~~Reactivation~~
Case 706A

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

MEMORANDUM AUG - 2 1982

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: William Miller (16)
Registration Division (TS-767)
and
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

SUBJECT: Safrotin EC Insecticide; EPA Reg.#11273-22; Propetamphos; PP#2H5349; Propetamphos in/on food handling establishments at 0.01 ppm. CASWELL#706A Accession Nos: 247475 - 247488

Recommendations:

1) The registration and requested tolerance in food handling establishments are not toxicologically supported.

2) The 3-generation rat reproduction/teratology study is only acceptable as Supplementary Data. Food consumption, body weight and toxic signs data were not reported for maternal and paternal animals. The definitions of fertility index, gestation index, viability index, lactation index I, and lactation index II were not presented in the report. There is no indication of maternal toxicity at 20 ppm in the diet. Maternal toxicity is required for teratologic evaluation. There was no visceral examination of fetuses in the teratologic portion of the study. The report does not describe the fetal variations, ossification retardations, and minor and major malformations. A rat teratology study which demonstrates maternal toxicity at the high-dose is required to be submitted.

3) The special rat cholinesterase carpet study is required to be submitted.

4) The 2-year rat chronic/oncogenic study is only acceptable as Supplementary Data. A summary table of the incidences of non-neoplastic lesions is required to be submitted. Additionally, explanation of the MTD for the study is required.

5) The chronic mouse study is considered as Supplementary Data. An explanation of the MTD of the study is required. There is a discrepancy in the report relating to the number of animals surviving the study and the number of animals at termination.

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The survival incidence at termination of the study is summarized as follows:

Group mg/kg/day	Week 92 Males	Females
Control I	14/50	24/50
Control II	31/50	28/50
0.05	6/10	7/10
1.0	21/50	27/50
6.0	28/40	23/40
21.0	32/50	24/50

The number of animals at terminal kill were:

Group mg/kg/day	Males	Females
Control I	14	27
Control II	31	28
1.0	21	26
6.0	28	23
21.0	29	20

The discrepancy has to be resolved.

Section F - Proposed Tolerance

A food additive tolerance of 0.01 ppm of propetamphos, [(E)-1-methylethyl 3-[[(ethylamino)methoxyphosphinothioyl] oxy]-2-butenoate] in or on food resulting from the application of Safrotin® EC Insecticide in food handling establishments is requested.

Review:

1) Propetamphos: Single dose rat pharmacokinetics: San 52-139-C^{14}, absorption, blood level, distribution and excretion in the rat (Sandoz report CBK 3034/81; 10/24/79)

Female Wistar strain rats weighing approximately 250 grams were used in all the studies.

The single oral dose was administered to each animal by gavage at the following concentrations:

Group Level mg/kg	Dose Concentration San 52-139-C^{14}/ml of PEG
0.6	0.15
6.0	1.5
16.0	4.0

For the determination of blood and tissue concentrations of radioactivity following either the 0.6 mg/kg or the 6 mg/kg dose, groups of 3 rats each were sacrificed at 1, 2, 4, 8, 24, 72, and 96 hours after the dose. Additional blood samples were obtained from separate groups of animals at 0, 0.5, 6, 12 and 48 hours after dosing. The animals were sacrificed by ether anesthesia and exsanguination. Blood samples were obtained using heparinized syringes. Organs and tissues were dissected from the carcass, placed in individual plastic bags, and frozen prior to weighing and analysis. Small tissues like bone marrow, cartilage, and ovaries were weighed onto 1 x 1 inch filter papers for the combustion assay as soon as possible after the dissection to minimize drying of samples.

To determine the excretion of radioactivity after an oral dose of SAN 52-139-C^{14}, 10 rats were dosed per dose level and quartered individually in Misco metabolism cages. Urine and feces were collected quantitatively at intervals of 0-12, 12-24, 24-48, 48-72, and 72-96 hours. Methanol and water cage washings were made at the end of the study period.

To determine the excretion of radioactive CO_2 in exhaled air, four rats were dosed per dose level and placed in Delmar glass metabolism cages. Twenty-five milliliters of an ethanolamine-methanol solution were used in each CO_2 trap. This trapping solution was changed frequently during the study.

The data were presented in terms of F-values, percent of radiolabeled C^{14} propetamphos recovered and ug equivalents/ml; where $F = \frac{\text{concentration (ug/ml of blood or g of tissue)}}{\text{Dose (ug/g of body weight)}}$.

Results:

Following the 0.6 mg/kg oral dose, at 30 minutes (first measurement) the mean level of radioactivity was F = 0.103. The blood concentrations then increased to an F = 0.118-0.120 at 4-8 hours, the F-value corresponding to an absolute concentration of 0.072 ug equivalents/ml of whole blood. The blood levels then declined gradually to F = 0.064 at 48 hours and 0.042 at 96 hours. The elimination of radioactivity from the blood was monophasic in nature with an estimated half-life of 60 hours.

After the 6 mg/kg oral dose, the blood levels were relatively lower than those seen after the 0.6 mg/kg dose. The maximum blood concentration of F = 0.106 (corresponding to 0.636 ug equivalents/ml) was measured at 4 hours after the dose. The blood radioactivity then declined slowly to F = 0.041 at 48 hours and F = 0.031 at 96 hours. The elimination of radioactivity was biphasic in nature with estimated half-lives of 12 and 110 hours.

Following the 0.6 mg/kg dose, the highest concentration of radioactivity for most tissues studied was observed at either 2 or 4 hours. Lung, spleen, bone marrow, ovaries, and possibly uterus however peaked at 8 hours, and the skin at 24 hours. Peak concentration of bone marrow, liver, lungs, uterus, kidneys, spleen, skin and ovaries were greater than that of blood. The decline of concentration of radioactive material from the tissues was gradual. At 96 hours after the dose, all the tissues investigated had measurable concentration of radioactive material.

The amount of radioactive material in the stomach at 1 hour after the dose was 59.0% of dose. This amount decreased to 7.9% at 8 hours and <1% at 24 hours. The small and large intestines contained small amounts of radioactivity during the entire study period, the amount ranged from 4.3 to 0.3% of the dose.

