UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

CASWELL #447AB

002490

DATE: November 15, 1979

SUBJECT: PP#8F2090 and Registrations 3125-322,-323,-324 - Petition for tolerances of oftanol 1-Methylethyl 2-[[ethoxy[1-methylethyl)amino] phosphinothioyl]oxy] benzoate and its cholinesterase inhibiting metabolites and registration for three formulations containing Oftanol.

FROM: Charles Frick C. Frick · 11/5/79
Toxicology Branch (TS-769)

TO: Ms. M. Mautz
Registration Division

THRU: Dr. Adrian Gross, Chief William H. Butler for M. Adrian Gross
Toxicology Branch (TS-769)

Petitioner: Mobay Chemical Corp.
Chemagro Agricultural Division
Kansas City, Missouri

Action Request: Tolerances of Oftanol of follows:

Crop	Tolerance
corn, forage and fodder	1.0 PPM
corn, fresh (including sweet) (K + CWHR)	0.1 PPM
corn, grain (including field and popcorn)	0.1 PPM
meat, fat and meat by-products of cattle, goats, hogs, sheep and poultry	0.1 PPM
milk and eggs	0.02 PPM

Use: Insecticide

Other Tolerances: EUP#8G2025 - No permanent tolerances (New Chemical).

Recommendation:

The submitted and in house toxicity data will support the tolerance and registration action request.

The NEL for this compound is 1.0 PPM based on a 2-year rat feeding study - cholinesterase inhibition effects. The safety factor utilized is 10x. For complete information regarding the NEL, ADI, MPI and TMRC, see computer printout at end of review.

All inerts in the formulations are cleared under Section 180.1001 (RCB review of PP#8G2025).

EPA FORM 1320-6 (REV. 3-76)

Toxicity Summary - The following includes extracts from review of PP#8G2025 by Mr. W. Greear 3/6/78: all studies at least core minimum unless noted.

Technical Material - SRA 12869, Oftanol, o-ethyl-o-(2-isopropoxy-carbonyl) phenyl isopropyl phosphoramidothioate.

1. Oral LD_{50} (in Lutrol)

	Species	Toxicity Category
38.7 mg/kg	rats, male	I
45.0	rats, male	I
28.0	rats, female	I
32.0	rats, female	I
127.0	mice, male	II
91.3	mice, female	II
150.0	rabbit, female	II

2. Four-Hour Inhalation LC_{50}

0.21 mg/L	rat, male	II
0.14	rat, female	II
>0.27	mice, male	II
0.23	hamster, male	II

3. Dermal Irritation (Draize)

P.I. = 0.21/8.00 (rabbit) IV

4. Eye Irritation (FHSLA)

Negative for Irritation (rabbit) IV

5. Dermal Sensitization (Guinea Pig)

Negative sensitization

6. Two-Year Feeding Study (Dog)

NEL = 2 PPM

7. Chronic Feeding (108 weeks) and Oncogenic Study in Mice

Negative oncogenic response
NEL = Not Determined

8. Two-Year Feeding and Oncogenic Study in the Rat

Negative oncogenic response
NEL = 1.0 PPM

9. Three-Generation Reproduction Study (Rat)

NEL = 1.0 PPM (ChE)

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10. Acute Dermal LD_{50} (Rabbit)

LD_{50} (Male) = 162 (80-328) mg/kg
LD_{50} (Female) = 315 (156-635) mg/kg

11. Acute Neurotoxicity Studies in the Hen

a. Oral Toxicity = LD_{50} = 20.9 (17.6-24.9) mg/kg
b. Intraperitoneal Toxicity, LD_{50} = 11.4 (9.4-13.9) mg/kg
c. Oral Toxicity following Pretreatment with atropine and 2PAM = Pretreatment of the hens with atropine and 2 PAM had a marked protective effect (LD_{50} > 45 mg/kg) - Core-Supplementary Data

12. Synergism Study with Malathion and EPN

Male Rat Oral Toxicity:

LD_{50} SRA 12869 = 95.9 mg/kg
LD_{50} Malathion = 1421 mg/kg
LD_{50} EPN = 44.5 mg/kg
LD_{50} (SRA 12869 + Malathion) = 217 mg/kg
LD_{50} (SRA 12869 + EPN) = 44.1 mg/kg
Effects are additive

