3-31-82

008163

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

MEMORANDUM

DATE: MAR 31 1982

SUBJECT: EPA Registration No. 59-ROU, PP OF2324, Request for a tolerance for permethrin in/on meat and meat byproducts, animal fat, whole milk and milk fat.

TOX Chem No. 652BB

FROM: John Doherty, TOXICOLOGY BRANCH, HED (TS-769) March 23, 1982

TO: F. D. R. Gee, PM (17), RD (TS-767)

THRU: O. E. Paynter, Ph.D., Branch Chief OEP 3/31/82
Toxicology Branch, HED (TS-769)

Background

The Burroughs-Wellcome Co. has requested the establishment of tolerances for their pesticide permethrin as follows:

0.025 ppm in lean red meat,
0.25 ppm in animal fat
.020 ppm in whole milk
0.20 ppm in milk fat

These residues may result from the use of their product ATROBAN(R) WP as a premise spray to control flies in and around beef, dairy, sheep, goat and horse barns.

RCB has not yet (as of February 1982) approved this petition. See reviews by J. H. Onley, Ph.D., dated September 16, 1980, and February 9, 1982.

Conclusions

(1.) RESIDUE CHEMISTRY BRANCH (RCB) has not recommended in favor of the proposed tolerances and the registrant has apparently agreed to submit a revised Section F. When the deficiencies indicated in the RCB memo are resolved and a revised Section F is submitted, TOXICOLOGY BRANCH will determine the effect of granting the proposed (or revised) tolerances on the TMRC and the ADI. As of now and unless an unexpected problem arises, there are no outstanding toxicological data requirements to register permethrin for this use.

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(2.) The product ATROBAN(R) WP has the signal word CAUTION consistent with the acute toxicity studies submitted to support the labeling.

However, this product contains the inert [redacted] in excess of allowable limits. For example, [redacted] but only [redacted] is allowable.

This problem must be resolved before the product is registered for use.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

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Studies Reviewed

The substance tested is 21Z73 (25% cis/75% trans permethrin) unless otherwise indicated. These studies are in EPA Acc. No. 099258 and 099263.

Study (see review for lab and date)	Results	Core Classification
1) Acute Oral LD_{50}-rats effects of solvents	see review p. 6	SUPPLEMENTARY
2) Acute Oral LD_{50}-rats effects of variation of cis/trans ratio	see review p. 6	SUPPLEMENTARY
3) Acute toxicity by various routes in the rat, mouse and chick	see review p. 7	SUPPLEMENTARY
4) Acute Dermal LD_{50}-rat (male)	> 2000 mg/kg	MINIMUM
5) Acute Dermal LD_{50}-rat (female)	> 2000 mg/kg	MINIMUM
6) Eye Irritation-rabbit	Not irritating	MINIMUM
7) Dermal Sensitization Study-guinea pig	Not a sensitizer	MINIMUM
8) 10-Day Oral (gavage)-rats	NOEL < 200 mg/kg for pharmacological effects on liver NOEL = 200 mg/kg for toxic response LEL = 400 mg/kg - for tremors, etc.	SUPPLEMENTARY
9) 10-Day Oral (gavage)-mice	NOEL = 400 mg/kg for pharmacological effects (increased liver weights) NOEL = 800 mg/kg for toxic response LEL = 1600 mg/kg - tremors, convulsions, death	SUPPLEMENTARY
10) 90-Day Oral Feeding-rats	NOEL = 2000 ppm LEL = 4000 ppm (hypersensitivity, decreased body weights, increased liver weights, possible thymic lesions)	MINIMUM

Study (see review for lab and date)	Results	Core Classification
11) 90-Day Oral Feeding-rats (40% cis and 60% trans)	NOEL = 200 ppm (?) LEL = 600 ppm (?), (increase in fat in renal tubules reported at 600 and 2000 ppm; toxicological significance of this finding, however, is questionable)	MINIMUM
12) Teratology-mouse	Not teratogenic or maternal toxic at 400 mg/kg (only dose tested)	MINIMUM
13) Teratology-rat	Not teratogenic or maternal toxic at 200 mg/kg (only dose tested)	MINIMUM
14) Teratology-rabbit	Not teratogenic or maternal toxic at 400 mg/kg (only dose tested)	MINIMUM
15) 3-Generation Reproduction-rats	NOEL = 180 mg/kg/day (highest dose tested) for any toxic effect	GUIDELINES
16) 6-Month Dog - capsule feeding	NOEL of 250 mg/kg (highest dose tested)	GUIDELINES
17) Dominant Lethal-mice (25% cis/75% trans)	Not mutagenic at 452 mg/kg (highest dose tested)	N/A
(40% cis/60% trans)	Not mutagenic at 285 mg/kg (highest dose tested)	N/A
18) Mouse Lymphoma	Not mutagenic at 47 µg/ml	N/A
Studies with ATROBAN (25% wettable powder)		
19) Acute Oral LD_{50}-rats	LD_{50} > 6000 mg/kg for males; 12,741.7 mg/kg for females (Tox. Cat. IV)	MINIMUM

Study (see review for lab and date)	Results	Core Classification
20) Acute Dermal LD_{50}-rabbits	LD_{50} > 5000 mg/kg Tox. Cat. III	MINIMUM
21) Acute Inhalation LC_{50}-rats	LC_{50} > 4.99 mg/l Tox. Cat. III	MINIMUM
22) Eye Irritation-rabbit	Mild irritation Tox. Cat. III	GUIDELINES
23) Dermal Irritation-rabbit	Draize score 0.65 (not irritating) Tox. Cat. IV	GUIDELINES

Review of Studies

1.) 21Z73 (25/75) effect of different solvents on the rat oral toxicity (tab 9)

Wellcome Research Laboratories, HEFG 75-4, February 17, 1975

Groups of female Wistar rats were dosed with 21Z73 permethrin by gavage. LD_{50}'s were determined with 6 rats per group and usually 4-6 groups per solvent as follows:

Solvent	LD_{50}
110% w/v neat	> 20090 mg/kg (HDT)
40% w/v corn oil	= 4672 mg/kg
40% w/v odorless petroleum distillate	> 8000 mg/kg (HDT)
40% w/v DMSO	> 8000 mg/kg (HDT)
20% w/v glycerol formal	> 5048 mg/kg (HDT)

SUPPLEMENTARY DATA: No necropsy or daily observations reported.