After the 6 mg/kg dose, uptake of radioactive material in the tissues was similar to that observed after the 0.6 mg/kg dose. Most of the tissues reached a maximum concentration at either 2 or 4 hours after the dose, with the exception of bone marrow, uterus, fat and cartilage. At the peak, the following tissues had higher concentrations than blood: bone marrow, lungs, liver, kidneys, uterus, ovaries, spleen and skin. Only heart, cartilage, brain, muscle, and fat had equal or lower concentrations of radioactive material than blood. The decline from the tissues was gradual with measurable concentrations observed in all the tissues at 96 hours after the dose.

At 1 hour after the dose, the stomach contained 77.3% of the dose which decreased to 1.4% within 24 hours. The small and large intestines contained small amounts of radioactivity which ranged from 4.4 to <0.1% of the dose during the study period.

The urinary excretion rate was highest in the 0-12 hour interval. The subsequent rates were such lower. During the 0-96 hour collection, 12.4% of the 0.6 mg/kg dose, 19.7% of the 6 mg/kg dose, and 38.4% of the 16 mg/kg dose were recovered in the urine.

The total radioactive dose in the urinary and fecal excretion plus the cage was amounted to 19.4 $\pm$ 1.0% of the 0.6 mg/kg dose, 26.5 $\pm$ 4.1% of the 6 mg/kg dose, and 55.2 $\pm$ 5.8% of the 16 mg/kg dose.

Following the 0.6 mg/kg dose, 49.5% of the dose was excreted during the initial 7 hour interval in the exhaled air $^{14}CO_2$. During the same time interval, a total of 46.0% of the 6 mg/kg dose was excreted by this elimination route. During the 0-48 hour collection interval, 34.6% of the 16 mg/kg dose was eliminated in the exhaled air.

Conclusion:

The radioactivity in the tissues like fat, bone marrow, etc. at hour after the dose may be the result of one or all of the following:

a) SAN 52-139 and/or its metabolite(s) bound to the cellular components.

b) Degradation of the carbon side chain of SAN 52-139 to C-2 and C-1 fragments during the biotransformation, followed by the incorporation of these fragments into the endogenous materials such as fatty acids, aminoacids, sugars, triglycerides, etc.

Of the above two possibilities, the second one is more likely because of the high proportion of the administered dose being eliminated in the exhaled air as $^{14}CO_2$.

Classification: Core-Minimum Data

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2) Propetamphos: Multiple Dose rat pharmacokinetics: Absorption, excretion, tissue distribution, and tissue binding (Sandoz report CBK#3051-81015; March, 1982)

Young adult male and female Wistar strain rats weighing about 250 grams were used in the studies as follows:

°Tissue Distribution Studies:

Three groups of 10 rats (5 male + 5 female)

°Blood Collection Studies:

10 rats (5 male + 5 female)

°CO_2 Collection Studies:

4 rats (2 male + 2 female)

Each animal received a single, oral dose (16 mg/kg for 2 days and 6.4 mg/kg for 5 days) of unlabeled propetamphos daily for seven conservative days. On day 8, a single oral radiolabeled propetamphos dose (6.4 mg/kg/64 uCi/kg) was administered as a solution in PEG-400.

Whole blood (50 ul) was collected serially from the tail vein from two groups of rats (5 females, 5 males) with the aid of a heparinized capillary tube. Upon collection, this tube was broken and placed in scintillation vial containing methanol (2 ml). The container was then shaken to extract all the blood from the tube, followed by addition of a scintillation cocktail. Collections were made at 0, 1, 2, 4, 12, 24, 72 and 120 hours following administration of the radioactive (last) dose.

From groups of 10 animals (5 females, 5 males) sacrificed at 24, 72 and 120 hours after the radioactive dose, the following tissues and organs were collected:

Liver	Heart	Blood
Lungs	Kidneys	Muscle
Spleen	Uterus	Fat
Brain	Gonad	Skin
Bone	Carcase	

All tissues and organs (whole) were collected in appropriately labeled preweighed plastic bags and frozen until analysis.

Results:

Tissue distribution showed no unusual accumulation of equivalent C^{14} - propetamphos.

Lung and skin had relatively higher levels than other tissues after 24 hours which is consistent with CO_2 respiration. Maximum levels found in total tissues were at 24 hours and represented 1% of the total dose.

Tissue binding studies showed nonfat residues to consist of 30% polar, 3% nonpolar and 35% non-extractable material for both male and female rats. Fat tissue residues for male and female rats consisted of 5% polar for both sexes, 45% nonpolar for males and 66% nonpolar for females, and 12% non-extractable material for males and 6% non-extractable material for females.

Conclusion:

The results indicate rapid metabolism of propetamphos and no bioaccumulation under steady state conditions.

Absorption of propetamphos is rapid and essentially complete.

Excretion of equivalent C^{14}-propetamphos consisted of essentially respired CO_2 at about 50%. Male rats appeared to have a higher metabolic rate than female rats.

Urinary and fecal exections were 40% and 7%, respectively. The percent recovery of administered dose eliminated by exhaled air, urinary, and fecal excretion is 94% for female rats and 99% for male rats.