13. Teratology Study Rat (Oral)

NEL for teratogenicity = 3 mg/kg/day

14. Dominant Lethal Test in the Male Mouse

Negative

15. Teratology Study Rabbit (Oral)

NEL for teratogenicity = 5 mg/kg/day highest level tested

16. Ames Test

Negative for Mutation

17. Oral Toxicity of SRA 12869 (ester chloride) in the rat

LD_{50} = > 5000 mg/kg

18. Acute Oral Toxicity of some Oftanol Metabolites - Oftanol Oxygen Analog; Des N-Isopropyl Oftanol Oxygen Analog; Des N-Isopropyl Oftanol

a. Oftanol Oxygen Analog (rat)

LD_{50} (male) = 38 (31-48) mg/kg
LD_{50} (female) = 17 (14-22) mg/kg

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b. Des N-Isopropyl Oftanol Oxygen Analog

LD_{50} (male) = 86 (69-108) mg/kg
LD_{50} (female) = 50 (44-56) mg/kg

c. Des N-Isopropyl Oftanol

LD_{50} (male) = 111 (83-148) mg/kg
LD_{50} (female) = 194 (155-244) mg/kg

NOTE: Additional information has been submitted since the review of PP#8G2025 to ~~ear~~ clarify the teratology and chronic rat feeding studies.

Toxicity Data Summary on Oftanol Formulations:

Formulation Oftanol 20% Granular

Acute Oral Toxicity in the Rat

LD_{50} (Male) = 212 mg/kg (183-246) Tox category II
LD_{50} (Female) = 189 mg/kg (166-215)
Category II

Dermal Irritation (Rabbit)

Mildly Irritating Tox Category III

Eye Irritation (Rabbit)

Slight Irritation Tox Category III

Acute Dermal Toxicity (Rabbit)

LD_{50} = > 2000 mg/kg Tox category III

Formulation Oftanol 15% Granular

Acute Oral Toxicity (Rat)

LD_{50} (Male) = 260 (198-342) mg/kg
LD_{50} (Female) = 202 (177-231) mg/kg
Category II

Acute Inhalation Toxicity (Rat)

LC_{50} = > 20 mg/L
Category IV

Eye Irritation (Rabbit)

Toxicity Category II

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Dermal Irritation (Rabbit)

P.I. = 0/8
Toxicity Category IV

Formulation Oftanol 6 Emulsifiable

Acute Oral Toxicity (Rat)

LD_{50} (Male) = 30 (13-72) mg/kg
LD_{50} (Female) = 27 (23-32) mg/kg
Toxicity Category I

Acute Dermal Toxicity (Rabbit)

LD_{50} (Male) = 252 (152-411) mg/kg
LD_{50} (Female) = 198 (109-291) mg/kg
Toxicity Category II

Eye Irritation (Rabbit)

Toxicity Category II

Dermal Irritation (Rabbit)

P.I. = 0/8
Toxicity Category IV

Acute Inhalation Toxicity (Rat)

LC_{50} (Male) = 1.950 (1.393-2.730) mg/L
LC_{50} (Female) = 1.650 mg/L
Toxicity Category II

Formulations:

Acute Oral Toxicity of Amaze 20% Granular in the rat.

Testing performed by Mobay Chemical Corp., Environmental Health Research, Stanley Research Center, Stilwell, Kansas, Reference#79A0R02

Test Material: Material was ground to a powder. Carbowax (Polyethylene Glycol-400) was used as the excipient for the test material and the dose levels were as follows:

Dose (mg/kg)	Dilutions (mg test material/ml)
102	816/40
150	1200/40
220	1760/40
323	2584/40
475	3800/40
698	5584/40

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Animals:

Sprague Dawley Derived Rats, Ten males and ten females were used for each dose level.

Protocol: The test compound was administered orally with a volume equivalent to 0.5% of the animal's body weight. Afuer dosing, the animals were observed twice daily over a 14-day period for mortality and signs of toxicity. Body weights were taken on days 0, 7 and 14. Necropsies were performed on all animals on day 14 or at the time of death. Mortality data was analyzed by the method of Carrol S. Weil, Biometrics, Vol. 8, No. 3, 1952.

Results:

Male Animals - At the lowest diarrhea was noted as a sign of toxicity. Toxicity at the other levels included diarrhea, lacrimation and tremors.

Female Animals - Signs of toxicity included diarrhea, lacrimation and tremors. The survivors at 102 and 150 mg/kg had an unthrifty appearance up until day 4 at 220 mg/kg unthrifty appearance were noted until day 13.

Gross Necropsies - The animals that died during the study had congested lungs and liver and some had congested kidneys. The animals that were sacrificed on day 14 did not have any gross lesions except one female at the 200 mg/kg level that had white nodules on the lungs.