2.) Cis/trans ratio and rat oral toxicity of the 21Z73 series (tab 5)

Wellcome Research Laboratories

Study No. HEFG 75-5 (no date; it is assumed the study was in 1975)

The LD_{50} of various cis/trans ratios of 21Z73 (permethrin) was determined using female Wistar rats. The test chemical was dissolved in corn oil and usually 4-5 rats per group were dosed. Dosing was by gavage.

Results

% cis	% trans	LD_{50} mg/kg	95% Conf. Limits
80	20	224.5	(200-252)
60	40	445.3	(397-500)
50	50	1000	(733-1363)
40	60	1260	(1000-1587)

Results

% cis	% trans	LD_{50} mg/kg	95% Conf. Limits
30	70	1684	(1257-2255)
20	80	ä 6000	(HDT)

SUPPLEMENTARY DATA: No daily observation report, no necropsy.

3.) 21Z73 (25/75) acute toxicity studies by various routes of administration in the rat, mouse and chick (tab 10)

Wellcome Research Laboratories, HEFG 75-8, March 27, 1975

The following LD_{50} data were generated using various methods of chemical administration and different test animals.

Species	Sex	Administration	Solvent	LD_{50}
Mouse	F	Immersion	Shellsol	None died when immersed in 10% solution.
Mouse	F	Oral	Corn oil	2690 mg/kg
Mouse	M	Oral	Corn oil	2500 mg/kg
Mouse	F	Intraperitoneal	Corn oil	> 12,800 mg/kg
Mouse	F	Intravenous	Ethyl cellosolve	400 mg/kg
Chick	M	Oral	Corn oil	> 4000 mg/kg
Rat	F	Oral	Corn oil	4672 mg/kg
Rat	M	Oral	Corn oil	1479 mg/kg
Rat	F	Dermal	Xylene	> 4000 mg/kg

Species	Sex	Administration	Solvent	LD_{50}
Rat	F	Intraperitoneal	Corn oil	> 3200 mg/kg
Rat	M	Intraperitoneal	Corn oil	> 4000 mg/kg
Rat	F	Intravenous	Ethyl cellosolve	356 mg/kg (> means highest dose tested)

SUPPLEMENTARY DATA: No necropsy or daily observation of behavior.

4.) 21Z73 - Dermal Toxicity in the Male Rat (tab 11)

Wellcome Research Laboratories

Study No. TL 22-77, HEFG 77-7, April 20, 1977

6 male rats/dose group were dosed with 1.25, 2.5, or 5.0 ml/kg (500, 1000, 2000 mg/kg of active ingredient) of 21Z73 (40% in xylene). Exposure time was for 24 hours and observation was for 14 days.

Results: No signs of toxicity resulted. Gross necropsy revealed signs of "old pneumonic lesions" but these were not definitely known to result from treatment with permethrin. LD_{50} > 2000 mg/kg.

CORE MINIMUM. The presence of the pneumonic lesions could mean a toxic effect. This study does not have an appropriate control group to ascertain that these lesions were not related to treatment of either xylene or permethrin.

5.) 21Z73 - Dermal Toxicity in the Female Rat (tab 12)

Wellcome Research Laboratories

Study No. TL 16-77, HEFG 77-5, March 28, 1977

(same as above)

LD_{50} > 2000 mg/kg, no lesions evident by gross necropsy observation.

CORE MINIMUM

6.) Ocular irritancy of 21Z73 in rabbits (tab 13)

Wellcome Research Laboratories

Study No. TL 16-74, HEFG 74-6, May 6, 1974

0.1 ml of 21Z73 (40% in corn oil) was instilled into the eye of each of 6 albino rabbits and observations were made for ocular irritancy.

No corneal opacity or conjuctival irritation developed.

CORE MINIMUM. Test chemical was dissolved in corn oil which is not a formulation or the technical product. Toxicity category IV.

7.) Guinea Pig Sensitization Study with 21Z73 using the "maximization" test method (tab 14)

Wellcome Research Laboratories

TL 13-74, HEFG 74-3, March 27, 1974

A guinea pig maximization test was conducted using 21Z73 dissolved in corn oil (1% permethrin) by the method described in "Allergic contact dermatitis in the guinea pig. Identification of contact allergens." Magnusson, B. and Kligman, A.M., 1970, C.T. Thomas, Springfield, Illinois, 1970. Ten guinea pigs were used for the test chemical. Five guinea pigs were used for vehicle control and positive control groups.

No evidence that 21Z73 is a skin sensitizer developed. The positive control (2,4-dinitrochlorobenzene) dissolved in corn oil (1%) responded by producing sensitization. CORE MINIMUM.

8.) 10-day cumulative oral toxicity study with 21Z73 in rats (tab 6)

Wellcome Research Laboratories, No. HEFG 74-10, June 13, 1974

Five groups of 6 female Wistar rats were dosed as absolute control, vehicle control, 200, 400, or 800 mg/kg/day of permethrin as 21Z73 (Batch #F) in corn oil. Administration was by gavage and there were 10 doses made. The rats were sacrificed 24 hours after the last dose.

Results

1. Clinical observations. The low dose group was reported as being identical to the control groups. The higher dose groups showed muscle spasms and hypersensitivity and 3/6 of the high dose group died as a result of the test chemical.

2. Bodyweights. The high dose group only was reported as being affected.

3. Haematology. No changes noted in the red and white cell counts determined.

4. Blood chemistry. There were "slight" increases in serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and lactic dehydrogenase to indicate that some liver damage may have resulted. It is apparent that the low dose group was affected.

5. Necropsy. No consistent changes related to the test chemical were noted in the organs by visual observation. Liver weights (relative) were statistically significantly higher in all groups in a dose related manner.

SUPPLEMENTARY DATA. 10 days is not a standard time interval. A NOEL of < 200 mg/kg for effects on the liver is noted. The NOEL for toxic response (tremors, etc.) is = 200 mg/kg.