Classification: Core-Minimum Data

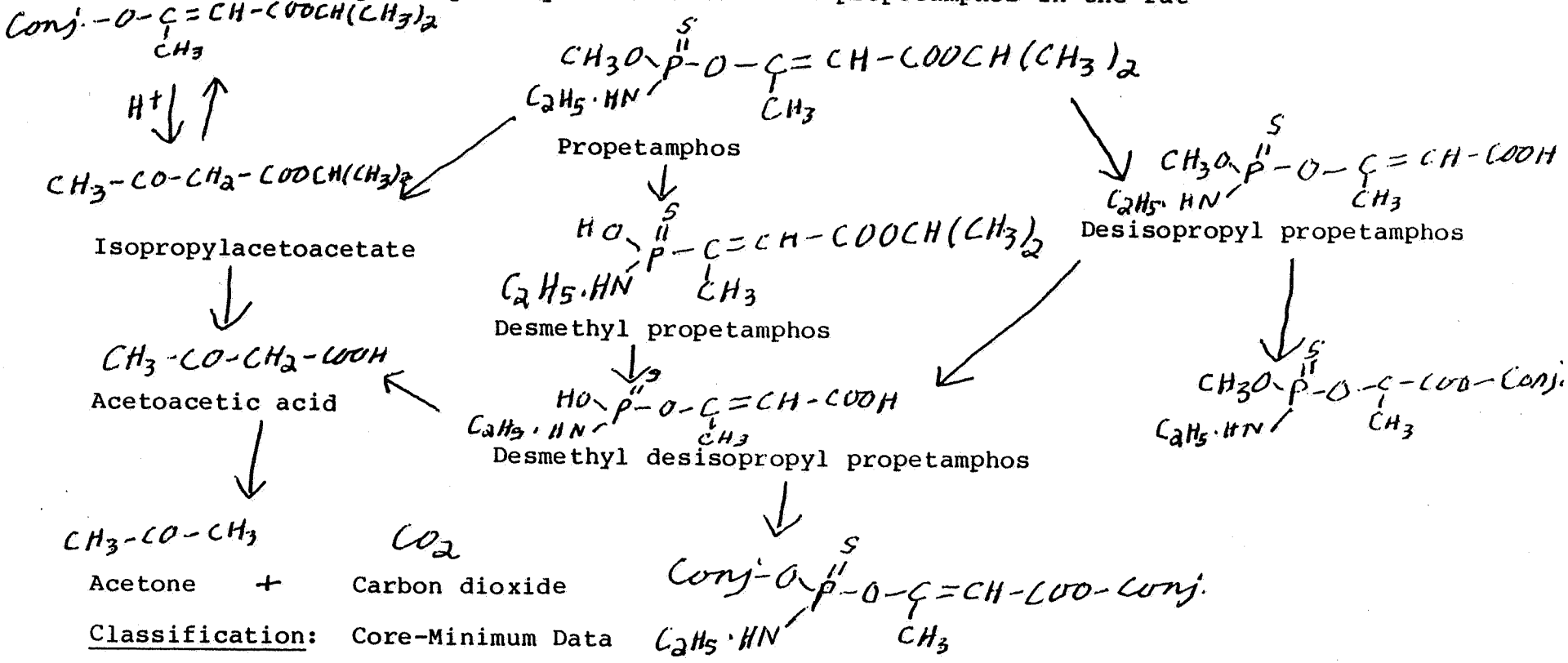
3) Propetamphos: Rat metabolism after single and multiple dose treatments (Sandoz report CBK 3055/82005W; March, 1982)

The radiolabeled material from Sandoz studies CBK 3034/81 and CBK 3051-81015 were identified by HPLC, TLC, Mass spectrometry, gas chromatography, and liquid scintillation counting in comparison to highly purified synthetic compounds.

Results:

Based on the results of the two previous studies and this study the registrant proposes the following metabolic fate for propetamphos in the rat:

Proposed pathway of metabolism of propetamphos in the rat



Classification: Core-Minimum Data

4) Propetamphos: 3-Generation Study in Rats (Sandoz Report No. 39/81; March, 1981)

Groups of 35 male and 35 female SPF Sprague-Dawley rats were administered 0 (control), 5, 10 and 20 ppm of technical propetamphos in the diet for three consecutive generations (F_0, F_1, F_2). Each parent generation was mated to produce two litters. Offspring from the second litters of the F_0 and F_1 parents (F_{1b} and F_{2b} litters, respectively) were selected to be parents for subsequent generations.

The first litter of each generation (F_{1a}, F_{2a} and F_{3a}) was reduced to 8 animals (4 males and 4 females) on day 4 post-partum if feasible. On day 21 p.p., the pups of ten mothers per dose group were x-rayed and their skeletons examined for abnormalities in case of relevant findings in the fetuses. The remaining pups were also observed for any abnormalities since birth up to day 21 p.p., then they were killed. Ten days later, the parent animals were again allowed to mate for the F_{1b}, F_{2b} and F_{3b}-generations. The observation of the copulatory plug was considered evidence of positive mating and the males were returned to single cages. In the 10 females of the teratogenicity test, the day the plug was found, was counted as day one of gestation. These ten pregnant females were killed by CO_2-asphyxiation and investigated for teratological findings.

For the teratological part, the following points were investigated:

Corpora lutea count
Number of implantation sites
Number of living and dead fetuses
Number embryonal fetuses
Number of resorption sites
Sex of individual fetuses
Average weight of placentae
Average weight of fetuses

The fetuses were dissected, eviscerated and subjected to skeletal cleaning and stained with Alizarin.

35 male and 35 female weanling animals per group of the F_{1b} and F_{2b}-generation were housed individually and received the treated diet for 100 or 120 days respectively. Then they were mated the same way as described above. Brother and sister pairings were avoided.

The animals of the F_{3b}-generation were observed for 21 days p.p. Then they were autopsied and the tissues of 10 animals per sex and dosage collected for histopathological investigation.

Samples of the following tissues were preserved in 4% Formaldehyde for subsequent processing:

lungs (right and left)	heart
liver	brain
spleen	spinal cord
kidneys (right and left)	sciatic nerve
stomach	sternum

The tissues were embedded in paraffin wax and the sections were stained with hematoxylin-eosine or SUDAN III.

In the reproduction study, the following data were collected for each litter and generation:

Identification of females and males mated

Fertility Index (%)

Gestation Index (%)

Viability Index (%)

Lactation Index I (%) 14 days

Lactation Index II (%) 21 days

Average number of pups per litter alive and stillborn, sex ratio

Average pup weight (g) on day 1, 4, 14 and 21.

Statistical analyses of the data were performed.

Results:

No food consumption, body weight, or toxic signs data were reported for maternal and paternal animals.

The tables below summarize the findings of the reproductive phase of the experiment.