Under the conditions of this study, the following LD_{50} values were determined for Amaze 20% granular.

Males: 212 mg/kg (183 to 246 mg/kg)
Females: 189 mg/kg (166 to 215 mg/kg)

Study Classification - Core-Guidelines

Toxicity Category: II

Eye & Dermal Irritation of Amaze 20% Granular

Test performed by Mobay Chemical Corp, Environmental Health Research, Stanley Research Center, Stilwell, Kansas.

Test Material: Material was applied in its granular form to the rabbits' backs for the dermal study, and was ground and applied in powder form to the rabbits' eyes for the eye irritation study. Animals used were the NZW Rabbit.

Protocol: Eye

The left eyes of nine rabbits were treated with 100 mg of test compound, right eyes served as control. Forty-five seconds after the test material was instilled, three rabbits had their treated eyes washed with water. On days 1, 2, 3, 4 and 7, the eyes were examined for lesions. The evaluations of the eye reactions were conducted according to the method of Draize in Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics, 1965.

Protocol: Dermal

The backs and sides of six rabbits were shaved. Four test sites were used on each animal - two sites were abraded on each animal. One-half of a gram of test compound was moistened with saline and applied on each test site under a one inch gauze patch and secured. After 24 hours the patches were removed and the test areas evaluated. Reactions were evaluated by Draize as noted above.

Results: Eye

Wash Group - Two of the three rabbits had slight amounts of erythema and discharge on day one. For the rest of the study this group appeared normal.

Non-wash Group - A slight redness was noted in five of the six animals on day 1 one animal had chemosis. On day 2 there was only one rabbit with slight erythema. All of the animals appeared normal throughout the rest of the study. Grades considered positive for irritation were not found in either group.

Study Classification - Core-Minimum

Toxicity Category: III

Results: Dermal

On day one slight erythema was noted on both abraded sites of four of the six rabbits. By 72 hours there was no irritation present. Under the conditions of this study, this formulation was found to be only mildly irritating to dermal tissue.

Study Classification - Core-Minimum

Toxicity Category: III

Acute Dermal Toxicity of Amaze 20% Granular to rabbits.

Study preformed by Mobay Chemical Corp., Environmental Health Research, Stanley Research Center, Stilwell, Kansas.

Test Material: Test compound was applied in a paste (saline) to rabbit backs. Animals used were New Zealand Rabbits.

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Protocol: Five male and five female rabbits had their backs shaved - material was applied and covered with gauze, then plastic, and secured with tape. After 24 hours, the wrappings were removed. Rabbits were observed for a 14-day period for mortality and signs of toxicity. Body weights were taken on days 0, 7 and 14. All animals were examined for gross lesions at the time of death or at day 14 sacrifice. Dose level (single) was 2000 mg/kg.

Results: The only sign of toxicity noted was diarrhea in the males on day 1. Weight gains were not extraodinary. No deaths occurred. Day 14 sacrifice, and gross and microscopic examination indicated no lesions.

The acute dermal LD_{50} when administered to rabbits under the conditions of this study was for male and female rabbits > 2000 mg/kg.

Study Classification - Core-Minimum

Toxicity Category: III

The Data on Formulations, Oftanol 15% Granular and Oftanol 6 Emulsifiable, have been previously reviewed (PP#8G2025; Bill Greear 3/6/78) and the results are summarized in TOX data summary list at the beginning of this review.

Effect of SRA 12869 on Reproductive Function of Multiple Generations in the Rats.

Testing performed by: Huntingdon Research Centre, Huntingdon, Cambridgeshire, England.

Protocol: Specific Pathogen free rats of the CFY strain following a period of acclimatization (Ca 7 days) were randomly allocated to the following groups:

Group	Treatment	PPM in diet	No. of Rats	
			Male	Female
1	control	-	20	20
2	SRA 12869	1	20	20
3	SRA 12869	10	20	20
4	SRA 12869	100	20	20

Concentration was expressed in terms of active ingredient - it was stated that the test material was supplied as 50% a.i.

The test compound was administered in the diet, and fresh batches of diet were mixed weekly. One sample of untreated rat diet and four subsequent samples of SRA 12869 were sent to Bayer AG of Germany for analysis.

