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TX2-1824

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

001424

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: February 2, 1982

SUBJECT: PP#1F2433. Terbufos (S-[[(1,1-dimethyl)thio]methyl]0,0-dietyl phosphorodithioate). EPA Reg.#241-238. A Petition for Permanent Tolerance in or on Cabbage, Broccoli, and Cauliflower for Terbufos and its Cholinesterase Inhibiting Metabolites at 0.20 ppm using Counter 15G Formulation. (Initial submission on 11/3/80, 1st Amendment on 6/17/81. and 2rd Amendment on 9/3/81).
CASWELL#131A Accession#099682 CFR No. 180.352

FROM: Amal Mahfouz, Toxicologist
Toxicology Branch/HED (TS-769) A. Mahfouz 2/3/82 2/11/82

TO: William Miller (16)
Registration Division (TS-767)

THRU: William Burnam, Acting Chief
Toxicology Branch/HED (TS-769)

Petitioner: American Cyanamid Company
Agricultural Research Division
P.O. Box 400
Princeton, New Jersey 08540

The current action for new uses of existing "old" pesticide, PP#1F2433, was evaluated in accordance with the "Interim Final Regulation Relating to Conditional Registration", published in the Federal Register on May 11, 1979, pages 27932-27945.. An assessment of the incremental risk due to this new use of Terbufos in or on cabbage, broccoli and cauliflower was conducted.

No new toxicology studies were submitted with this petition.

Conclusions & Recommendations:

The following studies are considered data gaps:

°One teratology study in a second species.
°A 2-year chronic feeding/oncogenic study in the rat.
°An 18-month or longer oncogenicity study in the mouse.

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The registrant should conduct and submit these studies for review within a reasonable time.

°The Toxicology Branch cannot support the proposed new use of Terbufos on cabbage, broccoli and cauliflower. The percent increase in the TMRC due to the new use has been judged unacceptable.

°The incremental dietary exposure from food uses has been assessed for the new use in or on cabbage, broccoli and cauliflower as follows (also see the computer printout attached).

Existing TMRC for published and unpublished tolerances (40 CFR 180.352)	= 0.0046 mg/day/(1.5 kg)
Increase in TMRC due to the new use.	= 0.00273 mg/day/(1.5 kg)
Percentage increase in TMRC due to the new use.	= 59.35

°A 100 fold safety factor was selected for PADI and MPI calculations based on a 6-month dog study (FDRL#1193) and a 0.10 ppm NOEL for ChE values. The presently calculated PADI < 0.0001 mg/kg/day and MPI = 0.0015 mg/day/60 kg man. Published tolerances utilize 303.41% of the PADI (see attached printout).

°Terbufos does not have an RPAR issued against it. Statutory prohibitions against conditional registration, therefore, are not applicable in this instance. However Counter 15G formulation is acutely toxic dermally (LD_{50} = 10.2 mg/kg), and should be classified in the restricted use Category.

Residue Chemistry Considerations:

°Toxicology Branch notes that the actual residues on the edible parts of cabbage, broccoli and cauliflower may be lower than the proposed tolerance of 0.2 ppm.

According to Residue Chemistry Branch (RCB) recommendation the initial request for 0.05 ppm tolerance in or on cabbage, broccoli and cauliflower was amended twice (0.135 ppm on 6/16/81 and further to 0.2 ppm on 9/31/81) to increase the proposed tolerance to 0.2 ppm in order to accomodate the high level of residues found in the wrapper leaves. The proposed tolerance of 0.2 ppm is based on the worst case calculations using data on cabbage. Fifty to sixty percent of the cabbage outer leaves are considered 'wrapper leaves' and contain most of the residues. (K.H. Arne (RCB) memos of 3/26/81 and 7/10/81).

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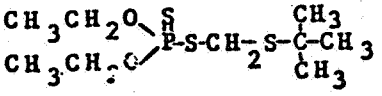
Substance Identification

Chemical Name: S-[[(1,1-dimethyl)thio] methyl]0,0-diethyl phosphorodithioate

Structure:



Synonyms:

Terbufos, Counter®, AC92100, ST-100, ENT-27920, C192100.

Purity of Technical Material: 85 to 88 percent

Physical and Chemical Properties: Technical

Technical Counter is a clear, slightly brown liquid, melting at slightly below - 15°C. Water solubility is 15 ppm. Soluble in acetone, aromatic hydrocarbons, and alcohol. Hydrolizes under alkaline conditions. Stable at room temperature for at least two years.

Referenced Petitions:

PP#3G1340 - 0.05 ppm - corn grain, fodder & forage.
PP#4F1496 - 0.05 ppm - corn grain, fodder & forage.
PP#5F1640 - 0.05 ppm - in or on sugar beets tops & roots
PP#6F1657 - 0.5 ppm - corn forage and fodder (field corn, popcorn and sweet corn). 0.05 ppm - corn grain (popcorn) and sweet corn (kernels plus cob with husks removed).

Refer also to the existing tolerances in CFR 40, 180.352 and the attached printout.

Formulation:

The formulation proposed for use on cabbage, cauliflower and broccoli is Counter 15G which is 15% (by weight) terbufos. The inerts are cleared under Section 180.1001.

The manufacturing process and the composition of technical Terbufos were discussed in the review of PP#4F1496 (memo of 10/7/74, R.D. Schmitt). Terbufos accounts for 85-88% of the technical material. The

[redacted]

Proposed Use

For control of cabbage maggots and flea beetles in broccoli, cabbage and cauliflower Counter 15G is to be applied to the furrows at seeding at a rate of 8-16 oz/1000 ft of row for any row spacing. (Equivalent to 1.1 to 2.2 lbs. a.i./A when the row spacing is 36 inches.) Counter 15G is not to be applied as a seed box application, i.e., it may only be applied directly to the soil. Label restrictions prohibit the use of treated plants for food or forage prior to normal harvest; field stripped leaves are not to be used for food ot forage and animals are not allowed to graze on any remaining plant material.

New Toxicity Studies Submitted

No new toxicity studies have been submitted with this petition.