Index / Dosage (ppm)	F_{1A}	F_{1B}	F_{2A}	F_{2B}	F_{3A}	F_{3B}
	%	%	%	%	%	%
Fertility - 0	86 (30/35)	94 (33/35)	80 (28/35)	74 (25/34)	70 (21/30)	70 (21/30)
5	83 (29/35)	83 (29/35)	83 (29/35)	86 (30/35)	63 (19/30)	67 (20/30)
10	80 (28/35)	86 (30/35)	77 (27/35)	80 (28/35)	67 (20/30)	73 (22/30)
20	80 (28/35)	83 (29/35)	79 (27/34)	82 (28/34)	53 (16/30)	70 (21/30)
Gestation - 0	100 (30/30)	100 (33/33)	100 (28/28)	100 (25/25)	95 (20/21)	95 (20/21)
5	100 (29/29)	100 (29/29)	100 (29/29)	100 (30/30)	95 (18/19)	100 (20/20)
10	100 (28/28)	100 (30/30)	100 (27/27)	100 (28/28)	100 (28/28)	100 (22/22)
20	100 (28/28)	100 (29/29)	100 (27/27)	100 (28/28)	94 (28/28)	95 (20/21)
Viability - 0	96 (403/418)	99 (322/327)	99 (320/323)	93 (159/171)	93 (218/234)	95 (124/130)
5	96 (342/357)	92 (278/302)	99 (373/376)	83 (222/267)	93 (202/217)	98 (92/94)
10	98 (321/328)	99 (295/299)	98 (293/298)	84 (199/238)	94 (202/215)	95 (137/144)
20	97 (318/329)	96 (255/267)	98 (300/306)	86 (191/222)	91 (152/168)	94 (95/101)
Lactation I - 0	86 (205/238)	63 (204/322)	99 (211/222)	69 (110/159)	96 (143/149)	79 (98/124)
5	84 (179/212)	76 (212/278)	97 (224/230)	31 (69/222)	98 (141/141)	85 (78/92)
10	90 (190/211)	82 (242/295)	99 (193/195)	51 (102/199)	92 (124/135)	98 (134/137)
20	83 (172/208)	65 (166/255)	95 (177/187)	63 (121/191)	89 (92/104)	99 (94/95)
Lactation II - 0	99 (205/207)	96 (204/212)	97 (211/217)	100 (110/110)	96 (143/149)	96 (98/102)
5	96 (179/187)	96 (212/220)	98 (224/229)	99 (69/70)	99 (141/142)	86 (78/21)
10	95 (190/200)	98 (242/248)	99 (193/195)	99 (102/103)	98 (124/127)	100 (134/134)
20	96 (172/179)	94 (166/177)	98 (177/180)	98 (121/123)	90 (92/102)	100 (94/94)

Index / Dosage (ppm)		F_{1A}	F_{1B}	F_{2A}	F_{2B}	F_{3A}	F_{3B}
Alive (mean per litter per group)	0	13.9	14.2	11.5	11.4	11.1	11.8
	5	12.3	14.4	13.0	14.1*	11.4	10.4
	10	12.1**	12.5	11.0	13.2	10.8	12.0
	20	11.8*	13.4	11.8	13.9**	10.5	9.2
Stillborn (mean per litter per group)	0	0.27	0.04	0.18	0.0	1.38	0.64
	5	0.62	0.19	0.17	0.21	1.36	0.11
	10	0.11	0.50**	0.19	0.22	1.60	0.58
	20	0.68	0.20	0.19	0.25	1.25	1.18
Sex ratio (Males) (Females)	0	0.93	1.21	0.72	0.84	0.95	0.73
	5	0.94	0.99	0.87	1.02	0.94	1.11
	10	0.87	0.94	0.70	1.10	1.04	0.84
	20	0.91	0.95	0.91	1.04	1.25	0.68
Weight day 1	0	6.8	6.7	6.7	7.6	6.3	6.3
	5	6.7	6.8	6.5	6.9**	6.3	6.9
	10	7.0	7.1	6.9	7.0*	6.5	6.8
	20	7.1	7.0	6.3	6.8*	6.4	6.7
Weight day 4 (before culling)	0	9.8	9.6	10.2	10.3	8.5	7.7
	5	9.7	10.2	10.0	8.5**	8.4	9.7**
	10	10.4	10.3	10.8	8.9**	8.8	8.7
	20	10.3	10.3	9.2**	6.8*	8.1	9.8*
Weight day 14	0	30.8	25.5	26.6	27.9	28.5	25.6
	5	31.1	26.6	27.6	23.8*	27.4	26.2
	10	31.7	27.2	29.3*	27.7	30.7	26.8
	20	31.9	27.9	27.7	25.6	28.8	27.5
Weight day 21	0	52.1	41.2	48.3	49.5	47.3	40.7
	5	50.8	42.3	46.9	43.2	46.4	44.7
	10	53.7	43.2	50.8	49.4	47.9	42.4
	20	54.2	47.2	50.4	46.8	49.3	46.1

*$p < 0.05$
**$p < 0.01$

The fertility values showed some differences in the F_{1a} and F_{3a} generations in both the test and control groups. Based on 904 historical control values, the mean value of fertility is 82.6% and a range of 65.7-100%.

With the exception of the low and high-dose group in the F_{3a} generation, the fertility values were within the historical controls.

These differences in fertility are not considered treatment related.

Gestation and viability values show no treatment-related effect. The values for viability in the F_{2b} generation are lower than controls, but are not dose-related and are not present in the subsequent generation.

The lactation I values of the F_{2b} low and mid-dose litters were lower than the controls, but the high-dose value was comparable to the controls. These differences in the low and mid-dose group are not considered treatment-related, since no effect was observed in the following generation or in other generations of the study. The effects at 5 and 10 ppm were attributed in the report to possible viral infection.

There were no remarkable differences in the lactation II values during the study.

In historical control data, the mean value for pups alive is 11.7 (10.2-13.3) based on a total of 8725 animals.

With respect to stillborn pups, the differences in the treated animals are comparable to differences in controls for the various litters.

In the historical control data of 8725 pups, the sex ratio was 0.94 (0.62-1.12). The control F_{1b} litters and the high-dose F_{3a} litters are different. These differences are not considered treatment-related.

Day 1 pup weights were lower than controls for each dose of the F_{2b} litters. This can be correlated to the findings of the lactation I values and may be due to the high control value of the weight of F_{2b} pups.

This finding is not considered treatment-related.

Day 4, day 14, and day 21 pup weights showed no treatment-related findings within the litters.