Animals of the F0 generation were maintained on their diets for 60 days prior to mating. They were then mated on a one male to one female basis for a period of 19 days. Resulting litters were reared to 21 days post partum when 20 of each sex were selected from each group to form the basis of the F1A generation. F0 animals and surplus pups were sacrificed and examined macroscopically. The F1A animals were reared on their respective diets to an age of 81 days and then mated for 19 days. The resulting litters were reared to 21 days post partum and 20 of each sex were selected to form the third

generation (F2A). The F2A animals were reared on the test diet till an age of 81 days and were mated for 19 days to produce the F3A generation. F[illegible] parents and F3A pups were sacrificed at weaning and examined macroscopically for abnormalities.

Modification of the above protocol has to be introduced; as 100 PPM insufficient F1A offpsring were obtained to form as second F1A generation. Initially all groups were remated with the intention that if sufficient animals were obtained at 100 PPM, the investigation would have been continued using the F1B rather than the F1A generation. However, since this second mating of the F0 generation again failed to provide sufficient F1B offspring at 100 PPM, the multi-generation animals which had been continued - continuation to the third generation was only possible at 1 and 10 PPM.

Results:

Signs and Mortalities - There were no signs of reaction and no death attributable to treatment.

Body Weight Change - F0 dams, during gestation, showed over 7% lag at 10 PPM and in excess of 9% in the 100 PPM group compared to control at 20 days. Post-partum weight changes at 10 and 100 PPM showed a significant lag-effect relative to the controls at the 14th day, and remained below controls at 21 days - Body Weight NEL = 1 PPM.

Mating Performance and Pregnancy Rate - Pregnancy rate was depressed at 100 PPM for the first mating in the F0 and F1A generations. The second mating pregnancy rates for the F0 generation showed biologically significant decreases at 10 PPM (30%) and 100 PPM (55%).

Gestation Period - The mean gestation period was comparable for all groups.

Cholinesterase Depression - RBC cholinesterase of both males and females of the F0 generation showed significant depression after treatment for 37 weeks at 10 and 100 PPM. Likewise, plasma ChE was depressed with the exception of males at 10 PPM. A slightly enhances ChE activity was reported for female RBC and plasma at 1 PPM.

Cholinesterase NEL = 1 PPM

Terminal Autopsy - The report states (without presenting data) that post mortem examination of all adult rats at termination of each generation revealed no macroscopic lesions attributable to treatment. Almost all litters born to dams fed a dietary level of 100 PPM were lost in the immediate neonatal period.

NEL for Reproductive Parameters is 1.0 PPM - Study Classification -Core-Minimum

Chronic Feeding and Oncogenic Study (Mice)

In a 108-week feeding experiment on male and female SPF mice, SRA 12869 was evaluated for tolerance, determination of its no-effect level and potential carcinogenicity.

The study was conducted by Beratungsforum Für Präventiumedizin and Umweltschuta Gmbh, Kattrepelsbrücke 1, 2000 Hamburgh 1.

Conduct of the feeding study was directed by Dr. med. Horst Brune, Biologisches Labs, Hamburg. The histopathological examinations were performed by Prof. Dr. U. Mohr, Director of the Institute of Experimental Pathology, Faculty of Medicine, Hannover University.

Compound Tested - Technical grade of SRA 12869, or Oftanol, or O-ethyl-o-(2-isopropoxy-carbonyl)phenyl isopropylphosphoramidothioate, with a purity of 89.3%. The compound was premixed with Wessalon S in a ratio of 1:1 and incorporated into the diet.

Animals Tested - The study was performed and SPF mice of the NMRI strain. At the start of the study, the male and female mice had average body weights of 28.3 gm and 25.7 gm, respectively, and were aged approx. 6-7 weeks.

Dietary Concentration
0 PPM - Control
1 PPM - Dose Group 1
10 PPM - Dose Group 2
100 PPM - Dose Group 3

The control group and the 3 dose groups each consisted of 40 male & 40 female mice. Each test group included 3 sub-groups each consisting of 10 males & 10 females used for cholinesterase activity measurements, hematological tests and clinical - chemistry tests.

It was stated that, samples of the test compound SRA 12869 used in the study were repeatedly taken and tested for unchanges quality and stability by the client.

The mice received food & water ad libitum. All the animals were inspected daily for signs of any alternations in their behavioral patterns or symptoms of ill-health.

Measurement of Bodyweight & Food Intake - The mice were weighed weekly during the first 8 weeks, and thereafter at 14-day intervals. Food intake ~~were~~ was measured for each mouse by weighing the uneaten portion of the ration twice weekly during the first 8 weeks, and thereafter once weekly.

Clinical Chemistry - Blood samples were withdrawn from the retroorbital venus plexus of the mice in the subgroups at the start of the feeding study and at intervals of 3 months.