9.) 10-day cumulative oral toxicity study with 21Z73 in mice (tab 20)

Wellcome Research Laboratories, HEFG-74-9, June 6, 1974

Six groups of 6 female CD-1 mice were dosed by gavage for 10 consecutive days with absolute control, vehicle control, 200, 400, 800 or 1600 mg/kg of permethrin (as 21Z73, batch WF, 40% in corn oil). The mice were sacrificed 24 hours after the last dose.

Results

1. Clinical signs were reported in the 1600 mg/kg dose group only. These included hypersensitivity with violent reaction to any stimulus. The symptoms appeared 1 hour after dosing and usually lasted for 4 hours. 3/6 mice in the high dose group died after the first dose. The mice apparently developed a tolerance to the poison because after successive doses the toxicity was reported as being less severe. Hypersensitivity was not observed in the surviving animals after the fifth dose. The other test groups were not reported as being affected.

2. Body weight. No differences noted.

3. Haematology and clinical blood chemistry (at termination). The changes noted were not considered important or of toxicological importance.

4. Absolute and relative liver weight was increased in the 800 and 1600 mg/kg dose test groups. Other organ weights were not statistically significantly different from controls.

SUPPLEMENTARY DATA. 10-day interval is not a standard interval, no histopathology. NOEL of 400 mg/kg is noted. Toxic responses, exclusive of liver weight changes, are noted at 1600 mg/kg only.

10.) 21Z73, rat oral 90-day study (tab 7)

Wellcome Research Laboratories, HEFG 76-1, February 25, 1976

Five groups of 36 Wistar rats (18 males and 18 females) were dosed (in the diet) as either 0, 200, 600, 2000 or 4000 ppm of 2:273 (25% cis 75% trans, batch WP) for 90 days. After 90 days, 10 rats of each sex from each group were sacrificed and examined. The remaining rats in each group were allowed 36 days to "recover" from any toxic effects of the test chemical.

Results

1. Clinical toxicity. The high dose males and females showed signs of hypersensitivity. The males adjusted to the chemical in the diet and after 3 weeks there were no signs of hypersensitivity. The signs persisted in the females until the permethrin was removed from their diets.

2. Body weights. [A defective watering system was stated as being responsible for initial reductions in body weight gain.] There were otherwise no reductions in body weight gain in the females or in the males dosed with 200, 600 or 2000 ppm. The high dose male group was affected but weight gain was improved after dosing was discontinued at 90 days.

3. Drug intake. The average daily intake of drug was 0, 17.0, 49.9, 179.6 and 357.4 mg/kg for males and 0, 18.5, 56.2, 176.5, and 356.7 mg/kg for females. The average daily food intake was not affected by the presence of permethrin in the diet. Food conversion rate was also not affected by the presence of permethrin.

4. Oestrus cycle (determined in females during the last 30 days of the 90-day feeding cycle). No effect of the test chemical.

5. Haematology and blood chemistry and urinalysis. A range of laboratory tests were performed on samples taken from 6 animals of each sex/group. The animals were bled from the orbit under light ether anesthesia at 14, 28, 56, 90 days (representing the dosing phase) and 126 days (36 days recovery phase). At 90 days, 10 additional animals of each sex and from each main group were bled in a similar manner from the orbit before killing. The following assays were performed on the samples:

Haematology

The packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total white cell (WBC) and differential counts were determined.

There were no consistant effects on the blood counts except for a possible decrease in white blood cell counts in the early weeks of the study in the high dose groups. This deviation is not conclusively linked to the presence of permethrin.

Blood chemistry

Fasting serum glucose (GLUC), urea nitrogen (UN), alkaline phosphatase (AP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), total protein (TP), albumin (ALB), sodium (Na) and potassium (K) were estimated. Hepatic aminopyrene demethylase was not assayed.

No consistent dose dependent deviations were noted for these determinations.

Urine analysis

Protein, pH, glucose, ketones and blood. No dose or drug related effects were noted.

6. Post mortem studies. Organ weights - the livers in the high dose groups were increased in weight, both absolute and relative, ($p < 0.05$), at the 90-day sacrifice period only. The liver weights were equivalent to controls at the 126 day sacrifice. The mid dose group females appeared to be affected also (17% absolute and 6% relative).

The male heart and lung weights appear to be affected (decreased) as follows (at 90 days). The inconsistency in the weights and lack of a clear dose response and there being no associated pathology indicate that these weight changes were not definitely related to ingestion of the test chemical.

	Heart		Lung	
ppm	Abs	Rel	Abs	Rel
Control	1.57	.374	2.19	.521
200	1.30	.379	1.86	.476
600	1.80	.405	2.45	.553
2000	1.27	.321	1.69	.438
4000	1.31	.356	1.56	.436

Gross necropsy - No dose related lesions were noted in the animals sacrificed at 90 or at 126 days.

Histopathology - (the rats in the control and 4000 ppm groups only) - Only a summary table was submitted, no individual animal pathology sheets were present.

The high dose group was associated with "pyknotic nuclei in the cortex of the thymus" - 6/10 animals (females) and 0/10 controls were

affected. The males were unaffected. The hearts of females had more animals affected with infiltration mononuclear cells in the high dose group (7/10) than in the controls (3/10).

CONCLUSION: This study is CORE MINIMUM. The limited number of test animals (10/sex) available for organ weight determinations and histopathological examinations at 90 days may have obscured possible effects. A NOEL of = 2000 ppm is supported. The LEL = 4000 ppm. Note - specific liver function test to assess the hepatic microsomal metabolizing system or electron microscopy of the smooth endoplasmic reticulum were not conducted.

11.) 27Z75, Rat Oral 90-day toxicity study (tab 8)

Wellcome Research Laboratories, HEFG-76-3, February 19, 1976

Five groups of 36 Wistar rats (18 of each sex) were dosed (in the diet) with 0, 60, 200, 600 or 2000 ppm of 27Z75 (40% cis, 60% trans permethrin) for 90 days. This experiment is essentially similar to the experiment with 21Z73 (see preceding section) except that the recovery animals were sacrificed on day 119 after allowing 29 days for recovery.

See the preceding review for parameters determined.

