



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

~~Reading~~  
Cas. 706A

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.

30 pages

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

<u>Group</u> <u>mg/kg/day</u>	<u>Week 92</u>	
	<u>Males</u>	<u>Females</u>
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:

<u>Group</u> <u>mg/kg/day</u>	<u>Males</u>	<u>Females</u>
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<sup>14</sup>, 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.

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

<u>Group Level</u> mg/kg	<u>Dose Concentration</u> San 52-139-C <sup>14</sup> /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<sup>14</sup>, 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<sub>2</sub> 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<sub>2</sub> trap. This trapping solution was changed frequently during the study.

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

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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.

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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  $^{14}\text{CO}_2$ . 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  $^{14}\text{CO}_2$ .

Classification: Core-Minimum Data

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<sub>2</sub> 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.

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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 excretions 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.

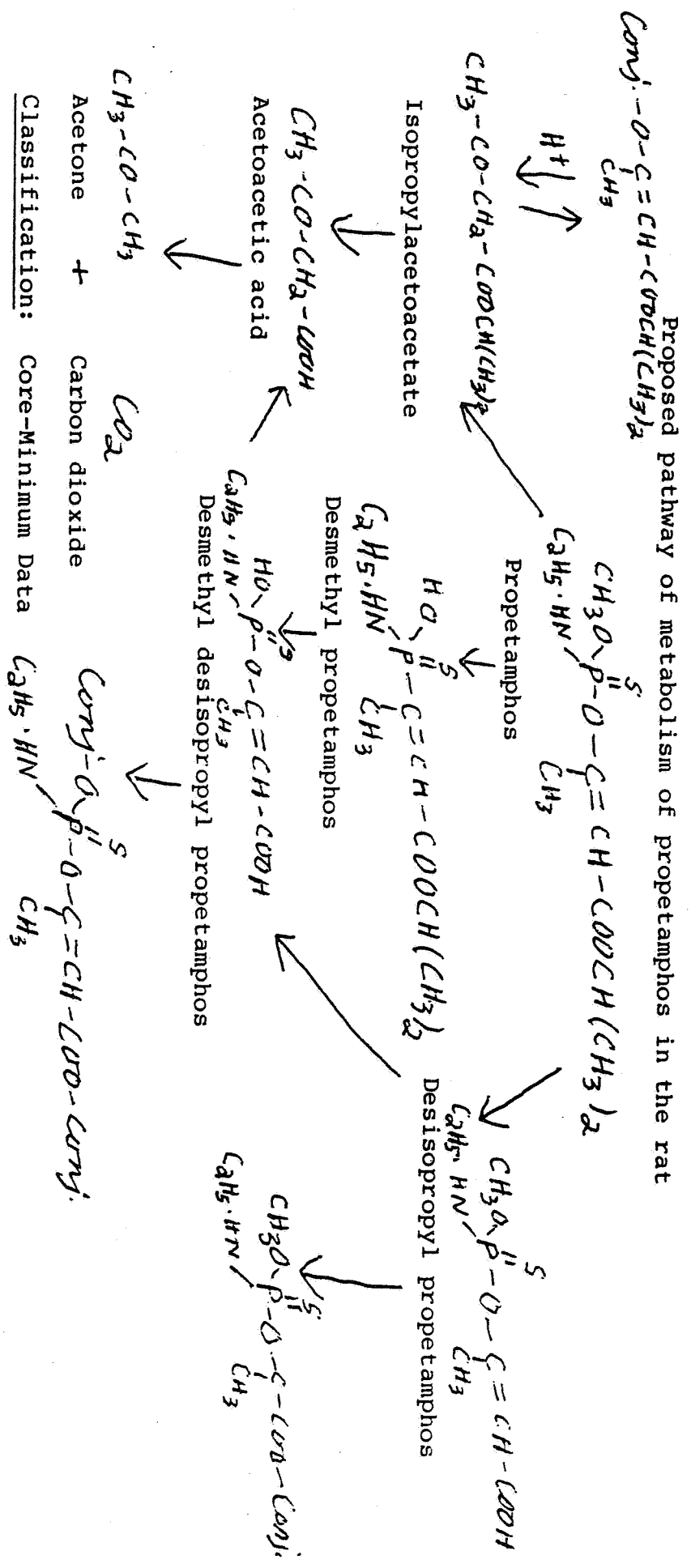
Classification: Core-Minimum Data

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:



