrifice by exsanguination under deep anesthesia. Those organs marked with XX were examined and also weighed. Those organs designated with an X were fixed in Bouin's fluid or 4% aqueous formaldehyde (however, there is no indication that these organs were examined).

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	X	.Aorta*	XX	.Brain*†
	.Salivary glands*	XX	.Heart*	X	Periph. nerve*#
X	.Esophagus*		.Bone marrow*		Spinal cord (3 levels)*#
X	.Stomach*	X	.Lymph nodes*	X	.Pituitary (Hypophysis)*
X	.Duodenum*	XX	.Spleen*	X	Eyes (optic n.)*#
X	.Jejunum*	XX	.Thymus*		Glandular
X	.Ileum*		Urogenital	XX	.Adrenals*
X	.Cecum*	XX	.Kidneys*†		Lacrimal gland#
X	.Colon*	XX	.Urinary bladder*	X	Mammary gland*#
X	.Rectum*	XX	.Testes*†	X	.Parathyroids*
XX	.Liver*†	X	Epididymides	XX	.Thyroids*
X	Gall bladder*#	XX	Prostate		Other
XX	.Pancreas*		Seminal vesicle	X	Bone*#(sternum, femur)
	Respiratory	XX	Ovaries*†	X	Skeletal muscle*#
	.Trachea*	X	Uterus*		Skin*#
XX	.Lung*	X	Ureters		All gross lesions and masses*

* Required for subchronic and chronic studies
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies

Note: thymus weights were only determined for the three males, and kidney weights were not determined for one male.
Ureters were taken from 3 females and 1 male.

Results:

Gross pathology:

There were the usual "slight alterations" in the lungs of several of the animals, and two monkeys also "showed slight redness or scarred recesses in the stomach fundus." All of the urinary bladders are reported (p. 14) as being "macroscopically normal."

Organ weights:

There was no indication of anything out of the ordinary for organ weights. It is stated (p. 15) that: "absolute organ weights varied individually to match the animals' differing body weights."

The mean urinary bladder weight (males and females combined) was 6.90 ± 1.74 (mean ± S.D.) grams; in terms of relative body weight the mean was 1.37 ± 0.39 g/kg. These are compared with "weights from previous studies with rhesus monkeys" which gave means of 6.62 ± 1.91 gm and 1.31 ± 0.36 g/kg respectively.

Histopathology:

From p. 16: "Histopathological examination of kidneys, ureters, and urinary bladders did not detect any indications of treatment-related organ alterations, in particular no urothelial hyperplasia was observed." The incidental findings which are reported (cellular or inflammatory cellular infiltration in 3/6 urinary bladders, 1/4 ureters and 2/6 kidneys) appear to have been minor and unrelated to dosing with Propoxur.

D. DISCUSSION:

The value of this study is somewhat limited, in part because of the lack of simultaneous control animals, the administration of the test material at only one dose level, and what appears to have been considerable variation in weights (3.37 - 10.03 kg), ages (2.5-6 yrs), and sources of the test animals (two having come from China, one from Charles River, U.S.A. and two having been bred at the laboratory).

A noteworthy finding was the occurrence of considerable (50% or more) plasma ChE inhibition at one hour after administration of the test material at 40 mg/kg. Whether this was the maximum level of plasma ChE inhibition is unknown. It is also noteworthy that there were possible (but not definite) indications that RBC ChE inhibition may have also occurred. From the symptoms of ChE inhibition that were present, 40 mg/kg is probably a maximally tolerated dose (MTD) level in the rhesus monkey when administered on a daily single dose basis.