Re-evaluation of Studies

One previously submitted and reviewed study, the rat 2-year chronic feeding/oncogenic study (Bio/dynamics 71R-725), was rereviewed (see review at the end of this action). This reviewer concluded that the NOEL for this study appears to be lower than 0.25 ppm for both systemic toxicity and ChE inhibition (RBC and brain). The study is classified as supplementary because of several discrepancies (see review, conclusion section).

In addition to the above study review, this reviewer also reclassified as supplementary the previously submitted and reviewed 18-month mouse carcinogenicity study (Bio/dynamics #71R-728) because only tissues from 15 animals/sex/group were microscopically examined (examinations at the low- and mid-dose levels involved only the liver, kidney, heart and lungs). This study is not informative about events which occur early during the study period because animals that died during the study were not examined.

This 18-month oncogenicity study in mouse is inadequate by design to detect incidence of rare tumors associated with Terbufos use.

The above reevaluations of the rat and mouse studies are recorded in the summary of Terbufos toxicity (table included with this action) and these two studies as presently classified (supplementary) are considered data gaps.

Summary of Prior Toxicity Studies

The following table (next 5 pages) summarizes the toxicity data that have been previously submitted and reviewed.

Pharmacology and Metabolism Studies

A rat metabolism study using ^{14}C labeled Terbufos was discussed in RBC review of PP#4F1496 and summarized in RBC review of this PP#1F2433 (K.H. Arne memo of 3/26/81) as follows:

"This study shows that the activity (^{14}C) was largely (83%) excreted in the urine when a single dose (0.8 mg/kg, equivalent to ca. 10 ppm in the diet of a rat) was administered. A relatively small amount (3.5%) was excreted in the feces. The maximum residues of cholinesterase inhibiting compounds were found in the liver six hours after dosing and totaled 0.08 ppm. In the kidney, at 12 hours past dosing, residues of hydrolysis products reached a maximum level of 0.9 ppm. All metabolites found in the rat had been previously identified in plant studies. There is no available ruminant metabolism study involving the use of radiolabeled material; however there is a 21 day feeding study of cows fed at a 2 ppm level in the dry diet. No detectable residues of cholinesterase inhibiting metabolites were found in samples of milk taken at days 7, 14, or 21 or in the edible tissues of the animal at sacrifice (day 21)."

"Considering the results of the available animal studies and the fact that TOX has determined that the hydrolytic products of terbufous are not of toxicological concern, we conclude that the fate of terbufos in animals is adequately understood. The residues of concern are the same for plants and animals."

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Summary of Terbufos Toxicity Data

Study	Formulation	Results	Toxicity Category	Core Classification
Acute Oral - Rat	Technical 96.7% 96.7% 85.5% 96.7%	LD_{50} (M) = 4.5 (2.6-7.7.) mg/kg LD_{50} (F) = 9.0 (5.2-15.3) mg/kg LD_{50} (M, fasted) = 1.6 (1.2-1.9) mg/kg LD_{50} (F, fasted) = 1.3 $\pm$ 0.2 mg/kg Vehicle: corn oil. Animals exhibited signs of acute ChE inhibition.	I I I I	No Core Classification
Acute Oral - Rat Consultox Labs. #CL75:37:11,31, Sample#911 April, 1975	Technical 86%	LD_{50} (M) = 1.5 mg/kg = 0.0015 mg/kg (100% mortality at 2.5 mg/kg) Vehicle: dimethyl sulfoxide Symptoms: excessive salivation, disoriented locomotion, piloerection and tremors; 100% mortality at 2.5 mg/kg; survivors usually recovered after 4 days.	I	Supplementary
Acute Oral - Rat Cannon Labs. #6E-3164 11/9/76	Technical 86%	LD_{50} (M&F) = 1.73 mg/kg Vehicle: corn oil Symptoms: respiratory depression, clonic convulsions, ptosis, decreased locomotion, piloerection, exophthalmous, lacrimation, hemorrhaging.	I	Minimum
Acute Oral - Mouse	Technical 96.7% 96.7% 85.5%	LD_{50} (M) = 3.5 (1.9-6.6) mg/kg LD_{50} (F) = 9.2 (6.0-14.0) mg/kg LD_{50} (F) = 5.0 (4.0-6.3) mg/kg Vehicle: corn oil Animals exhibited signs of acute ChE inhibition.	I I I	No Core Classification
Acute Oral - Dog	Technical 96.7% 96.7%	LD_{50} (M) = 4.5 (2.2-9.0) mg/kg LD_{50} (F) = 6.3 mg/kg Vehicle: gelatin capsule Animals exhibited signs of acute ChE inhibition.	I	No Core Classification
Acute Oral - Rat	Counter 15G	LD_{50} (M, fasted) = 11.7 (9.0-15.3) mg/kg Vehicle: corn oil Animals exhibited signs of acute ChE inhibition	I	No Core Classification

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Study	Formulation	Results	Toxicity Category	Core Classification
Acute Dermal - Rabbit	Technical 96.7%	LD_{50} (M) = 1.1 (0.82-1.4) mg/kg	I	No Core Classification
Y	85.5%	LD_{50} (M) = 1.0 (0.67-1.3) mg/kg	I	
Acute Dermal - Rabbit	Counter 15G	LD_{50} (M) = 10.2 (7.7-13.4) mg/kg		No Core Classification
Acute Inhalation - Rat	Technical	LC_{50} (M) > 1.99 mg/L/7 hr	II	No Core Classification
Primary Skin Irritation-Rabbit	Technical 85.5%	0.25 ml/24 hrs: Death of all animals with intact or abraded skin.		No Core Classification
Primary Eye Irritation - Rabbit	Technical 85.5%	0.1 ml: All animals died within 24 hrs.		No Core Classification
Primary Skin Irritation-Rabbit	Technical 96.7%	0.5 ml/24 hr death to all animals in 24 hrs.		No Core Classification
Primary Eye Irritation - Rabbit	Technical 96.7%	0.1 ml/24 hr death to all animals within 2-24 hrs.		No Core Classification
Primary Skin Irritation-Rabbit	Counter 15G	0.5 g/24 hr.: Death of all animals (abraded and intact skin)		No Core Classification
Primary Eye Irritation - Rabbit	Counter 15G	0.1 g: Death of all animals within 72 hrs.		No Core Classification
Mutagenicity - Ames tests including metabolic activation American Cyanamid Lab.				
-S. typhimurium (TA98, TA100, TA1535, and TA1537) and E. coli (WA-2-uvrA⁻) tests	Technical	Negative		
-S. typhimurium (TA1535, TA100, TA1537, and TA98) and E. coli (WP-2-uvrA) tests	Technical	Negative		
-S. typhimurium and E. Coli; Rosenbrang DNA repair	Counter (AC-2374-15-2)	Negative		