Classification: Core-Minimum Data

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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<sub>0</sub>, F<sub>1</sub>, F<sub>2</sub>). Each parent generation was mated to produce two litters. Offspring from the second litters of the F<sub>0</sub> and F<sub>1</sub> parents (F<sub>1b</sub> and F<sub>2b</sub> litters, respectively) were selected to be parents for subsequent generations.

The first litter of each generation (F<sub>1a</sub>, F<sub>2a</sub> and F<sub>3a</sub>) 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<sub>1b</sub>, F<sub>2b</sub> and F<sub>3b</sub>-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<sub>2</sub>-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.

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35 male and 35 female weanling animals per group of the F<sub>1b</sub> and F<sub>2b</sub>-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<sub>3b</sub>-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	F1A	F1B	F2A	F2B	F3A	F3B
<u>Dosage (ppm)</u>	%	%	%	%	%	%
<u>Fertility</u> - 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)
<u>Gestation</u> - 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)
<u>Viability</u> - 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)
<u>Lactation</u> - 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)
<u>Lactation</u> - 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)
<u>Lactation</u> - 0						
5						
10						
20						

Index		F1A	F1B	F2A	F2B	F3A	F3B
Dosage (ppm)							
<u>Alive</u> (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
<u>Stillborn</u> (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
<u>Sex ratio</u> ( 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
<u>Weight</u> <u>day 1</u>	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
<u>Weight</u> <u>day 4</u> (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*
<u>Weight</u> <u>day 14</u>	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
<u>Weight</u> <u>day 21</u>	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<sub>1a</sub> and F<sub>3a</sub> 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<sub>3a</sub> 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<sub>2b</sub> 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<sub>2b</sub> 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<sub>1b</sub> litters and the high-dose F<sub>3a</sub> litters are different. These differences are not considered treatment-related.

Day 1 pup weights were lower than controls for each dose of the F<sub>2b</sub> 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<sub>2b</sub> 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	Male	Female	Placenta (g)	Fetus (g)
<u>F<sub>1B</sub></u>	$\bar{x}$	$\bar{x}$							
	s.d.	s.d.							
	$\bar{x}$	$\bar{x}$							
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
<u>F<sub>2B</sub></u>	0	18.7 (1.9)	14.2 (4.2)	14.0 (4.2)	-	0.2	8.3	0.9	3.2
	5.0	16.7 (1.2)	13.6 (1.0)	13.6 (1.0)	-	-	5.7	1.0	4.8
	10.0	18.4 (1.8)	14.7 (3.1)	14.6 (3.1)	-	0.1	7.7	0.9	3.7
	20.0	19.3 (1.9)	15.0 (3.0)	14.7 (3.1)	-	0.3	8.4	0.9	3.6
<u>F<sub>3B</sub></u>	0	17.6 (1.7)	15.4 (2.8)	15.1 (2.7)	-	0.3	6.7	0.9	4.2
	5.0	16.8 (2.8)	15.2 (2.8)	14.6 (3.0)	-	0.6	7.4	1.0	4.9
	10.0	16.6 (2.0)	14.2 (3.1)	13.8 (3.2)	-	0.4	7.4	1.0	4.8
	20.0	17.4 (3.0)	14.8 (3.0)	14.6 (3.1)	-	0.2	7.3	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<sub>1b</sub> 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<sub>2b</sub> 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<sub>3b</sub> 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<sub>2b</sub> 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<sub>3b</sub> 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.