Significant findings:

A NOEL of 200 ppm is supported. At 600 and 2000 ppm, the kidneys of males developed increases in the fat content of renal cortical tubules although no other evidence of kidney dysfunction was reported.

This effect on the kidney is considered unusual and of questionable toxicological significance since no similar effect was noted in other chronic feeding studies with permethrin. Other parameters investigated were considered to be within natural variations and no consistent patterns of effects to suggest a dose related toxic response to the test chemical were noted.

CORE MINIMUM.

12.) Foetal toxicity study of 21Z73 (NRDC 143) in the mouse (tab 22)

Wellcome Research Laboratories, Beckenham, No. BPAT 74/12, March 1976

Three groups of virgin female mice (CD1 strain approximately 3 weeks of age) were bred and then grouped as environmental control (20 mice, no treatment), diluent control (corn oil, 23 mice) and test group (22 mice, 400 mg/kg 21Z73 permethrin in corn oil). The test chemical was administered orally by stomach tube on days 6 - 15 inclusive of pregnancy and the pups were delivered by Caesarian section on day 18 after sacrifice of the dams by ether.

The fetuses were examined by one of three investigative procedures (a) open dissection, (b) Wilson's section technique, (c) Staples and Schnell's Alizarin red S staining method for examination of skeletal morphology.

Results

A. Maternal effects. There were no effects of treatment of the dams with 21Z73 with respect to body weight gain, behavior or implantations per dam. One dam in the group treated with 21Z73 died but no apparent cause of death was noted.

B. Fetal effects. No significant effects in the number of live and normal fetuses, litter size, fetal weight, or sex ratio. Examination of the fetuses did not show evidence of a teratogenic effect of 21Z73.

Conclusion. CORE MINIMUM. A single dose level of test chemical was used and no positive control was included. No evidence that 21Z73 (permethrin) was teratogenic was reported or evident from this experiment.

13.) ✓ Foetal Toxicity Study of 21Z73 (NRDC 143) in the Rat (tab 21)

Wellcome Research Laboratories, Beckenham, March, 1976. Study No. BPAT 74/10

Three groups of virgin female rats (Wistar, 3 months of age) were bred and then grouped as environmental control (22 rats, no treatment), diluent control (23 rats, corn oil) and test group (23 rats, 200 mg/kg 21Z73 in corn oil). The test chemical was administered daily by stomach tube on days 6-16 inclusive of gestation and pups were delivered on day 20 (following sacrifice of dams by chloroform inhalation).

The fetuses were examined by one of three investigative procedures (a) open dissection for examination of the organs of neck, thorax and abdomen; (b) Wilson's technique; (c) Staples and Schnell's Alizarin red S staining method for examination of skeletal morphology.

Results

A. Maternal effects. There were no differences among the three groups related to corpora lutea, implantations, live foetuses, early deaths, or late deaths. An unusual finding among the dams was that two of the test group (21Z73) dams died and one was cannibalized. No deaths were reported in the other groups.

B. Fetal effects. No significant differences in the number of live and normal fetuses, litter size, fetal weight or fetal sex ratio. A single fetus in the test group (treated with 21Z73) was malformed (including brachymelia and tetradactyly and other malformations). This single

malformed fetus, although in the group treated with permethrin, was 1 of 9 in the litter and the other 8 were essentially normal.

Conclusion: This test is CORE MINIMUM. Only a single dose was used and no positive control was run. No evidence that 21Z73 is a teratogen in the rat was presented.

14.) Foetal Toxicity Study of 21Z73 (NRDC 143) in the rabbit (tab 23)

Wellcome Research Laboratories, Beckenham, March, 1976. Study No. BPAT 74/19

Female rabbits of the Dutch strain (about 8 months old) were grouped as environmental control (13 rabbits), diluent control (14 rabbits), and test group (15 rabbits) and were artificially inseminated. The diluent control and the test group received corn oil or 21Z73 (400 mg/kg) in corn oil daily by stomach tube on days 6-18 inclusive of pregnancy. The dams were sacrificed on day 28 and the uterine tissue and pups examined.

Results

A. Maternal. The pregnancy rate for this experiment was poor but this deficiency could not be attributed to administration of 21Z73. For example, 9 dams in the environmental control group, 6 dams in the diluent control group and 7 dams in the test dose group were pregnant and had litters available for examination. One female in each group died.

There were no adverse effects on the dams related to maternal benavior, body weight or implantations.

B. Fetal Analysis. The environmental control group had 50, the diluent control group had 29 and the test group had 28 live fetuses available for examination. There were no abnormal fetuses, or intergroup differences reported. Fetal examination was by (a) open dissection, (b) Wilson's technique, (c) skeletal examination.

Conclusion: This study is CORE MINIMUM. Too few dams had pups for examination (there should be 12 pregnant dams in each test group). No positive control was included. No evidence that 21Z73 (permethrin) is teratogenic is presented by this study.

15.) A multigeneration reproduction study of 21Z73 (permethrin) in the rat (tab 28)

The Wellcome Foundation Company (Beckenham), Study No. BPAT 79/3 Date of issue, January, 1979

Four groups of 20 male and 20 female Wistar strain rats (COBS, 6 weeks of age) were selected as the P generation. They were dosed as 0, 5.0

mg/kg, 30.0 mg/kg and 180.0 mg/kg per day with permethrin (21Z73, Batch ZJ lot no. C8165-106) as an admixture in the diet.

The P generation was used to produce the F_1A and F_1B generations. The F_1B generation was used to produce the F_2A and F_2B generations. F_2B offspring were used to produce F_3A and F_3B generations. Each litter was culled to 8 (4 males and 4 females when necessary and possible). F_A generations were kept until weaning and examined. The last generation was given a foetal examination and the F B pups were processed as a teratogenicity study.

Results

1. Achieved dosage. Chemical analysis of the test diets revealed that slightly less than the desired dosage was actually attained. This was attributed to mixing procedures. The deficit is not considered serious enough to offset the conclusion of this study. For example, the achieved dosages were 0, 4.9, 28.1, and 174.0 mg/kg for males and approximately similar for females.