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Study	Formulation	Results	Toxicity Category	Core Classification
Delayed Neurotoxicity - Chicken Bio/dynamics Inc., 72S-788	Technical	Negative at 0.01 mg/kg (no clinical evidence of residual neurotoxicity)		No Core Classification
31-Day Subacute Oral - Rat	Technical	NOEL (ChE) = 0.5 ppm		No Core Classification
31-Day Subacute Oral - Mouse	Technical	NOEL (systemic toxicity) = 4 ppm		No Core Classification
30-Day Subacute Oral - Dog	Technical	NOEL (PChE) 0.01 mg/kg NOEL (Brain ChE) < 0.25 mg/kg		No Core Classification
28-Day Oral for ChE determination - Dog Cyanamid #A77-158 10/19/77	Technical	NOEL (RBCChE) = 0.05 mg/kg PChE levels are reduced to as much as 21% of control (NOEL was based on RBC ChE values)		No Core Classification
30-Day Subacute Dermal - Rabbit	Technical	Dosage: 0.004 to 0.10 mg/kg (3 dose levels) Slight edema and erythema (subsided at termination) for both abraded and intact skin. No symptoms; ChE not determined.		No Core Classification
30-Day Subacute Dermal - Rabbit	Counter 15G	Dosage: 0.2 to 5.0 mg/kg (3 dose levels) Slight edema and erythema (subsided at termination) for both abraded and intact skin. No symptoms except death of 3/4 females and 3/4 males at 5.0 mg/kg dose within 6 to 20 days of study. ChE values not determined.		No Core Classification
90-Day Oral Feeding - Rat Bio/dynamic #71R-725		NOEL (RBCChE and Brain ChE) = 0.25 ppm, LEL = 1.0 ppm Systemic NOEL not determined because the highest dose was changed from 2 ppm to 4 ppm after 5 weeks of treatment.		No Core Classification

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Study	Formulation	Results	Toxicity Category	Core Classification
Teratogenicity - Rat IBT#B1374-(B)	Technical	Negative at 0.15 mg/kg/day. Valid.		Not Classified
3-Generation Reproduction - Rat Bio/dynamics Inc., #71R-727	Technical	NOEL > 1.0 ppm		Not Classified
6-Month Feeding - Dog FDRL; 1193	Technical	Dosage: 0.0025, 0.01, 0.04 mg/kg/day; 6 days/wk for 26 wks. NOEL > 0.04 mg/kg/day (= 1.6 ppm) for systemic toxicity NOEL = 0.0025 mg/kg/day (=0.1 ppm) for ChE and LEL = 0.010 mg/kg/day (26%, p = 0.01 for PChE and 14% for RBCChE)		Acceptable
2-Year Chronic Feeding/ Oncogenicity in rat Bio/dynamics #71R-725	Technical	Dosages in ppm: 0.25, 0.1 and 4.0 (2.0 → 4.0 → 8.0 → 4.0) for F and 8.0 (2.0 → 4.0 → 8.0) for M. RCB and brain ChE were significantly inhibited at 1 ppm (20% Inb. for Brain 43% for RBC in F) and above. Mortality: 24% (M), 27% (F) at high-dose, 19% mid-dose (M) and 10% low-dose (M) above controls. Incidence of ocular lesions i.e. exophthalmia, film on eye, and loss of lacrimation was noted in the high-dose females during the first year of the study period (exophthalmia was also noted in the low- and mid-dose females and at a lower incidence in the control females); and corneal scarring and cataracts were noted in in both males and females of the high-dose group but also seen at a lower incidence in the low-, mid-dose and control groups. However, no microscopic examination was done on animals that died or were sacrificed moribund during the study; or for the 3-month interim sacrifices.		Supplementary

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Study	Formulation	Results	Toxicity Category	Toxicity Classification
		Only 5 animals/sex in the control group and 10 animals/sex in the treatment groups were microscopically examined and organ weights determined. Also lesions noted microscopically in the high dose group at high incidence (i.e. bone marrow hyperplasia) were not examined at the lower doses.		
18-Month Carcinogenicity Mice Bio/dynamics #71R-728	Technical	Dosages: 0.5, 2.0, and 8.0 ppm Negative oncogenic at 8.0 ppm Symptoms: alopecia and signs of disturbed balance. Ocular lesions: exophthalmia in males, corneal cloudness and opacity, and eye rupture. Only 15 animals/sex/group were histologically examined at termination (examination of the low and mid-dose groups included only liver, kidneys, heart and lungs).		Supplementary
Metabolism - Rat	Technical ^{14}C	Dosage: 0.5 mg/kg, equivalent to ca. 10 ppm in diet. 83% excreted in urine and 3.5% in feces. Maximum residues of ChE inhibiting compounds were found in liver (0.08 ppm) 6 hrs after dosing. Residues of hydrolysis products reached a maximum in the kidneys after 12 hrs (0.9 ppm). All metabolites found in the rat had been previously identified in plant studies.		Not Classified
21-day feeding - cow	Technical	Dosage 2 ppm in dry diet. No detectable ChE inhibiting metabolites in milk at days 7, 14, or 21. No detectable ChE inhibiting metabolites in edible tissues at sacrifice.		Not Classified

Data Gaps

°One teratology study in a second species.
°A 2-year chronic feeding/oncogenic study in rats.
°An 18-month or longer oncogenicity study in mice.