Blood Test - Erythrocytes, leucocytes, hemoglobin content were monitored and a differential blood count performed.

Liver Function Tests - The activities of alkaline phosphatase and glutamate-pyruvate transaminase were measured.

Urinalyses - The urine of the mice in the sub-groups was collected at three-month intervals by caging in metabolism cages. Testing was done semiquantitatively with Ames Multistix for protein, glucose, hemoglobin, bilirubin and pH.

Cholinesterase Activity - The blood was taken from the sub-group animals and cholinesterase activity was measured in plasma and erythrocytes - Brain cholinesterase activity was also measured.

Necropsy - The mice that died during the study were dissected and macroscopically examined. All the mice alive at the end of the 106 weeks were killed and the organs macroscopically examined.

For the histopathological examination, the tissues were fixed in 10% formalin solution and assessed by Dr. U. Mohr, Institute of Experimental Pathology, Medizinische Hochschule Hannover.

The following were embedded in paraffin wax:

Brain
pituitary
eyes
thyroid
heart
aorta
trachea
lung
liver
spleen
pancreas
esophagus
stomach
S. intestine
L. intestine
kidneys
adrenals
urinary bladder
testes
epididymides
seminal vesicle
prostate
ovaries
uterus
skeletal muscle
femur
Nervous ischiadicus
sternum
female mammary gland
cervical lymph nodes
tumors

The 5-8 µ sections were routinely stained with hematoxylin and eosin.

Protocol Changes - The sub-groups used for measurements of cholinesterase activity were maintained and assayed for a period of 24 weeks, and the sub-groups used for the liver function tests were run for 9 months.

Results

General Examination - During the study the mice treated with test compound did not differ in their physical appearance and behavioral patterns from the controls. No differences were seen in any of the groups with respect to need for food or drink, activity and condition of coat.

Food & Test Compound Intake - There was no indication of any dose-related differences in food intake and test compound intake.

Body Weights - No dose-dependent differences were noted.

Mortality - No dose-dependent differences were noted.

Blood Tests - No significant differences between the treated groups and the controls with respect to the erythrocyte counts, leucocyte counts and hemoglobin levels at anytime during the feeding experiment. The values of the differential blood counts likewise that there were no significant difference between the treated groups and the controls with respect to the proportions of the different cell types; also no pathological changes were seen in the different blood cell types.

Liver Function Test - There was no evidence of the test compound having a dose-related effect on Liver Function.

Urinalyses - There was no clinical-chemical evidence of kidney damage due to dietary administration of the test-compound.

Cholinesterase Activity - The study indicated that plasma cholinesterase activity was not affected in the 1 PPM group. However, the dietary levels of 10 PPM and 100 PPM caused significant depression of plasma cholinesterase activity in both sexes after only two-weeks in the study. At 10 PPM depression was 74.0% in males and 77.7% in females. At 100 PPM, activity was depressed by 88.6% in males and by 92.2% in females.

Erythrocyte cholinesterase activity was not significantly depressed at any dose level in either the male or female animals.

Brain cholinesterase activity was within the range of variation of the measurement technique in both sexes at the dietary levels of 1 PPM and 10 PPM. At the dose level of 100 PPM, the test compound caused appreciable depression of brain cholinesterase activity (46.4% in males and 30.6% in females).

It was concluded that the NEL of the test compound on plasma cholinesterase activity is 1 PPM - (for a 24 week testing period) comparison of the treated groups with the control group indicated that the spontaneous rate of tumors was not increased by dietary administration of the test compound-the number and type of tumors were similar in the test and control group.

Summary of test results;

Table: XI Number of tumor bearing animals in each group (SRA 12869). (Less than 3 tumors in the groups have not been taken into account; differences were calculated from comparison with the controls).

Male Group	Dose ppm	Effective No. of Animals	Tumor bearing Animals (TBA)	Total No. of Tumors	Tumors per TBA	± SD	Lung Adenoma	Mal. Lymphoma
1	0	37 (1)	20	25	1.25	0.55	6	13
2	1	37 (0)	21	26	1.24	0.54	10 (0.26)	7 (0.12)
3	10	39 (0)	21	30	1.43	0.87	7 (0.84)	6 (0.046)
4	100	38 (0)	24	28	1.17	0.38	10 (0.29)	8 (0.174)