Inspection of the achieved dosage for each generation showed that there were large variations in the mean drug intake in the high dose group. Changing the feed hoppers was given for the reason for the changes in the P generation. In other cases the high values were associated with lactation.

2. For each generation and for each sex body weight, food intake, drug intake, general behavior and well being, deaths, and pregnancy rate were determined. For each set of dams, the body weight, general behavior and well being, deaths, gestation period, maternal body weight, were determined. For the offspring, filial data, growth and survival were noted. At weaning, the sex ratio and body weight and general examination of their appearance were noted. For the F_2B generation the number of corpora lutea, implantations, post implantation losses and abnormalities were determined.

In the foetal toxicity aspect of this study F_2B generation, there was an internal examination of the pups, examination by Wilson's serial slice technique, and skeletal preparation.

There was no pattern of development of toxicity signs in any of the above indexes for evaluating the effects of permethrin in the reproduction study. All abnormalities were reported as being spurious in occurrence and did not show indications of dose response relationships.

This study is CORE GUIDELINES, a NOEL of 180 mg/kg/day is supported.

16.) Permethrin oral administration to dogs for 6 months (tab 29)

The Wellcome Foundation, Ltd., Berkhamsted, Study No. HEFG 78-14, December 1, 1978

Four groups of 4 male and 4 female Beagle dogs (20-22 weeks old at the start of the experiment) were dosed daily for 6 months with 0, 10, 50, or 250 mg/kg/day of permethrin (technical 94.5% w/v, 25% cis 75% trans, batch ZJ) by gelatin capsule. The amount administered was determined based on the current body weight of the dog. The testing laboratory asserted a NOEL of 250 mg/kg/day.

Results

1. Clinical and overt toxicity - observed daily - no effects of test chemical noted.

2. Body weight and food intake - no effects of test chemical noted.

3. Opthalmoscopy - determined on days - 6, 28, 91 and 173. The pupil was dilated with 1% tropicamide and examined by opthalmoscope. No effects of test chemical noted.

4. Electrocardiography - recorded on days 35, 91 and 174. No effects of the test chemical were noted.

5. Hematology and Clinical Biochemistry - Approximately 8.5 ml of blood was withdrawn from the jugular vein on days -14, -7, 0, 14, 56, 112 and 180.

No consistent adverse effects on either hematology or clinical biochemistry were noted.

The following haematology parameters were measured:

packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), differential white blood cell count, and prothrombin.

The following clinical biochemistry parameters were measured:

glucose, urea, sodium (Na^+), potassium (K^+), bilirubin (BILI), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (AP), creatine phosphokinase (CPK), total protein, albumin, #, β_1, β_2, and #2-globulin.

6. Urinalysis - Samples of urine were aspirated from the bladder of each animal with a needle and syringe during post mortem examination. Each sample was tested with BM8 Boehringer test sticks for nitrite, pH,

blood, glucose, ketones, urobilinogen, bilirubin and protein. A sample of spun deposit was examined under the microscope.

No consistent dose related effects were noted on the urinalysis.

7. Plasma antipyrine determinations - on the day the dogs were sacrificed. No test chemical effect was noted.

Post mortem analysis. The dogs were sacrificed between days 180-183 by intraperitoneal injection of pentobarbitone sodium.

1. Organ weights (brain, liver, pituitary, spleen, heart, lungs, adrenals, testis, ovaries, thyroids and kidneys).

Some weight changes were noted but the greatest magnitude was only 17% different from the controls. Thus, it is concluded that no consistent adverse effects on organ weights were noted. Since only 4 dogs/sex/dose group were used, organ weight differences would have to be very large to be noticeable.

2. Gross Necropsy - No animal by animal data sheets were presented. The data are in a summary table (Table 28) which shows the animal number of dogs which had gross lesions together with the type and degree of intensity of the lesion.

No test chemical effects were reported.

3. Histopathology - (all animals examined) The data are presented in a table (Table 31) showing the organ examined and the status for each dog (by number). Nearly all tissues of the nearly 40 tissue types examined were reported being within normal limits or having commonly occurring lesions that did not show dose or test chemical dependence.

A special neuropathology analysis of the dorsal root ganglia, trigeminal ganglion and proximal root, peripheral nerves (several), spinal cord and brain also did not show evidence of test chemically related pathology (control and high dose groups only).

Conclusion. This study is CORE GUIDELINES. A NOEL of 250 mg/kg/day is supported.

17.) 21Z73. Dominant lethal study in male mice (tab 26)

Wellcome Research Laboratories, Berkhamsted, HEFG-75-10, November 27, 1975

Three groups of 10 male mice (CD 1 sexually mature) were dosed as vehicle control, 452 mg/kg of permethrin, and 679 mg/kg of trimethylphosphate (TP) in corn oil. The permethrin tested was 21Z73 batch ZB (% purity was not stated). Trimethylphospnate is a positive

control. The dose level for both permethrin and the positive control were 1/5 of their LD_{50}'s in mice. Dosing at the above dose levels was orally for 5 consecutive days. Immediately after the 5th dose, the males were allowed to mate with three untreated virgin females. New females were presented to the males each week for a total of 6 weeks.

The females were dissected 14 days following the mid-week of their exposure to the males. Pregnancy rate and average number of implants per male were used as an index for a positive dominant lethal study.

Results

1. The males showed some signs of hypersensitivity following the 4th and 5th doses.

2. The permethrin treated males impregnated the females at a rate of 83% or usually better. The controls showed a rate of 63% or better. TP, the positive control, was clearly affected in the first matings (7%) but by week 4 the rate was up to 72%. No adverse effect of permethrin was noted.

3. In all weeks the percentage of dead implants for permethrin treated mice was equivalent to or less than the controls. The TP control group had more deaths than the controls. No effect of permethrin on the percentage of dead implants was noted.

An additional study tested permethrin (27Z75, Batch 669965) at 285 mg/kg/day in an essentially similar procedure (The Wellcome Foundation, HEFG-76-2, February 17, 1976). There was also no evidence of dominant lethal effects.

Conclusion: These studies show that permethrin as 21Z73 (25% cis, 75% trans) and 27Z75 (40% cis and 60% trans) are negative in a sufficiently conducted dominant lethal toxicity study.