The table below summarizes the findings of the teratologic portion of the study:

Generation Dosage ppm	CL		IP		LF		FD	RS	LF		Placenta (g)	Fetus (g)
									Male	Female		
F_{1B}	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$							
0	18.0	(2.5)	15.2	(2.2)	15.2	(2.2)	-	-	5.4	9.8	0.9	5.6
5.0	21.0	(7.0)	17.0	(2.9)	16.6	(3.0)	0.4	-	5.4	11.2	0.9	5.5
10.0	21.0	(4.1)	16.2	(1.6)	16.0	(1.9)	0.2	-	5.4	10.6	0.9	6.0
20.0	17.4	(5.4)	14.4	(4.2)	14.2	(4.1)	0.2	-	5.2	9.0	0.9	5.4
F_{2B}												
0	18.7	(1.9)	14.2	(4.2)	14.0	(4.2)	-	0.2	8.3	8.3	0.9	3.2
5.0	16.7	(1.2)	13.6	(1.0)	13.6	(1.0)	-	-	5.7	5.7	1.0	4.8
10.0	18.4	(1.8)	14.7	(3.1)	14.6	(3.1)	-	0.1	7.7	7.7	0.9	3.7
20.0	19.3	(1.9)	15.0	(3.0)	14.7	(3.1)	-	0.3	8.4	8.4	0.9	3.6
F_{3B}												
0	17.6	(1.7)	15.4	(2.8)	15.1	(2.7)	-	0.3	6.7	6.7	0.9	4.2
5.0	16.8	(2.8)	15.2	(2.8)	14.6	(3.0)	-	0.6	7.4	7.4	1.0	4.9
10.0	16.6	(2.0)	14.2	(3.1)	13.8	(3.2)	-	0.4	7.4	7.4	1.0	4.8
20.0	17.4	(3.0)	14.8	(3.0)	14.6	(3.1)	-	0.2	7.3	7.3	1.0	5.0

Legend: CL = Corpora lutea
IP = Implantations
LF = Living fetuses
FD = Fetus deaths
RS = Resorptions

No treatment-related findings at any dose level were noted with respect to corpora lutea, implantations, live fetuses, dead fetuses, resorptions, placenta weight and fetus weight.

In the F_{1b} litters, the skeletons were examined. In the controls and in the high dose group, all fetuses had 13/13 ribs (right and left). In the low dose group, three out of 32 fetuses inspected had 14/13 ribs, the others 13/13 ribs. In the middle group 75 animals had 13/13 ribs, two 12/13 ribs, another one 12/12 ribs, whereby the 13th rib was punctiform. Two fetuses had 14/13 ribs.

In the F_{2b} litters, skeletal examination revealed the following. In the controls, 137 fetuses had 13/13 ribs, one fetus 12/12 and two 14/13 ribs. In the low dose group all 136 fetuses had 13/13 ribs, in the middle dosage 144 fetuses had 13/13 ribs, one 13/14 and another one 14/13 ribs. In the high dosage, 138 fetuses had 13/13 ribs, one 13/12 ribs, three 13/14 ribs and one 14/13 ribs; in the fetus with the 13/12 ribs, the 4th right rib showed only the ossification center and was not fully developed; three others showed a rudimentary rib 14 and in one case, an additional rib was observed between rib 3 and 4; additionally, in one fetus the 8th rib on the right side showed only one tenth of the normal length.

With respect to the F_{3b} litters, in the controls, 149 fetuses had 13/13 ribs and two 14/13. 144 fetuses of the low dose group showed 13/13 ribs, two 14/13 ribs. 13/13 ribs were observed in 135 animals of the middle group, one fetus each had 11/13, 13/14 and 14/13 ribs. In the high dose group 145 fetuses had 13/13 ribs, one 12/12.

In the F_{2b} generation, there were one minor and 2 major anomalies (not described in the report). The historical controls of 8731 animals showed an average of 0.24% (0.0-1.11) and 0.08% (0.0-0.45) of such (not described in report) anomalies.

No treatment-related effect was observed in ten litters per dose group which were necropsied after 21 days.

Histopathological examination of 10/sex/dose of F_{3b} weanlings did not show any treatment-related effects.

Conclusion:

Food consumption, body weight and toxic signs data were not reported for maternal and paternal animals in the reproduction and teratologic portions of the report.

The definitions of fertility index, gestation index, viability index, lactation index I and lactation index II were not presented in the report.

There is no indication of maternal toxicity at 20 ppm in the diet. The NOEL for reproduction may be 20 ppm. The report does not describe the teratologic variations (only ribs!), ossification retardations, and minor or major malformations.

Additionally, there was no visceral examination of the fetuses in the teratologic portion of the study. The report does not describe the fetal variations, ossification retardation, and minor and major anomalies.

A rat teratology study which demonstrates maternal toxicity at the high-dose is required to be submitted.

Classification:

Reproduction: Supplementary Data
Teratology: Supplementary Data

5) Evaluation of 52.139 in the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay (LBI Project No. 21001; July, 1981)

The test material, 52.139, was insoluble in WME medium at 3.3 ul/ml but dissolved easily in dimethylsulfoxide (DMSO) at a concentration of 100 ul/ml. DMSO was chosen as the vehicle for the study and stock solutions were prepared just prior to use in the assay. The stocks were prepared by serial dilution with DMSO and these stocks were subsequently diluted 1:100 into WME medium containing 1% fetal bovine serum to prepare the test concentrations. The treatments were initiated by replacing the media in the cell cultures with media containing the desired concentrations of test material. A cloudy appearance, due to undissolved test material, was observed in the cultures at concentrations from 100 nl/ml to 500 nl/ml, and a fine precipitate occurred at 1000 nl/ml. The test material appeared to remain completely soluble in WME at 50 nl/ml and lower concentrations.

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Results:

Test Condition	Concentration	UDS grains/ nucleus	Avg. % nuclei with > 6 grains	Avg. % nuclei with > 20 grains	Survival at 20-23 hrs %
Solvent control	1% DMSO	0.49	0	0	100.0
Positive Control (2-AAF)	0.05 ug/ml	11.84	70.0	17.0	97.8
Test Material:					
52.139	100.0 nl/ml	NUCLEAR GRAINS NOT COUNTABLE			0
52.139	50.0 nl/ml	1.13	2.0	0	53.0
52.139	25.0 nl/ml	0.74	0.7	0	60.8
52.139	10.0 nl/ml	0.59	0	0	77.6
52.139	5.0 nl/ml	0.55	0.7	0	86.1
52.139	2.5 nl/ml	0.71	0.7	0	95.9
52.139	1.0 nl/ml	0.73	1.3	0	98.0
52.139	0.5 nl/ml	0.81	0.7	0	97.5
52.139	0.25 nl/ml	0.85	2.0	0	100.8

Conclusion:

Propetamphos was not mutagenic in the UDS assay.