Female Group	Dose ppm	Effective No. of Animals	Tumor bearing Animals (TBA)	Total No. of Tumors	Tumors per TBA	± SD	Lung Adenoma	Mal. Lymphoma	Thyroid Adenoma	Ovarian granulos cell tumor
1	0	36 (0)	24	29	1.21	0.51	5	5	1	9
2	1	36 (2)	21	32	1.52	0.81	4 (0.69)	12 (0.06)	1 (-)	4 (0.11)
3	10	38 (1)	23	31	1.34	0.65	3 (0.39)	12 (0.08)	0 (-)	8 (0.64)
4	100	36 (1)	24	32	1.33	0.56	4 (0.69)	8 (0.39)	3 (0.32)	7 (0.53)

Significance limits are 0.05 (= 5%) and 0.01 (= 1%)

p-values in parenthesis
SD: standard deviation

Chronic Toxicity Study on Rats - (Two-Year Feeding & Oncogenic Study)

Testing performed by Bayer AG Institute Fur Toxikologic; Report No. 6979. Report signed by Drs Ernst Bonhard and Eckhard Loser Histopathology examinations by U. Mohr, Hannover.

Compound Tested - The toxicity study reported herein was conducted with a sample of technical grade SRA 12869 or O-ethyl o-(2-isopropoxycarbonyl) phenyl isopropylphosphor amidothioate or Oftanol, Isofenphos, Fenisophos, Bay 92114. Batch No. 1603/73 using a 50% premix prepared with Wessalon S.

Animals Tested - Were SRF rats (Wistar strain). The animals were about 28 to 32 days old at the start of the testing and had an average body wt. of 50 gm for the males and 49 gm for females.

The animals were housed singly and room temperature was kept constant (24°C). The rats received fresh pulverized Altromin R feed (made by Altromin GmbH, Lage/Lippe) each. The animals had constant access to food and water.

Protocol

The following dietary concentrations were employed:

0 (contol), 1 PPM, 10 PPM, 100 PPM - The test group consisted of 50 male and 50 female rats that received the test compound. The control group comprised 100 males and 100 females. It was stated, that for laboratory investigations, 5 additional rats were used per group and sex.

The test animals were inspected daily, and any changes or symptoms were recorded. The animals were weighed weekly during the first 26 weeks, and thereafter at 14-day intervals. The amount of food consumed weekly was measured by weighing the uneaten potion of the food.

Parameters Measured

1. Clinical Chemistry: Chemical tests were performed on 5 male and 5 female rats of each test group at intervals of 1, 3, 6 and 12 months on test and on 10 males and 10 females of each group at the end of the 24 month testing period.

a. Blood tests included determinations of the following:

1. hemoglobin
2. hematocrit level
3. erythrocyte and leucocyte count
4. raticulocyte and thrombocyte count
5. differential blood count
6. thromboplastin time
7. Glucose and cholesterol concentrations

b. Liver function tests included:

1. alkaline phosphatase
2. SGOT
3. glutamate dehydrogenase
4. SGPT
5. bilirubin
6. total protein

c. Urinalyses and kidney function tests included:

1. glucose
2. protein, blood and pH
3. ketone bodies and protein content
4. Urea and creatinine

d. Measurement of cholinesterase activities:

Plasma and erythrocyte cholinesterase activities were measured in 5 male and 5 female rats of each group at intervals of 1, 2, 4, 13, 26, 52, 78 and 105 weeks of the test period. The procedure used was the hydroxamate method described by PTLZ and EBEN, 1967. Brain cholinesterase activity was measured in 10 male and 10 female rats of each group at the end of test (modification of method of ELLMAN 1961).

2) Post Mortem

a. Posts were performed on the rats which died during the study and were grossly examined.

Rats still alive at the end of the 24-month testing period were sacrificed by exsanguination. They were then dissected and macroscopically examined. The thyroid, heart, lung, liver, spleen, kidneys, adrenals and gonads were weighted.

b. For the histological examination the following tissues were fixed in Bouin's solution:

brain
pituitary
eyes
lymph nodes (cervical)
salivary glands
thyroid
thymus
heart
aorta
lung
adrenals
urinary bladder
epididymides
seminal vesicle
femur
Nervous ischiadicus
trachea
liver
spleen
pancreas
esophagus
stomach
intestines
kidneys
testes
prostate
ovaries
uterus
skeletal muscle
bone marrow
sternum

Results: For the statistical analysis of this study, the non-parametric ranking test of Wilcoxon 147, was used.

a) General Appearance and Behavior - The animals fed concentrations of 1 and 10 PPM did not differ in their physical appearance and behavioral patterns from the controls. They showed no differences with respect to activity, condition of coat, and need for food or drink. It was stated that the animals at the 100 PPM test level showed typical symptoms of cholinesterase depression during test week 1.