18.) Mutagenicity of BW 21Z73 in L5178Y/$TK^{+/-}$ mouse lymphoma cells with and without exogenous metabolic activation (tab 25)

Burrougns-Wellcome, Research Triangle Park, N.C., No. TTEP/77/0001, dated January 5, 1977

Permethrin (as BW 21Z technical) was tested in the $TK^{+/-}$ ô $TK^{-/-}$ mutagen assay with and without metabolic activation by rat liver S-9 liver homogenate (from Aroclor 1254 pretreated rats).

Samples of the results are as follows:

In the experiments without metabolic activation, ethyl methanesulfonate (EMS) was used as a positive control. In these experiments 47 ?g/ml of permethrin resulted in 47% survival of mouse lymphoma cells and the

number of induced mutants was below background. EMS at 620 ?g/ml resulted in 25% survival and 17 times the background rate for induced mutants.

In the experiments with metabolic activation, 2-acetylaminofluorene (2-AAF) was used as a positive control. Permethrin treated samples at 47 ?g/ml resulted in 25% cell survival and the number of mutants were equivalent to background. 2-AAF at 50 ?g/ml resulted in 2.9% survival but 3.6 times background rate for induced mutants.

Conclusion. Permethrin is considered as being negative in this study.

19.) A Single Dose Oral LD_{50} Study in the Rat with ATROBAN (BW 0021Z73) 25% Wettable Powder (tab 15)

Burroughs-Wellcome, RTP, No. TTEP/79/0017, April 2, 1979

Male and female CD rats (10 per sex per group) were fasted and dosed with 1200, 2400, 3600, 4800, and 6000 mg/kg of ATROBAN (25% WP of Permethrin) suspended in 0.25% agar and observed for 14 days.

Following treatment, the rats displayed a sensitivity to noise, tremors and jerking movements. Other signs included chromodacryorrhea, diarrhea, and urinary incontinence. These effects were present on days 1 and 2 of the study.

The LD_{50}'s calculated were:

> 6000 mg/kg for males

12,741.7 mg/kg $\pm$ 10,796 mg/kg for females

Toxicity Category IV

CORE MINIMUM. No necropsy was reported.

20.) A Single Dose Dermal LD_{50} Study in the New Zealand White Rabbit with ATROBAN (BW 0021Z73) 25% Wettable Powder (tab 16)

Burroughs-Wellcome, RTP, TTEP/79/0014, March 30, 1979

Four groups of 8 New Zealand White Rabbits (4 per sex per dose group) were prepared and dosed with 500, 2000, 3500 or 5000 mg/kg of ATROBAN and exposed for 24 hours.

No deaths occurred during the 14-day observation period (LD_{50} > 5000 mg/kg). No clinical signs of toxicity or no signs of irritation were reported.

Toxicity Category III

CORE MINIMUM. No necropsy reported.

21.) Acute Rat Innalation Toxicity Study (LC_{50}) with ATROBAN(R) 25% Wettable Powder (BW 0021Z73) (tab 17)

Burroughs-Wellcome, RTP, TTEP/79/0031, July 26, 1979

Four groups of 20 rats (10 males, 10 females, Charles River CD(R)) were exposed to atmospheric nominal concentrations of 10.72, 13.45, 33.85 or 133.46 mg/l for four hours and then observed for 14 days for reactions. Exposure was in a 160 liter cubical container and the dust atmosphere containing ATROBAN was generated by using "calibrated cups."

The atmospheric concentration was also determined gravimetrically using a fiberglass filter technique. By this method, the atmospheric concentrations were determined to be 0.76, 0.57, 3.54 and 4.99 mg/li. Particle size analysis indicated that usually 68% or more of the particles were 7.0 micrometers or smaller.

Results: No test animals died. The LC_{50} is > 4.99 mg/li (or by nominal concentration 133.46 mg/li). Toxicity category III. (The lower value was used in assigning toxicity category.)

CORE MINIMUM.

22.) A Primary Eye Irritation Study in the New Zealand White Rabbit with ATROBAN(R) (BW 0021Z73) 25% Wettable Powder (tab 18)

Burroughs Wellcome, RTP, TTEP/79/0019, April 3, 1979

Two groups of 9 rabbits (males and females) were dosed with either 100 mg of ATROBAN(R) or 26 mg ATROBAN(R) directly into the conjunctival sac. The eyes of 3 from each treatment level group were washed with lukewarm tap water.

6/9 rabbits (including 1 washed rabbit) developed corneal opacity which persisted well beyond the 7 day observation period when 100 mg/kg were instilled.

No corneal involvement resulted when 26 mg of ATROBAN(R) were instilled.

CORE GUIDELINES. Toxicity Category III. Toxicology Branch considered the instillation of 100 mg of powder into the rabbit's eye an unrealistic exposure because of the physical nature of the material. The Toxicity Category III assignment is based on the instillation of 26 mg of powder.

23.) A Primary Dermal Irritation Test in the New Zealand White Rabbit with ATROBAN (BW 0021Z73) 25% Wettable Powder (tab 19)

Burroughs-Wellcome, RTP, TTEP/79/0012, March 13, 1979

Six rabbits (3 male and 3 female) were prepared and administered 500 mg of ATROBAN and contact was for 24 hours. Observation was for 7 days after dosing.

Results: a Draize score of 0.65 resulted.

CORE GUIDELINES. Toxicity Category IV.

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

MEMORANDUM

DATE: MAR 3 1982

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Permethrin: EPA Reg. No. 59-ROU and PP 0F2324. Review of Rat Chronic Feeding/Oncogenesis Study Submitted by the Burroughs-Wellcome Company.

TOX Chem. #652BB

FROM: John Doherty 2/22/82
Toxicology Branch/HED (TS-769)

TO: F. D. R. Gee, PM #17
Registration Division (TS-767)

Background:

The Burroughs-Wellcome Company has submitted a rat 2 year chronic feeding /oncogenesis study as a part of the requirement to support registrations and tolerances for Permethrin. The study has been reviewed as follows.

Overview:

Wistar rats (60 males and 60 females per dose group) were dosed with Permethrin (25% cis, 75% trans isomers) for 103 weeks (males) or 104 weeks (females) at dose levels of 0, 10, 50, or 250 mg/kg/day.