Classification: Acceptable

6) Mutagenicity Evaluation of SAN 52.139 in the Saccharomyces cerevisae Reverse Mutation Induction Assay (LBI Project#20998; August, 1981)

The test compound was examined for mutagenic activity in a series of in vitro yeast assays employing Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Arochlor-induced rats.

The dose range employed for the evaluation of this compound was from approximately 0.42 ul/ml to 125 ul/ml is test volumes of about 1.2 ml/well.

Results:

Strain S138

		Plate	Negative Control	Test Material Concentrations 1	2	3	4	5	6	7	8	Positive Control
NONACTIVATION TEST CONDITIONS	MUTANT COUNTS	1	0	1	0	0	1	1	0	0	1	25
		2	0	0	1	1	0	4	0	0	0	22
		3	1	1	2	4	1	4	2	0	0	22
		4	0	1	0	0	1	4	0	0	0	24
		Total	1	3	3	5	3	13	2	0	1	93
	SURVIVOR COUNTS ON DILUTION PLATES	1	59	50	30	72	51	64	67	63	50	65
		2	63	55	45	69	72	53	68	66	52	68
		3	45	49	61	64	70	53	61	67	52	54
		4	47	50	58	79	55	43	51	56	44	201
		Total	214	204	194	284	248	213	247	252	198	388
ACTIVATION TEST CONDITIONS	MUTANT COUNTS	1	4	2	7	1	6	0	2	0	0	13
		2	2	3	0	0	0	1	3	3	0	8
		3	0	3	4	1	3	2	5	0	0	16
		4	1	3	0	3	4	3	0	1	1	20
		Total	7	11	11	5	13	6	10	4	1	57
	SURVIVOR COUNTS ON DILUTION PLATES	1	56	66	61	45	33	45	56	30	44	17
		2	59	63	60	51	36	53	54	39	43	14
		3	52	62	61	54	39	35	45	45	44	12
		4	46	56	59	47	35	24	50	37	59	10
		Total	213	247	241	197	143	157	205	151	190	53

Strain S211

		Plate	Negative Control	Test Material Concentrations 1	2	3	4	5	6	7	8	Positive Control
NONACTIVATION TEST CONDITIONS	MUTANT COUNTS	1	2	1	3	2	0	2	0	0	4	703
		2	3	3	2	4	3	8	2	2	4	562
		3	1	0	3	3	3	5	1	1	3	525
		4	0	4	3	3	2	0	2	2	2	292
		Total	6	8	11	12	8	15	5	5	13	2082
	SURVIVOR COUNTS ON DILUTION PLATES	1	152	101	122	162	116	102	125	128	93	78
		2	136	128	128	126	90	110	81	160	89	69
		3	123	101	153	153	109	107	93	124	82	37
		4	113	136	158	158	101	105	97	107	73	104
		Total	524	384	487	599	416	424	396	519	337	288
ACTIVATION TEST CONDITIONS	MUTANT COUNTS	1	4	4	4	7	2	3	8	6	6	5
		2	2	4	4	3	1	4	6	4	6	14
		3	1	2	3	2	4	3	0	3	3	5
		4	2	4	4	3	2	2	–	–	–	3
		Total	9	14	15	15	9	12	19	17	20	27
	SURVIVOR COUNTS ON DILUTION PLATES	1	44	123	115	93	87	90	95	108	104	130
		2	91	85	120	93	95	85	91	132	92	157
		3	80	110	135	123	94	80	112	95	101	75
		4	108	79	81	86	82	63	78	89	85	87
		Total	323	397	451	395	358	318	376	424	382	449

The test material concentrations were as follows:

Code	Concentration Per Well (1.5 ml vol.)
1	0.5 ul
2	1.0 ul
3	10.0 ul
4	25.0 ul
5	50.0 ul
6	75.0 ul
7	100.0 ul
8	150.0 ul

Conclusion:

Propetamphos was not mutagenic in this assay.

Classification: Acceptable

7) Mouse Micronucleus Assay in CD-1 Mice (LBI, Sandoz AGRO DOK CBK 3163/78; October, 1978)

Doses were selected based on a preliminary toxicity test. The concentrations employed in the toxicity screen were chosen from a range covering the reported LD_{50} value for rats (provided by the sponsor). The following concentrations of test and control compounds were employed. Solvent control - 0.15 and 0.2 ml DMSO/mouse/day. Positive control - 0.6 mg/kg of TEM and test compound doses were 0.0009 ml/kg and 0.009 ml/kg.

A subchronic dosing regimen was used, consisting of a split dose with volumes administered 24 hours apart. The treatment covered a 30-hour period. Six (6) hours after the last dose, the animals were killed with CO_2 and the adhering soft tissue and epiphyses of one both-femurs removed. The bone marrow was aspirated from the bone into centrifuge tubes containing 5 ml fetal calf serum (one tube for each animal).

The tubes were centrifuged to pellet the tissue, the supernatant was drawn off and portions of the pellet were removed to slides and spread; the slides were then air dried. The slides were stained in May-Gruenwald solution and Giemsa followed by clearing in distilled water.

One thousand (1000) polychromatic erthrocytes per animal were scored. The frequency of micronucleated cells were expressed as percent (%) micronucleated cells based on the total polychromatid erthrocytes present in the socred optic field. The normal frequency of micronuclei in this mouse strain is 0.5%.

The negative control consisted of the solvent vehicle for the test compound. It was administered to the animals is 0.15 or 0.2 ml doses which were equivalent to the maximum amount given the experimental animals. Triethylene Melamine (TEM) was used as the positive control substance. It was given in an acute administration of 0.6 mg/kg.

Results:

Test	Concentration[a]	Route of Administration	Total Number of PCFs Scored[b]	Mean Percent Micronucleic M	F	C[c]	Slide ID Numbers[d]
Solvent Control (DMSO)	0.2 ml/male mouse/day	PO	8,000	0.5	0.6	0.6	6298-9-12
	0.15 ml/female mouse/day	PO					
TEM	0.6 mg/kg	IP	8,000	4.0	3.3	3.6**	6298-13-16
Propetamphos Tech 91.8%	0.0009 ml/kg	PO	8,000	0.5	0.5	0.5	6298-5-8
Propetamphos Tech 91.8%	0.009 ml/kg	PO	8,000	0.3	0.7	0.5	6298-1-4

[a]Chemical administered as a two day split dose.