PADI Calculation

Due to the data gaps stated above a PADI is calculated based on a 6-month dog feeding study (FDRL#1193). The study was reviewed by W.E. Parkins on 2/6/73 and D. Ritter on 4/2/73. The PADI is based on a NOEL for ChE of 0.10 ppm (0.0025 mg/kg/day) and a 100 fold safety factor:

$$\text{PADI} = 0.0025 \text{ mg/kg/day} \times \frac{1}{100} = 0.000025 \text{ mg/kg/day}$$

MPI for 60 kg person = 0.000025 mg/kg/day x 60 kg = 0.0015 mg/day/60 kg bw

According to the computer printout (attached) the PADI and MPI are as follows:

Older rat mg/kg	NOEL	S.F.	ADI mg/day/kg	MPI mg/day/kg
0.003	0.1 ppm	100	0.0000	0.0015

Published tolerances utlize 303.41% of the PADI; the current action would have utilized the PADI to 485.32%.

RPAR Criteria

The compound is not on the RPAR list and there are no pending regulatory actions against the compound to this reviewer's knowledge.

Additional Information

Counter 15G is acutely toxic dermally, LD_{50} = 10.2 (7.7-13.4) mg/kg consequently this formulation should be is classified in the restricted use category.

CFR 180.342 Terbufos 2/3/82

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File last updated 2/3/82

ACCEPTABLE DAILY INTAKE DATA

Dog	NOEL	S.F.	ADI	RPI
mg/kg	ppm		mg/kg/day	mg/day(60kg)
0.003	0.10	100	0.0000	0.0015

Published tolerances

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Sugar,cane&beet(154)	0.050	3.64	0.00273
Corn,grain(66)	0.050	1.00	0.00075
Corn,sweet(40)	0.050	1.43	0.00107

RPI	THRC	% ADI
0.0015 mg/day(60kg)	0.0046 mg/day(1.5kg)	303.31

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Current Action PP# 1F2433.

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Cabbage,sauerkraut(22)	0.200	0.74	0.00221
Cauliflower(27)	0.200	0.07	0.00021
Broccoli(19)	0.200	0.10	0.00031

RPI	THRC	% ADI
0.0015 mg/day(60kg)	0.0073 mg/day(1.5kg)	485.32

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o/o increase in TMRC
48.043
4.585
6.739

Review:

A Three and Twenty-Four Month Oral Toxicity and Carcinogenicity Study of AC 92,100 in Rats. Bio/dynamics Inc., Project No. 71R-725, Submitted by American Cyanamid on July 31, 1974.

I. Material and Methods

Test Substance

AC 92,100 premix, a greenish colored mixture containing 100 ppm (0.01%) of active ingredient.

Animals

Three hundred males and 300 females weanling rats were obtained from Blue Spruce Farms (Altamont, New York) on March 7, 1972 for this study. The baseline weight range (last weight prior to treatment) was 138.8 ± 2.3 g for males and 122.0 ± 2.6 g for females.

The animals were divided into five groups of 60 males and 60 females each. The rats were assigned to group by weight in an attempt to equalize mean group by weight prior to compound administration. Each animal was given a number and housed individually in an elevated wire mesh cage. The teeth were cut routinely; and water and test diets were available ad libitum.

The study was initiated on March 27, 1972. Interim sacrifice and necropsies of five rats/sex/control group and 10/rats/sex/treatment group were conducted at 3 month (June 20-27, 1972); all surviving rats were sacrificed and necropsied at 24 month (termination) on April 4-24, 1974.

Dosage

AC 92,100 was offered daily in the diet to the rats. The treated feed was prepared weekly by mixing AC 92,100 premix at appropriate amounts to give the following concentrations:

Group	Dosage Level (ppm)	Number of Rats/Group Males	Females
I (control)	0.0	60	60
II (control)	0.0	60	60
III (low)	0.25	60	60
IV (mid)	1.00	59**	61**
V (high)	2.0/4.0/8.0 (M)* 2.0/4.0/8.0/4.0 (F)*	60	60

*Group V (high-dose) was initially treated at 2.0 ppm then the dosage was elevated first to 4.00 ppm and then to 8.0 ppm at the beginning of the sixth and twelfth weeks respectively. The dose level of the females was decreased back to 4.0 ppm during the sixteenth week of treatment.

**One rat in Group V (mid-dose) was found to be a female placed with the male group and was accordingly transferred to the female group.

Samples of prepared test diet were not analyzed for test material content. However the actual compound consumption was calculated from the food consumption data.

Observations

The rats were observed daily for signs of overt toxicity, morbidity and mortality. Body weights were recorded once pretest (one week prior to treatment), weekly through the first 3 months, bi-weekly from 3 to 6 months, monthly from 6 to 24 months, and terminally after fasting.

Food consumption was measured and reported on the same schedule as body weight measurements were made.

Compound consumption was calculated from the food consumption data and reported also on the same schedule.

Observation for tissue masses was done weekly.

Ophthalmoscopic examinations were done on months 23 (for the high-dose group) and month 24 (for the control, low and mid-dose groups). However this reviewer notes that an interim report concerning eye observations in this study was submitted with PP#3G1340 (a proposed tolerance on corn; amendment of 3/15/73) and reviewed by D. Ritter.

Clinical

These investigations were done on 3 animals/sex/control group and 6 animals/sex/treatment group. The following parameters were evaluated:

1. Hematology
2. Urinalysis
3. Clinical Chemistry
4. Cholinesterase Activity

Hematology and urinalysis were performed on months 3, 6, 12, 18 and 24, and clinical chemistry on month 3 and 24.

Erythrocyte and plasma cholinesterase activities were determined on months 3, 6, 12, 18 and 24; however erythrocyte cholinesterase was also measured during the first month of treatment. Brain cholinesterase was measured on month 3 and 24.

1. Hematology

Hematology parameters included the following:

Hematocrit (HCT)
Hemoglobin (RBC)
Erythrocyte count and morphology (RBC)
Total and differential leucocytes (WBC)
Clotting time (CLOT X)

2. Urinalysis

Urine was tested for the following:

Gross appearance
Protein
Occult blood
Glucose
Ketones
Refractive Index
pH
Bilirubin

3. Clinical Chemistry

The parameters evaluated were as follows:

Serum glutamic pyruvic transaminase (SGPT)
Alkaline phosphatase (ALK-PHOS)
Fasting glucose (GLU)
Blood urea protein (BUN)

4. Cholinesterase Activity

The cholinesterase activity was determined by Michel method (1949). Cholinesterase values (ΔpH) were reported for the following systems:

Plasma (PChE)
Erythrocytes (RBC ChE)
Brain (B ChE)

Gross Pathology

After 3 months of compound administration, 5 animals/sex/control group and 10 animals/sex/treatment group were sacrificed using ether (for animals used in the hematology and clinical chemistry tests) or chloroform (remainder of animals). Surviving rats were also sacrificed as above and necropsied at the end of the 24 month study period.