b) Food Consumption - In the 1 and 10 PPM male rats and the female rats in the 1, 10 and 100 PPM was comparable to the control animals. The male rats of the 100 PPM group consumed slightly less food than the control group.

c) Body Weights - The male and female rats of the 1 and 10 PPM groups made about the same body weight gains as the controls. Both sexes of the 100 PPM group had significantly lower body weights than the controls throughout the feeding experiment.

d) Mortality - Rate did not appear to be related to compound injection.

e) Clinical Chemistry

1. Blood Profile - Nothing extraordinary noted.

2. Liver Function Test - Nothing extraordinary noted.

3. Urinalyses and Kidney Function - Nothing extraordinary noted.

4. Blood Sugar and Cholesterol - None of the animals had levels within the pathological range.

5. Measurements of plasma, erythrocyte and brain cholinesterase activities - data indicated that neither erythrocyte nor plasma cholinesterase activity was depressed significantly in the male and 100 PPM caused dose-dependent depression of cholinesterase in plasma and erythrocytes in both the males and females. The level of depression remained almost constant throughout the study. Brain cholinesterase activity in male and female rats was depressed significantly only in the 100 PPM dose group.

Post Mortem

a. Autopsies on rats which died during study - It was stated that, no pathological alterations attributable to the test compound were seen in any of the rats that has received dietary concentrations of 1 to 10 PPM of test material.

b. Autopsies on rats sacrificed at end of study - As above, no pathology that could be attributed to test material was noted.

Organ Weights

In the male rats of the 100 PPM group, the mean organ weights, with the exception of the testes, were significantly lower than those of the controls. The female rats of the 100 PPM group has significantly lower thyroid weights and significantly higher spleen and adrenal weights, Histopathological examination did not reveal any pathology in these organs that could be correlated with the test compound.

Tumors

The type, localization and incidence of the benign and malignant tumors observed provide no indication of this compound having a carcinogenic effect.

The following is a summary of tumor incident.

Dose in ppm	MALE 0	1	10	100	FEMALE 0	1	10	100
Numbers of rats with benign tumors	15	12	14	9	38	21	11	15
Number of rats with malignant tumors	6	5	9	3	20	6	9	8
Total number of rats with tumors	20	17	20	11	52	26	19	18
Number of benign tumors	16	12	16	9	45	28	12	17
Number of malignant tumors	6	5	10	3	21	8	11	9
Total number of tumors	22	17	26	12	66	36	23	26
Number of histopathologically examined rats	93	48	45	48	94	48	45	47

The NEL demonstrated in this experiment is 1.0 PPM based on erythrocyte cholinesterase depression.

Study Classification - Core-Guideline

Chronic Toxicity - Two-Year Feeding Study in the Dog

Study performed at Bayer AG Institute Fur Toxikologic, Oct. 25, 1977 by K. Hoffmann and G. Kaliner - Report Nos.: 7072, SRA 12869. Submitted by Chemagro Agricultural Division Mobay Chemical Corporation.

Material Tested

SRA-12869, common name is isofenphos, trade name is Oftanol. Chemical name is O-ethyl-o-(2-isopropoxy-carbonyl)phenyl isopropylphosphoroamidothioate. Technical grade compound is 89.3% and it was necessary to prepare a 50% premix with Wessalon S in order to administer the test compound with the animal food.

Animals Tested

Thirty-two pure-bred Beagle dogs which at the start of the study were 22 to 29 weeks old and weighted between 5.9 and 11.0 kg. All animals were housed individually. Food consumption was controlled and actual consumption measured.

Protocol

8 animals per test group (4 M & 4 F)

Control Group	0 PPM
Group I	Male dogs: 3 PPM from Week 1-83 2 PPM from Week 84-104
	Female dogs: 3 PPM from Week 1-104
Group II	15 PPM
Group III	75 PPM from Week 1-53 150 PPM from Week 54-99 300 PPM from Week 100-104

The following parameters were investigated:

1. General Examination
2. Ophthalmascopic Examination
3. Hematology
4. Clinical Chemistry
5. Cholinesterase Activity (Plasma, RBC, Brain)
6. Urinalysis
7. Food & Test Compound Intake
8. Body & Organ Weights
9. Gross & Histopathology

Results

Clinical Findings - The dietary levels of up to and including 75 PPM were well tolerated. The dogs receiving these dose levels showed no differences from the controls in physical appearance and behavioural patterns. It was not until after the dietary concentrations has been raised from 75 to 150 PPM that symptoms associated with the test compound's cholinesterase - inhibitory effect were noted toward the end of the second treatment year. When the concentration was raised from 150 to 300 PPM as from Week 100, the symptoms became intensified especially among the males; one dog in this group died in week 104 and another had to be sacrificed in a moribund condition.