[b]Only Polychromatic Erythrocytes (PCE) scored.

[c]Combined results from male and female animals.

[d]Numbers refer to the slides scored in this study. Each number has a "male" and "female" slide.

Analysis

Data analyzed by t-statistic.
Each animal constituted a data point.

*Significant at p = < 0.05

**Significant at p = < 0.01

Conclusion:

Propetamphos was not mutagenic in this assay.

Classification: Acceptable

8. Salmonella Mammalian Microsome in vitro Mutagenicity Test on SAN 52-139 (EG & G Mason Research Institute Study#025-695-424-1; May 9, 1980)

Technical propetamphos was tested at concentrations ranging from 0.1 to 10 ul per plate (trplicate plates) in Salmonella typhimurium strains TA-1538, TA-1537, TA-1535, TA-100 and TA-98 with and without metabolic activation. Metabolic activation included male rat liver microsomes induced by Aroclor 1254. Positive controls were tested.

Results:

Propetamphos was not mutagenic under the conditions of the assay.

Classification: Acceptable

9. Two-Year Chronic Feeding Study in Rats (Sandoz Report#AGRO DOK CBKI 5214/81; June 22, 1981)

Groups of 55 male and 55 female OFA rats (a Sprague-Dawley strain) were fed propetamphos in the diet at 0 (controls), 6, 12 and 120 ppm for 2 years. The animals were observed daily for symptoms and behavior. Body weight and food consumption was recorded weekly for the first 56 weeks and monthly thereafter. Hematology, blood chemistry, and urinalysis were investigated after 4, 8, 13, 27, 52, 91 (males only, termination), 104 and 109 weeks (females only, termination). Brain cholinesterase was also evaluated of 8/sex/group at termination.

Hematology included: hematocrit, hemoglobin, erythrocyte counts, total and differential leucocyte counts, reticulocyte counts; platelet count at half year interval and at termination. The indexes MCV, MCH and MCHC were also calculated. EDTA was used an anticoagulant.

Blood chemistry of the blood plasma (Li-heparinate as anticoagulant): fasting glucose, BUN, total protein, total cholesterol, cholinesterase, alkaline phosphatase, transaminases GPT and GOT and also erythrocyte cholinesterase, albumin, total bilirubin, lactate dehydrogenase, creatinine, calcium and potassium were introduced after six months on diet in the test battery.

Urinalysis (24 hours urine): volume, pH, specific gravity, protein, glucose, blood pigments, ketones and microscopic examination of sediments.

All animals from all dose levels which died during the study, killed moribund or sacrificed at termination received a gross necropsy and histopathological examination. Selected organs weighed at termination for 10/sex/group included: spleen, kidneys, liver, heart, brain, testes.

Representative samples of the following organs, tissues and of all tumors or anomalies of all died or sacrificed rats were fixed in 4% formaldehyde and processed histologically:

aorta	cerebellum
pancreas	cerebrum
large and small intestine	testes/ovaries
duodenum	pituitary
urinary bladder	ischias nerve
skin (mamma region)	lymph nodes
heart	spleen
liver (3 sections/ various lobes)	adrenals
	kidneys
lungs	prostate/uterus
stomach	seminal vesicle
esophagus	skeletal muscle
parathyroid	thymus, if present
thyroids	sternum (bone marrow)
salivary glands	eyes
trachea	bone marrow (femur)
tongue	all tumors and lesions

Statistical analyses of the data were performed and the level of significance was $p < .05$.

Results:

Males and females of the 120 ppm groups had red belly marks, alopecia, weakness and hyperflexia. Because of the high mortality rate in male rats, the males were terminated in week 91. Surviving females were terminated in week 109.

Survival was greater than 50% for both sexes of all groups at 18 months.

The following number of animals died or were sacrificed moribund during the study (including week 90 for males and week 109 for females).

ppm in diet	Males	Females
0	43/55	35/55
6	37/55	36/55
12	41/55	37/55
120	15/55	18/55

Both males and females of the high-dose group had a significantly lower mortality than the other groups.

Mean body weight gain of males and females of the high-dose group were significantly decreased in comparison to the controls and other groups up to week 67 for males and up to week 82 for females. Food consumption was unaffected by treatment.

Hematology, clinical chemistry and urinalysis data showed no treatment-related effects. However, BUN values in males increased beyond the normal range in all groups (though less in high-dose males). Creatinine was also elevated in mid-dose males (week 52) and in all treated and control groups in week 78 and 91.

No significant decrease in plasma or RBC cholinesterase inhibition was present in low-dose (6 ppm) rats as shown below:

CHOLINESTERASE ACTIVITY (% deviation vs. control)

Group/Month

Male (ppm)	1	2	3	6	12	18	21	24	25
				Plasma-ChE					
6	+1	+3	-9	-13	+1	-18	-2		
12	-3	+6	-14	-15	-4	-29	-2		
120	-30	-18	-33	-41	-23	-55	-49		
Female (ppm)									
6	+8	-3	-9	-17	-14	-6		-18	+8
12	-4	-2	-17	-23	-7	-11		-32	-23
120	-42	-55	-63	-63	-66	-55		-68	-52

RBC-ChE

Group/Month

Male (ppm)	1	2	3	6	12	18	21	24	25
6	-4	-4	-2	-11	-8	-4	-13		
12	-14	-20	-17	-21	-9	-12	-14		
120	-37	-42	-46	-48	-25	-37	-39		
Female (ppm)									
6	-5	-6	-7	-11	-12	-7		-9	-9
12	-16	-16	-17	-21	-18	-13		-12	-14
120	-42	-41	-41	-41	-41	-35		-37	-32

A moderate inhibition (-34 to -40%) of brain cholinesterase was noted in the high-dose rats only.

No treatment-related effect was noted with respect to organ weights.

No summary tables of the incidences of non-neoplastic lesions were reported. The incidence, type and distribution of benign and malignant tumors was similar in all groups.

The oncogenic potential was negative.