Gross necropsy was also performed on all rats that died spontaneously or were sacrificed moribund during the study.

Heart, kidneys, liver (fresh weights); and thyroids (after fixation) from 5 animals/sex/control group and 10 animals/sex/treatment group were weighed at month 3 and 24 and the organ/body weight ratios computed.

The following tissues were fixed in 10% buffered neutral formalin except the eyes and testes (fixed in Bouin's solution):

Adrenals (2)
Aorta
Bone marrow (sternal)
Brain (2 sections)
Eye (with optic nerve)
Heart
Intestine
  colon
  duodenum
  ileum
Kidneys (2)
Lacrimal glands (2) (Month 24 only)
Liver (2 sections)
Lung
Lymph node
  mesenteric
Mammary glands (inguinal)
Nerve (peripheral)
Ovary
Pancreas
Pituitary
Prostate
Salivary gland
Seminal vesicles (Month 24 only)
Skeletal muscle
Skin
Spinal cord (thoracic)
Spleen
Stomach
Testis
Thyroid
Urinary bladder
Uterus
Gross lesions (including a section of normal-appearing portion of same organ)
Tissue masses

Histopathology

Hematoxylin and eosin were used for staining the tissues. Histological examination was done on all tissues (as specified in the above section) from 5 animals/sex/control group and 10 animals/sex/high-dose group at months 3 and 24. Liver, kidneys, lungs and heart and grossly abnormal tissues were also evaluated histologically from 10 animals/sex/low- and mid-dose groups at months 3 and 24.

Statistical Analysis

F-test and Student's t-test were used for comparison of the hematology and clinical chemistry parameters, and organ weights and organ/body weight ratios of animals sacrificed at month 3 for interim examination.

Dunnett test was used for comparison of body weight, food consumption, organ weights and organ/body weight ratios of animals sacrificed terminally at month 24.

Chi-square method was used for analysis of the mortality and ophthalmology data.

II. Results

General Observations

The report states that the following signs were observed at comparable incidence in all animals, including the control groups: irritability, abnormal respiratory sounds (gurgle, wheeze), alopecia, urinary staining of the urogenital fur, epistaxis, chromodacryorrhea, mucous nasal discharge and encrustation on head.

Clinical signs of cholinesterase inhibition i.e. muscle tremors, excessive salivation, hyperactivity and tachycardia, were first observed in the high-dose females when the dosage was temporarily increased from 4.0 ppm to 8.0 ppm (weeks 12-15, month 4). However the incidence of these signs decreased markedly from month 4 through month 18 and disappeared from month 18 through month 24. In addition, a few females of the mid-dose group (1.0 ppm) exhibited two or more of these clinical signs during month 5 and 6. None of these signs was observed in any other animal group except 8 high-dose males (8.0 ppm) during the last 3 months of treatment (muscle tremors).

Ocular lesions were noted during the study and at termination. These lesions are discussed in the section below on ophthalmology.

Ophthalmology

Ophthalmologic findings noted during observations for general signs included films on eyes, ocular opacity ; and exophthalmos (excessive bulging or protruding of the eyes to the extent that the sclera of the eye was visible without manual retraction of the eyelids).

These findings were first noted to occur in, and at that time were limited to, the high-dose females receiving the temporarily increased dosage of 8.00 ppm AC 92,100 (weeks 12-15, month 4). However, the incidence and degree of occurrence of exophthalmos was greater in the high-dose females throughout the study and rats in all female groups, including controls were noted to exhibit this finding from month 4 through month 15. The incidences at which these findings occurred decreased throughout the remainder of the study.

This reviewer notes that an 11-month status report on eye observations was submitted on 3/15/73 (amendment to PP#3G1340) and reviewed by D. Ritter on April 2, 1973. The review indicated that exophthalmia affected only female rats, that the incidence of exophthalmia was time and dose-related and that control rats also showed a similar but numerically reduced incidence of this syndrome (see table below, from April 2, 1973 review):

	% of female rats affected with exophthalmos								
WK	11	12	13	14	16	19	26	32	42
Control	0.0	0.0	0.0	0.9	0.0	1.8	9.1	10.9	9.1
0.25 ppm	0.0	0.0	0.0	6.0	22.0	4.0	26.0	14.0	18.0
1.0 ppm	0.0	0.0	0.0	2.0	64.7	19.6	58.8	25.5	10.2
4-8-4 ppm*	0.0	16.7	66.0	91.7	82.6	26.1	20.5	34.1	23.3

*Dose increased to 8 ppm on day 77, but reduced back to 4 ppm on day 105 due to severe toxicity.

The reviewer stated that "the high dose group showed severe involvement fairly early, (91.7% at week 14), with the middle and low dose groups showing later and less severe reaction to insult. Interestingly, the control group showed a mild but definite increase in incidence of the exophthalmos. Other ocular damage that could be related to treatment was confined to the high dose group, with seven occurances of corneal opacity; lower dose incidences were comparable to those of the controls" and finally concluded that a no-effect level of toxicity has not been demonstated thus far in this study insofar as the eye is concerned.

It is noted that exophthalmia is uncommon in rat colonies and its presence in the control group in this study is hard to explain.

Ophthalmoscopic examinations performed terminally on all surviving rats revealed corneal scars and cataracts in rats of all groups. The incidences of these findings in both sexes of the high-dose group (8 ppm for males and 4 ppm for females) were greater than those noted in the combined control group. The incidence of corneal scars was statistically significant ($p < 0.001$) in the high-dose females. The incidence of cataracts in the high-dose group was not statistically significant when computed for each sex separately. However the level of relative risk (RR) was similar in each sex (RR = 2.84 for males and 2.80 for females), thus the pooled cataract effect for all animals (M&F) of the high-dose group was statistically significant at $p < 0.02$ (Mr. Bertram Litt review of the statistical analysis, 1/25/82).