1. The study has been assigned a Core Guidelines classification.

2. Based upon the data as submitted, no oncogenic effect for Permethrin was conclusive in this experiment.

   There were no adenomas or carcinomas in the lungs.

   There were only three liver tumors randomly occurring in a total of 480 test rats.

   There were a total of six tumors in the brain and nerve tissue occurring randomly in all of the test groups.

   The most frequently occurring tumors in females were mammary gland fibroepithelial tumors and pituitary adenomas. These did not show evidence for a dose response effect in either an increase in frequency or earlier time of onset.

The most frequently occurring tumors in males were pituitary and thyroid adenomas and skin neoplasms. These did not show a dose reponse for their frequency of occurrence. Malignant lymphoma occurred only in the mid (4 rats) and high (3 rats) dose group males but these occurrences were considered incidental.

3. A NOEL of 10 mg/kg/day is noted for any effect.

4. There are no outstanding issues related to this study at this time.

Review of Study

2IZ: Potential Toxicity and Oncogenicity in Dietary Administration to Rats for a Period of 104 Weeks.

Life Sciences Research (The Wellcome Foundation Ltd.), July 2, 1980, Study No. HEFG 80-33.

NOTE: This review contains information that is stored in EPA Accession Nos. 243977, 243978, 243979 (original study final report), 070303, 070304, 070305, and 070306 (addendum to the final report consisting of histopathology data) and 070307 (verification of tissues examined microscopically).

Charles River Wistar rats were (obtained from the Charles River Co., Margate, Kent, U.K.) randomly selected and placed into four groups of 60 males and 60 females per dosing group. These rats were administered dosages in the diet of 0, 10, 50, or 250 mg/kg/day of 2IZ (permethrin 25% cis and 75% trans isomers, the exact purity of the test chemical was not stated) for 103 (males) and 104 (females) weeks. Satelite groups of 15 rats/sex/dose level were also included for clinical evaluations at predetermined times throughout the study. The rats were approximately five weeks of age at the start of the feeding phase of the experiment.

This experiment was designed as both a chronic feeding and oncogenesis study.

The test animals were housed 5/cage. The rats were allowed to feed ad libitum (Spratt's Laboratory Diet No. 2). The test chemical was adjusted in the diet so that the desired doses were attained based on changes in body weight and in food consumption.

At termination, the surviving rats were sacrificed by carbon dioxide inhalation.

Results:

General Health (Observations were made daily)

1. Palpable swellings were noted in 275 rats but these were reported as being equally distributed among all dose levels.

Rats with Palpable Swellings [1]

	Males	Females
Control	37	37
Low	22	44
Mid	30	41
High	28	36

[1] (number of rats out of 60/group)

These swellings included mammary gland fibroepithelial tumors, cysts, sarcomas, fibromas, various ulcers and other miscellaneous lesions. No dose relationship for the frequency of occurrence was established for these lesions (see also p. 8 of this review).

Toxic signs - Body tremors were noted in the high dose group in ten males and 5 females. No signs of unusual chronic or infectuous diseases were reported as occurring in the test animals.

2. Mortality: The following table shows the fate of the rats with respect to spontaneous death, moribund sacrifice and survival:

	Male			Female		
Dose Group	Spont.	Moribund	Survivors	Spont.	Moribund	Survivors
Control	17	18	25	5	17	38
10 mg/kg	22	25	13	3	17	40
50 mg/kg	17	23	20	7	11	42
250 mg/kg	21	27	12	6	14	40

The study was originally scheduled to run for 104 weeks. Mortality in the male high dose group reached 80% at 103 weeks and the remaining males were then sacrificed.

Although survival was poor in the low and high dose male test groups, 50% of the test animals received their diets for 100, 95, 96 and 93 weeks for the control, low, mid and high dose male test groups respectively. The animals that died prior to scheduled termination

were necropsied and subjected to histopathology examination and no unusual causes of death could be found for the animals dosed with the test chemical.

The study report contains the conclusion that administration of 21Z at 250 mg/kg/day exerted a significant adverse effect upon survival in male rats, but not in females. Toxicology Branch does not concur with this conclusion. The pattern of deaths among the males in the high dose group indicates an early phase (less than one year) when eight rats in the high dose group died followed by a period which shows no particular relationship between test chemical in the diet and death. Moreover, the rats did not lose weight or show other serious signs of toxicity to support a dose related increase in deaths.

3. Body weight, food and water consumption, dietary analysis and achieved dosage - Body weights and food consumptions were determined weekly for the first 26 weeks, biweekly for weeks 27-78 and monthly for weeks 79-103. The diets were fortified according to the body weights and food consumption data. No differences in body weight, food consumption or water intake were noted throughout the study among the dosed groups. The achieved dosage was reported as being within the expected dietary levels. Control diets were not analyzed for the presence of Permethrin.

The following table shows that mean body weight for all groups and by sex was approximately the same when determined at 0, 52 and 103 weeks. No trend for decreases in weight gain due to ingestion of the test chemical were noted.

	Males				Females			
EEK	CONTROL	LOW	MID	HIGH	CONTROL	LOW	MID	HIGH
0	109(60)	109(60)	108(60)	108(60)	104(60)	105(60)	104(60)	105(60)
52	736(59)	742(56)	730(59)	732(52)	375(58)	387(58)	383(60)	384(60)
103	685(25)	658(13)	714(20)	720(12)	466(39)	482(40)	473(43)	475(40)

Mean group body weight (number of rats in group).

4. Opthalmoscopy (at weeks pretest, 7, 26, 51, 78 and 103). No compound related lesions noted.

5. Haematology, blood chemistry and urinalysis, (10 male and 10 female rats from the satelite groups were examined at weeks 6, 26, 52, 78 and 103. For the interim determinations usually only the control and high dose groups were tested).

[Haematology included: packed cell volume, Hb concentration, RBC count, WBC count (total and differential), prothrombin time, platelet count and reticulocyte count.

Blood chemistry included: urea, glucose and total and specific protein concentrations, electrophoretic proteins, alkaline phosphatase, SGPT, SGOT, aspartic aminotransferase activity and Na^+ and K^+ concentrations.