Conclusion:

The oncogenic potential was negative. The NOEL for ChE inhibition was 6 ppm. The NOEL for chronic toxicity was not determined since no summary tables of the incidences of non-neoplastic lesions were reported. Additionally, an explanation of the MTD of the study is required.

Classification: Supplementary Data

10. Lifetime Oral (Diet) Carcinogenicity/Toxicity Study in the Mouse on SAN 52-139 (WiL Project No. 79218; March 30, 1982)

Eight hundred (800) weanling Charles River CD-1 mice were randomly divided into six groups as follows:

Group No.	Dose Level mg/kg/day	Number of Males	Number of Females
I (Control I)	0.0	80	80
II (Control II)	0.0	80	80
III (Sub Low)	0.05	10	10
IV (Low)	1.0	80	80
V (Mid)	6.0	70	70
VI (High)	21.0	80	80

Routinely 50 males and 50 females were placed on study per group for toxicity and carcinogenicity assessment. Ten animals per sex, per group were also placed on study for a 12-month interim necropsy, as well as an additional ten animals for the 18-month interim necropsy, excluding Group III (0.05 mg/kg/day of SAN 52-139). For assessment of cholinesterase activity in plasma, erythrocyte, liver and brain, another ten animals were placed on study, excluding Group V. Additionally, Group III at 0.05 mg/kg/day dose level, with only ten males and ten females, was placed on study in order to evaluate a NOEL for cholinesterase activity. All animals on study were observed for daily clinical signs and mortality. All animals, excluding those animals placed on study for cholinesterase activity assessment, were palpated weekly for the presence or absence of masses. Body weights were recorded for all animals weekly through week 13, and every other week thereafter to termination. Individual food consumption was recorded weekly through Week 13 and every other week thereafter for 25 animals/sex/group, except for Group III, where all ten animals had food consumption quantitated.

Ten animals/sex/group, excluding Group III, were routinely bled via the retro-orbital sinus for a routine hematologic (hematocrit, hemoglobin, erythrocyte count, leukocyte count) and clinical chemistry (BUN, glucose, alkaline phosphatase, SGOT, SGPT, total protein) evaluation at 6, 12, and 18 months into the initiation of the study, and prior to termination of the study. Animals placed on study for cholinesterase activity (10 animals/sex) were similarly bled in week-1 and weeks 2, 6, 12, 24, 50, 77 and 86 for plasma and erythrocyte cholinesterase activity. As this

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latter group of animals expired or were sacrificed at termination of the study, liver and brain were excised and cholinesterase activity was assessed. All animals placed on study, excluding cholinesterase animals were necropsied irrespective as to type of death. Similarly, a histopathologic examination was performed on tissues stained with H&E and one section of liver stained with Oil Red O.

The following is the list of tissue collected at the 12 month, 18 month, terminal sacrifice and for animals found dead or sacrificed moribund.

adrenals
aorta
heart
lungs and bronchi
trachea
esophagus
thymus
respiratory lymph nodes
thyroids & parathyroid
pituitary
head, nasal cavity
salivary gland
mandibular lymph node
axillary lymph node
brain
liver
gall bladder
stomach, duodenum
spinal cord
sternebrae, vertebrae
larynx
jejunum, ileum & cecum
colon
mesenteric lymph nodes
spleen
pancreas
kidneys
bone, femur
prostate
bladder, urinary
ureters
vagina & ovaries
uterus
testes
skeletal muscle
sciatic nerve
skin
mammary gland
eyes and optic nerve
costochondral junction, rib
masses or lesions
rectum

Statistical analyses of the data were performed.

Results:

The animals available at terminal sacrifice summarized as follows:

Group mg/kg/day	Terminal Sacrifice Males	Females
Control I	14/50	27/50
Control II	31/50	28/50
0.05	6/10	7/10
1.0	21/50	26/50
6.0	28/40	23/40
21.0	29/50	20/50

There were no treatment-related effects on body weight, or toxic signs. A decrease in food consumption was noted for males and females at the high-dose. No consistent treatment-related effects were noted in hematology, clinical chemistry or organ weights.

There was a significant and dose-related decrease in plasma, RBC, liver and brain cholinesterase activity at 1, 6 and 21 mg/kg/day dose levels. The NOEL for inhibition of cholinesterase activity was 0.05 mg/kg/day.

The distribution of bronchoalveolar tumors is presented in Table I.

TABLE I

Lung

Dosage Level (mg/kg/day)	0		0		1		6		21	
Sex	F	M	F	M	F	M	F	M	F	M
No. Examined	70	70	68	70	69	70	60	59	70	70
No. in Group	70	70	68	70	69	70	60	59	70	70
Bronchoalveolar adenoma	7	5	4	5	2	11	6	10	5	5
Bronchoalveolar carcinoma	10	13	10	16	12	18	4	12	10	11
Bronchoalveolar adenoma + carcinoma	17	18	14	21	14	26	10	22	15	16

Bronchoalveolar adenomas were present at significant increased incidences in the lungs of low- and mid-dose group male mice. However, the relatively lower incidence in the high-dose group male mice and the lack of a dose-related trend is suggestive of a spontaneous and not a treatment-related effect.

Non-neoplastic lesions indicative of a treatment-related effect were hepatic vacuolization of the liver in high-dose males, interstitial nephritis in high-dose males and females, vacuolization of the brain in high-dose females and neural vacuolization of the sciatic nerve in high-dose females.

Conclusion:

The oncogenic potential is negative. The cholinesterase inhibition NOEL is 0.05 mg/kg/day. The NOEL for chronic toxicity is 6.0 mg/kg/day. Additionally, an explanation of the MTD of the study is required. There is a discrepancy in the report relating to the number of animals surviving the study and the number of animals at terminal kill as follows:

The survival incidence at termination of the study is summarized as follows:

Group mg/kg/day	Week 92 Males	Females
Control I	14/50	24/50
Control II	31/50	28/50
0.05	6/10	7/10
1.0	21/50	27/50
6.0	28/40	23/40
21.0	32/50	24/50

The number of animals at terminal kill were:

Group mg/kg/day	Males	Females
Control I	14	27
Control II	31	28
1.0	21	26
6.0	28	23
21.0	29	20

This discrepancy has to be resolved.

Classification: Supplementary Data

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8/11/82

TS-769:th:TOX/HED:WDykstra:7-19-82:card 5

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