Incidence of corneal scarring and cataracts in the mid- and low-dose groups were comparable to the combined control group.

Corneal scarring may be associated with a history of previous exophthalmia in these animals or to hyperactivity.

The following table demonstrates the ocular lesions noted at termination.

		Number with Corneal Scars/No. Alive (%)				Number with Cataracts/ No. Alive (%)			
	Dosage	Males		Females		Males		Females	
Group	(ppm)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
I	0.00	5/38	13.2	1/39	2.6	2/38	5.3	3/39	7.7
II	0.00	1/38	2.6	1/41	2.4	3/38	7.9	7/41	17.1
I and II	0.00	6/76	7.9	2/80	2.5	5/76	6.6	10/80	12.5
III	0.25	2/28	7.1	0/38	0.0	1/28	3.6	3/38	7.9
IV	1.00	2/24	8.3	0/40	0.0	0/24	0.0	3/40	7.5
V	8.0(M)	5/24	20.8			4/24	16.7		
	4.0(F)			9/21	42.9***			6/21	28.6

***$p < 0.001$

Although the above table reflects no difference in corneal scarring between males of the low and mid-dose groups and the combined control group, the number of males examined in these two treatment groups was 26% and 37% respectively lower than the number of males examined in the control group, and no data were available on animals (males or females) that died or were sacrificed moribund during the study. Consequently these terminal ophthalmoscopic data alone are inadequate to reflect a no effect level for ocular lesions.

Mortality

Mortality rates increased above the control mortality in the high-dose group for both sexes throughout the study and for mid-dose males from month 18 to month 24.

Data for mortality rates at months 18 and 24 are presented in the following table:

Group	Dosage (ppm)	No. Died/No. Initiated (less interim sacrifice) 0-18 Months Males No.	(%)	Females No.	(%)	0-24 Months Males No.	(%)	Females No.	(%)
I	0.00	6/50	10.9	3/50	5.5	18.50	32.7	16/50	29.1
II	0.00	9/50	16.4	6/50	10.9	24/50	43.6	20/50	36.4
I and II	0.00	15/100	13.6	9/100	8.2	42/100	38.0	36/100	32.7
III	0.25	6/50	12.0	5/50	10.0	24/50	48.0	13/50	26.0
IV	1.00	13/49	26.5	9/50	17.6	28/49*	57.1	17/50	33.3
V	8.0(M)&(4.0(F)	15/50*	30.0	21/50***	42.0	31/50**	62.0	30/50**	60.00

*p < 0.05 **p < 0.01 ***p < 0.001

The above table reflects higher mortality rates (up to 10%) in control no. II for both females and males than control no. I; the mortality data in the treatment groups were compared to the average mortality rate of the two control groups.

Mortality rates for both males and females of the high-dose group increased significantly (p < 0.05 to p < 0.001) during the second year of the study period. Data for males reflected an increase of 14%, 16% and 24% in the total mortality as noted above as compared to the average control value for month 12, 18 and 24 respectively; the female reflected an increase of 28%, 34% and 27% above the average control value for months 12, 18 and 24 respectively.

The mid-dose level caused 13% and 19% increase in male mortality above the average control values for month 18 and 24 respectively. The increase was statistically significant (p < 0.05) at month 24 only. The female mortality was comparable to the combined control value.

The low-dose level caused a slight increase of 10% above the average control value in male mortality at termination (month 24). The female mortality was comparable to the combined control value.

Body Weight and Food Consumption

Mean body weight and food consumption values (gram/rat/week) were significantly lower (p < 0.05 to p < 0.01) than the control values for the high dose rats throughout the study period (decrease in mean body weight values was noted as early as week 5 of the study, and as early as week 35 for the mean food consumption value). However the food consumption values in gram per kilogram body weight per day were comparable to the control values.

The mid- and low-dose values for body weight and food consumption were comparable to the control values.

The following table presents the mean body weights and average food consumption at week 103:

Group	Dosage (ppm)	Average Food Consumption g/rat/week (M)	(F)	Average Food Consumption g/kg bw/day (M)	(F)	Mean Body Weights (g) (M)	(F)
I	0.0	149.6	126.0	35.7	52.0	587.3	352.8
II	0.0	147.8	125.0	34.5	53.8	591.2	335.4
I and II	0.0	148.7	125.5	35.1	52.9	589.2	343.7
III	0.25	150.9	122.1	37.0	45.9	580.7	352.5
IV	1.00	159.4	116.5	36.7	47.4	573.7	357.0
V	8.00	120.4**	-----	38.6	----	422.7**	------
	4.00	------	102.2**	----	50.7	------	280.4**

**p < 0.01

Compound Intake

The compound intake were calculated from the food consumption values. The calculated average Terbufos intake was 80.0% to 84.2% of the nominal dosages for all male groups and 106% to 111% of the nominal dosages for females.

Clinical Evaluation

1. Hematology

Hematology data were reported for months 3, 6, 12, 18 and 24. No significant differences between control and treatment groups were noted for HCT, HGB, RBC, WBC and CLOT X except occasionally:

- HCT and RBC values for the low-dose males were slightly depressed ($p < 0.05$) on month 3, and RBC value was slightly elevated ($p < 0.05$) on month 12.

- HCT was slightly elevated ($p < 0.05$) for the mid-dose females on month 3 and RBC was also slightly elevated ($p < 0.05$) on month 18.

- WBC was slightly elevated ($p < 0.05$) for the high-dose females on month 3.

- Slight changes in the clotting time were noted in the female group. Clotting time was slightly increased on month 3, 6 ($p < 0.05$) and 12 for the high-dose females and on month 6 ($p < 0.01$) for the low-dose females. However the clotting time was slightly decreased on month 3 for both the low-dose ($p < 0.05$) and mid-dose ($p < 0.01$) females.

2. Urinalysis

Urinalysis was reported for months 3, 6, 12, 18 and 24. Higher levels of protein were noted in males on month 12 for all treatment groups especially at the mid-dose level. Occult blood was also noted more often in these mid-dose males than any other group. Also on month 24 all treated males had higher level of protein than the controls.