Results:

Test Condition	Concentration	UDS grains/nucleus	Avg. % nuclei with		Survival % at 20-23 hrs
			> 6 grains	> 20 grains	
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:

Test Material	Concentration	NUCLEAR GRAINS	NOT COUNTABLE	Survival %
52.139	100.0 n1/ml	1.13	2.0	0
52.139	50.0 n1/ml	0.74	0.7	53.0
52.139	25.0 n1/ml	0.59	0	60.8
52.139	10.0 n1/ml	0.55	0.7	77.6
52.139	5.0 n1/ml	0.71	1.3	86.1
52.139	1.0 n1/ml	0.81	2.0	95.9
52.139	0.5 n1/ml	0.85	0	98.0
52.139	0.25 n1/ml			97.5
				100.8

Conclusion:

Propetamphos was not mutagenic in the UDS assay.

Classification: Acceptable

6) Mutagenicity Evaluation of SAN 52.139 in the *Saccharomyces cerevisiae* 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 in test volumes of about 1.2 ml/well.

Results:

Strain S138

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

6

Strain S211

NONACTIVATION TEST CONDITIONS	MUTANT COUNTS	Plate	Negative Control	Test Material Concentrations								Positive Control																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
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NONACTIVATION TEST CONDITIONS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										

The test material concentrations were as follows:

<u>Code</u>	<u>Concentration Per Well (1.5 ml vol.)</u>
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<sub>50</sub> 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<sub>2</sub> 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 erythrocytes per animal were scored. The frequency of micronucleated cells were expressed as percent (%) micronucleated cells based on the total polychromatid erythrocytes present in the scored 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:

<u>Test</u>	<u>Concentration<sup>a</sup></u>	<u>Route of Administration</u>	<u>Total Number of PCFs Scored<sup>b</sup></u>	<u>Mean Percent Micronucleic</u>			<u>Slide ID Numbers<sup>d</sup></u>
				<u>M</u>	<u>F</u>	<u>Cc</u>	
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

<sup>a</sup>Chemical administered as a two day split dose.

<sup>b</sup>Only Polychromatic Erythrocytes (PCE) scored.

<sup>c</sup>Combined results from male and female animals.

<sup>d</sup>Numbers refer to the slides scored in this study. Each number has a "male" and "female" slide.

Conclusion:

Propetamphos was not mutagenic in this assay.

Classification: Acceptable

Analysis

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

\*Significant at  $p = < 0.05$

\*\*Significant at  $p = < 0.01$

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
lungs	kidneys
stomach	prostate/uterus
esophagus	seminal vesicle
parathyroid	skeletal muscle
thyroids	thymus, if present
salivary glands	sternum (bone marrow)
trachea	eyes
tongue	bone marrow (femur)
	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).

<u>ppm in diet</u>	<u>Males</u>	<u>Females</u>
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

<u>Male (ppm)</u>	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:

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<u>Group No.</u>	<u>Dose Level mg/kg/day</u>	<u>Number of Males</u>	<u>Number of Females</u>
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

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

Statistical analyses of the data were performed.

Results:

The animals available at terminal sacrifice summarized as follows:

<u>Group</u> <u>mg/kg/day</u>	<u>Terminal Sacrifice</u>	
	<u>Males</u>	<u>Females</u>
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

<u>Dosage Level</u> <u>(mg/kg/day)</u>	<u>0</u>		<u>0</u>		<u>1</u>		<u>6</u>		<u>21</u>	
	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>
<u>Sex</u>										
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	<u>11</u>	6	<u>10</u>	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:

<u>Group</u> <u>mg/kg/day</u>	<u>Week 92</u>	
	<u>Males</u>	<u>Females</u>
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:

<u>Group</u> <u>mg/kg/day</u>	<u>Males</u>	<u>Females</u>
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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