Food consumption and food intake times were not affected by administration of test compound at concentrations of up to and including 150 PPM.

There were no noteworthy differences in body weight gains between the dogs fed test compound at levels of up to and including 150 PPM and the controls. It was not until the level was raised to 300 PPM that body weight depressions were noted in all males and in 3 of the 4 females of Group III.

Opthalmoscopic examination of the eyes provided no indication of any treatment-related variations from the physiological norm in either the transparent media or on the Fundus oculi.

Hematological tests were performed before the start of the feeding experiment and in Treatment Weeks 14, 27, 39, 53, 66, 79, 92 and 104. The following were measured: heamtocrit level, hemoglobin, medium cell hemoglobin, erythrocyte count, reticulocyte count, thromabocyte count, sedimentation rate and thromboplastin time. Nothing extraordinary noted with the possible exception noted in Dog C 537/Group III just before the dog died; it had a marked increase in the number of stab leucocytes, accompanied by a reduction in the number of segmented cells. The 7% "other" cells were made up of 4% myclocytes and 3% immature. At the same time, a substantitally accelerated sedimentation rate was noted in this animal. The explantation given was that these alterations are considered to have been due to an accelerated decomposition of the blood and a reactively increased formation of new blood cells from the bone marrow.

Clinical Chemistry was performed in the same intervals as hematology. The following were measured: glucose, urea, creatinine, total protein, GOT, GPT, Alkaline phosphate and cholesterol. Nothing extraordinary noted with the following exceptions: alkaline phosphatase activity level in the Group II dogs either did not decline to the control level, or increased still further as the treatment progressed. This effect is attributed to the increase of the test compound in the diet from 75 to 150 PPM as from Treatment Week 54.

Plasma cholinesterase activity depression was observed in animals with dietary concentrations of 15 PPM and above. The dietary concentration of 3 PPM of test compound did not cause any marked depression of plasma cholinesterase activity in female animals. In the male dogs the 3 PPM level showed some depression. The reduction of plasma cholinesterase in the male dogs in Group I were seen to have slightly exceeded the 20% tolerance at some of the testing intervals. For this reason, the dietary concentration of test compound administered to the male animals in Group I was reduced from 3 PPM to 2 PPN in 84th week. The results were such that in the last 20 weeks of treatment the plasma cholinesterase levels in the male dogs returned to normal and did not differ substantially from the control group in weeks 92 and 103.

Erythrocyte cholinesterase activity was not reduced in either male or female dogs at dietary levels of up to and including 15 PPM.

Urinalyses - Nothing extraordinary noted.

Gross Pathology - No consistent pathology or dose relationship was noted. The following tissues were examined and weighted: brain, lung, liver, spleen, kidneys, pituitary, thyroid, adrenals, testes, heart, ovaries, prostate gland and pancreas.

Absolute & Relative Organ Weights - Nothing extraordinary noted.

Histopathology - The following tissues were taken from all animals and fixed in Bouin's solution, embedded in Paraplast, stained with hemalun and eosin:

Small Intestine
Large Intestine
Heart
Testes
Pituitary
Liver
Lung
Stomach
Spleen
Epididymides
Adrenals
Kidneys
Esophagus
Ovaries
Prostate Gland
Thyroid & Uterus

In addition the following tissues from the control dogs and the dogs of the highest dose group (300 PPM) were similarly processed and examined: eyes, aorta, fasciculi optici, gall bladder, brain, urinary bladder, bone and bone marrow, mesenteric lymph nodes, Nervous ischiadicus, pancreas, parotis, skeletal muscle and thymus.

In three of the four male dogs in the 300 PPM dose group of the esophagus showed erosions of variable microscopic distension and the Propria mucosae showed cell infiltration and proliferation. Some alterations seen in the brain stems in two dogs at the high dose level (300 PPM) were interpreted to be foci of softening (degenerative processes) the intensity of this finding in the high dose group might be treatment related. No other consistent or dose related pathology was noted.

Conclusion

Because of the wide dosage difference between systemic effects compared to cholinergic effects, a safety factor of 10x reflecting cholinergic toxicity is employed.

Base on this study the NEL = 2PPM

Study Classification - Core-Guideline

TOX/HED:th:RD Initial WWOODROW:11-15-79