No difference between control and treated females was noted except for occasional appearance of occult blood in the urine of few animals of the low- and high-dose groups on month 12 and 24.

3. Clinical Chemistry

Values for SGPT, ALK-PHOS, GLU, and BUN determinations were reported for months 3 and 24. No difference was noted between the control and treatment groups except in the high-dose rats for the following values:

- Alkaline phosphatase was low ($p < 0.05$) for males, 26.3 IU (limits 30-500 IU) on month 3.

- Glucose level was low ($p < 0.01$) for females, 67.2 mg/dl (limits 80-200 mg/dl) on month 3. Also BUN value was high ($p < 0.01$) but within the normal range, 20.00 mg/dl (limits 9-30 mg/dl) for females at month 3.

4. Cholinesterase Activity

Cholinesterase determinations for PChE and RBC ChE were reported for months 3, 6, 12, 18 and 24; RBC ChE was also determined on the first month of treatment. BChE values were reported for months 3 and 24.

PChE, RBC ChE and BChE values were highly and significantly depressed ($p < 0.05$ - $p < 0.01$) in both males and females of the high-dose group throughout the study (except for PChE values on month 3, 6 and 24 in males and month 12 in females where the depression in activity was low or nominal.

The mid-dose group reflected a high and significant depression ($p < 0.05$ - $p < 0.01$) in RBC ChE activity on months 6, 18 and 24 in males and months 1, 3 and 24 in females; the rest of the RBC ChE values reflected a moderate to low inhibition. BChE was moderately but significantly depressed ($p < 0.01$) in females at termination. PChE values at this mid-dose level were erratic and varied between depression and activation in both males and females.

The low-dose group reflected a slightly depressed level of activity for RBC ChE for both males and females ($p < 0.05$), and BChE in females. PChE values were erratic and varied between slight depression to high and significant ($p < 0.01$) activation.

The terminal PChE, RBC ChE and BChE values (pH and % Inhibition (% I) are presented in the following table:

Group	Dosage (ppm)	PChE pH Male	PChE pH Female	PChE % I Male	PChE % I Females
I & II	0.00	0.642	1.037	00	00
III	0.25	0.655	1.243	00	00
IV	1.00	0.627	1.102	2	00
V	4.00	-----	0.703**	--	32
	8.00	0.508		21	--

Group	Dosage (ppm)	RBCChE pH Male	RBCChE pH Female	RBCChE % I Male	RBCChE % I Females
I & II	0.00	0.407	0.435	00	00
III	0.25	0.357	0.368*	12	15
IV	1.00	0.273**	0.248**	33	43
V	4.00	-----	0.153**	--	65
	8.00	0.155**	-----	62	--

Group	Dosage (ppm)	BChE pH Male	BChE pH Female	BChE % I Male	BChE % I Females
I & II	0.00	1.448	1.537	00	00
III	0.25	1.495	1.435	00	7
IV	1.00	1.415	1.353**	2	12
V	4.00	-----	0.645**	--	58
	8.00	0.547**	-----	62	--

*p < 0.05 **p < 0.01

This reviewer notes that the potentiometric method (Michel, 1949) used in this study for ChE determinations is mainly accurate for RBC ChE determination. This method is known to be non-specific for plasma and brain homoginates as they may include other esterases not subject to the same kind of inhibition as acetylcholinesterase. Consequently the pH would reflect the activity of these other active esterases.

In conclusion the NOEL of ChE inhibition should be based on RBC ChE values in this study. RBC ChE NOEL is lower than 0.25 ppm (12 to 15% inhibition $p < 0.05$).

Tissue masses

External tissue masses were noted during the first 9 month of the study in one male and one female of the mid-dose and 3 males and 5 females of the high-dose. However the incidence of tissue masses externally observed was similar in the control and treatment groups during the second year of the study. The study did not clearly identify the animals affected and it is not clear if these animals died before termination or were included among the animals selected for microscopic examination at termination.

Tissues of 10 animals/sex/treatment group and 5 animals/sex/control were microscopically examined at termination (the examination at the low- and mid-dose groups included few major organs and the lesions noted at gross necropsy). These data indicated no difference between the control and treatment groups. The kind of tumors noted in both control and treatment groups was common in normally aging rat colonies.

Although this study reflects a negative oncogenic potential for Terbufos, an inadequate number of animals were microscopically examined in the high dose and controls.

Necropsy

Esophageal dilation was noted at necropsy in some high-dose animals dying between 3 and 6 months. This finding was associated with the increase in dosage from 4.0 ppm to 8.0 ppm (the animals exhibited severe cholinergic symptoms; in addition, exophthalmos and film on eyes were noted in females). This esophageal dilation is probably due to Terbufos administration and its action on the contractability of the esophageal muscles.

Mean organ weights and mean organ to body ratios of liver, kidney, heart and thyroids were evaluated at necropsy at the 3 month interim sacrifice and at termination on month 24.

Liver: Except for the high-dose males and females where mean liver weights decreased at termination (significantly in males $p < 0.01$), mean liver weights and liver to body weight ratios increased in all treatment groups at the 3 and 24 month necropsies. The increases were statistically significant at month 3 for both the low-dose females (liver weight, $p < 0.05$) and the high-dose females (liver to body weight ratio $p < 0.05$).

Kidneys: The high-dose mean male kidney weights and organ to body weight ratio decreased at termination ($p < 0.01$ for kidney weight). The mean female kidney weights in this group was similar to the control at termination but there was a significant increase ($p < 0.05$) in the organ to body weight ratio. The low and mid-dose levels reflected a trend toward increased weight and organ/body weight ratio at termination. Data at the interim 3 month sacrifice were similar to the control but with a significant increase ($p < 0.01$) in the mean kidney/body weight ratio in the high-dose females.

Heart: Mean heart weights decreased in both males and females of the high-dose at termination; the decrease was significant in males ($p < 0.01$). The high-dose females reflected a trend toward decreased mean heart weight at the 3-month interim sacrifice, however the mean heart/body weight ratio was significantly elevated ($p < 0.05$). All the other data were similar to the control data or reflected a trend toward increased mean heart weight and heart/body weight ratio at both the 3 and 24 month necropsies.

Thyroid: Changes in mean thyroid weights were not statistically significant in any group. However a trend toward decreased mean thyroid weights was noted in the high-dose males and females at both the interim and terminal necropsies; and a trend toward increased mean thyroid weights and thyroids/organ weight ratio was noted in males of the 2 lower doses at month 3 and in females at month 24. All other determinations were similar to the control group.

The noted increase in the mean organ/body weight ratios for the organs evaluated at the high-dose level may not be biologically significant because the mean body weights of males and females at this dosage were significantly depressed ($p < 0.05$-$p < 0.01$). The noted decrease in the mean absolute organ weights in this high-dose group may also be associated with the significant decrease in body weights.

Organ weight changes were associated with increased incidence of microscopic changes in the treatment groups as compared to the control animals. The changes are discussed under the histopathology section below.

Histopathology

Microscopic changes were noted at termination in liver and heart of all three treatment groups (low-, mid- and high-dose levels) as compared to the control group. The liver, in males and females, reflected an increase in bile duct hyperplasia. The heart reflected an increase of myofibril degeneration and lymphocytic myocarditis in males (see table below).

Microscopic changes at termination were also noted at a higher incidence in the kidneys and lungs of the high-dose group as compared to the control group. Higher incidence was noted in chronic interstitial nephritis in females, and localized mineralized foci in males. Incidence of lung granuloma (lypoid) also increased in both males and females and bronchopneumonia in females (see table below).

Table reflecting the increased microscopic lesions in organs examined:

		Control I	Control II	Low-dose	Mid-dose	High-dose
Liver						
Bile duct hyperplasia	(M)	0/5	0/5	7/10	5/10	4/10
	(F)	0/5	0/5	8/10	8/10	4/10
Heart						
Myofibril degeneration	(M)	1/5	1/5	4/10	5/10	4/10
Lymphocytic myocarditis	(M)	1/5	1/5	4/10	5/10	4/10
Kidneys						
Chronic interstitial nephritis	(F)	2/5	3/5			8/10
Localized mineralized foci	(M)	1/5	2/5			6/10
Lungs						
Granuloma (lypoid)	(M)	0/5	0/5			5/10
	(F)	2/5	1/5			5/10
Bronchopneumonia	(F)	2/5	0/5			5/10

Histological examinations of other tissues at termination was only done in the high-dose group. Lesions noted in these tissues were not further investigated in the low and mid-dose groups. The following findings were noted in the high-dose group:

Bone marrow hyperplasia in 4/10 males and 2/10 females as compared to none in the control group. Thyroid medullary cell hyperplasia in 2/10 males as compared to none in the controls. Both lesions (in bone marrow and thyroid) are considered to be reversible, therefore their importance is limited.

Finally microscopic examinations of the 3 months interim sacrifices or of animals that died during the study were not recorded in this report or in the previously submitted 3 and 6 months interim status reports (reviewed by W.E. Parkins on 2/6/73).

Discussions and Conclusions

This two year feeding/oncogenic study in rats indicates that Terbufos is toxic to rats at the mid- and high-dose levels (1.0 ppm, and 4.0 ppm for (F) and 8.0 ppm for (M) respectively). Systemic changes were also noted at the low-dose level (0.25 ppm) but apparently not significant. The calculated compound intake during the study was 80 to 84% of the nominal dosages in males of all groups.

The toxic effects noted in the treatment groups as compared to the combined control groups are the following:

1) Highly significant increase in mortality rate at termination in both males and females, of the high-dose (24%, $p < 0.01$ and 27%, $p < 0.01$ respectively) and males of the mid-dose (19%, $p < 0.05$) as compared to the combined control groups.

2) Highly significant and dose-dependent increase in RBC ChE inhibition throughout the study at termination (12, 33 and 62% for males, $p < 0.05$-0.01 and 15, 43 and 65% for females, $p < 0.05$-0.01 in the low-, mid-, and high-dose groups respectively). BChE was highly inhibited termination in at the high-dose males and females (62%, $p < 0.01$ and 58%, $p < 0.01$ respectively), and moderately in the mid-dose females (12%, $p < 0.01$). PChE was moderately inhibited in the high-dose males and females at termination (21% and 32%, $p < 0.01$ respectively).

3) Statistically significant body weight depression and food consumption in all animals of the high-dose.

4) Statistically significant decrease in liver and thyroid weights in both males and females of the high-dose group, and heart, kidney and kidney/body weight ratio in the high-dose males (at month 24).

5) Histopathological changes in liver of all treated animals, heart of all treated males, kidney, lungs and bone marrow of the high-dose animals.

6) Statistically significant ocular scarring ($p < 0.001$) in high-dose females at termination (biologically significant ocular scarring in males), and cataracts in both males and females of the high dose at termination. Ocular scarring may be related to hyperactivity and/or the exophthalmos syndrome previously noted in females in this study. Exophthalmos has been noted in all female animals, including the controls, during the first year of study. The incidence of exophthalmos was low in the control animals and dose-related in the treated females. However the available data in this final report indicate that exophthalmos subsided in all groups during the second year of study.

This study appears to reflect the following NOELs:

NOEL < 0.25 ppm (lowest dose level) for systemic toxicity (see necropsy & histopathology sections pages 13 & 14). NOEL for RBCChE < 0.25 ppm.

Due to the following discrepancies noted in this study, no definitive conclusion could be reached.

1) The number of animals microscopically examined at termination is inadequate. Only 5/60 animals per sex per control group and 10/60 animals per sex per treatment group were examined (Complete histological examination was done on animals of the control and high-dose groups).

2) No microscopic examination was reported for animals that died or were sacrificed moribund during the study; or for the 3-month interim sacrifices.

3) No microscopic examination was done in the mid- and low-dose groups for lesions which were noted terminally at an unusually high incidence in the high-dose group i.e. bone marrow hyperplasia (4/10 males 2/10 females as compared to none in the control group).

4) The study is inadequate by design to fully assess Terbufos oncogenic potential because an inadequate number of animals were microscopically examined in the high dose and control (5/60 animals per sex in the control groups and 10/60 animals per sex in the high dose group). Consequently the oncogenic potential of this compound needs to be further investigated.

Classification: Core Supplementary

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