Urinalysis included: volume, pH, specific gravity, reducing substances, glucose, protein, ketones, bile pigments and urobilin and microscopic examination of sediments.]

No consistent dose related variations in these parameters were noted. Occasional statistically significant deviations were reported.

For example, in males at 103 weeks, the α_2 globulins in the blood were reported as being statistically significantly decreased at all doses as follows:

	Control	Low	Mid	High
Relative α 2 Globulin concentration (Units not stated)	0.8	0.6[b]	0.6[a]	0.6[a]
S. D.	0.2	0.2	0.1	0.1

[a] Significantly different from controls, $p < 0.05$
[b] Significantly different from controls, $p < 0.01$

Toxicology Branch does not consider this change to be related to the presence of the test chemical because there is no progressive decrease in α 2-globulin with increase in dose level of the test substance.

Also, the α 2-globulins were assayed by a method which involves electrophoresis, staining the strips and densitometric analysis. Such an involved procedure might be expected to give the variations as found with the α 2-globulins.

6. Gross Necropsy (all animals). Analysis of the data submitted indicated no dose dependent increases in grossly observable lesions. The correlation between gross necropsy observations and histopathological findings was considered acceptable by this reviewer.

7. Organ weights (on terminally sacrificed animals only): The absolute organ weights and organ weights relative to body weights were calculated for adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary gland, spleen, testes, thyroid glands (after fixing) and uterus. The following selected statistically significant changes were noted.

In males, liver weights were increased (19% absolute and 12% relative) for the high dose group only and this is considered to be related to the ingestion of the test chemical.

Adrenal weight was also apparently affected in the high dose test group. Exclusion of two test animals in the high dose group (rats No. 201 and 218) with massive tumors in the adrenals resulted in elimination of the apparent increase. There was no corresponding increase in the number of animals with adrenal tumors to indicate that the tumors were due to the test chemical.

In females, high dose group lung weight was decreased (16% absolute and 19% relative) and kidney weight was decreased (8% absolute and 15% relative). These depressions were not considered to be related to ingestion of the test chemical. For example, when lung weights of two control group females which had massive cysts (rats 258 and 279) are eliminated, the difference noted in the high dose test group is no longer statistically significant.

The pituitary gland (mid dose groups) and heart (low dose group) showed incidental increases in weight but these changes were not considered related to ingestion of the test chemical.

8. Histology - Thirty-five tissues or organs were routinely saved for microscopic examination. In addition, any anatomical area that appeared to have been related to the cause of death or to be diseased was saved for examination.

The tissues were stained with haematoxylin and eosin. Tissues from all animals dying during the study feeding period from all dose groups were examined microscopically. The protocol called for microscopic examination for only the controls and high dose group survivors, except that tissues showing abnormal masses and presumptive neoplasms from all dose groups were to be examined. Eventually all liver and thyroids were examined.

Information related to the number of tissues examined and the identification of the tissues examined for the low and mid dose groups is contained in an addendum to the study, EPA Accession No. 070307.

Histopathological findings were reported in the following tissues and organs (*examination called for in the study protocol).

Adrenals*
Aorta*
Arteritis (arteries)-bile duct
Bone*
Bone marrow
Brain*
Cervix
Cervical musculature
Epididymal fat pad
Epididymides
Eyes and optic nerve*
Kidneys*
Liver*
Lungs*
Lymph Nodes (unspecified)
Lymph Nodes (axillary)
Lymph Nodes (cervical)*
Lymph Nodes (iliac midiales)
Lymph Nodes (inguinal)
Lymph Nodes (mesenteric)*
Lymph Nodes (pancreatic)
Lymph Nodes (renal)
Lymph Nodes (thymic)
Mammary gland*
Mesenteric blood vessels
Mesenteric fat
Mesenteric mass
Ovarian fat pad
Ovaries*
Pancreas*
Parathyroids
Gastro-intestinal tract
Oesophagus*
Duodenum*
Ileum*
Jejunum
Caecum
Colon*
Rectum
Harderian gland
Heart*
Peritonitis
Pituitary gland*
Preputial gland
Prostate gland*
Salivary gland* (parotid)
Salivary gland (mandibular)
Salivary gland (sublingual)
Sciatic nerve*
Seminal vesicles*
Skeletal muscle*
Skin* and subcutis
Spleen*
Stomach*
Testes*
Thymus*
Thoracic connective tissue
Thyroids*
Tongue*
Trachea*
Urinary bladder*
Uterus*

NOTE: Tissues that were diagnosed as autolysed were classified as "too autolytic for useful study", "autolytic" without additional pathological findings, or "autolytic" with various pathological findings. The number of tissues designated as "too autolytic for useful study" for any given organ was not considered to be so great that an oncogenic evaluation could not be made. Fourteen control and seven high dose males were affected with at least some tissues that were "too autolytic for useful study". The tissues that were usually affected were the gastro-intestinal tract, the eye, seminal vesicles annd occasionally others.

The following three tables summarize the development of neoplasms in the rats on this study.

Rats with one or more neoplasms

Dose Group	Sex: Males	Sex: Females
Controls	36(38)/60*	47/60
10 mg/kg	27/60	50/60
50 mg/kg	37/59	46/60
250 mg/kg	30(31)/60	44(45)/60

*number of rats with one or more tumors/number of rats evaluated histologically; the number in () was reported by the registrant, the other numbers were determined by J. Doherty based on data in the addendum to the final report (EPA Acc. No.s 070303,-4,-5, and -6)

This table does not indicate a dose related increase in the number of rats with neoplasms.

Total number of neoplasms

Dose Group	Sex: Males	Sex: Females
Controls	57/60*	74/60
10 mg/kg	42/60	79/60
50 mg/kg	71(72)/59	74(75)/60
250 mg/kg	39/60	72/60

*number of tumors/number of rats evaluated histologically

The above information was obtained from the "Addendum to the final report".

The numerators are based on determinations made by J. Doherty, the number in ( ) is the number reported in the study report.

This table indicates that there is a slightly higher occurrence of neoplasms in the mid dose male group. This apparent increase is discussed further below.

Table of frequently occurring neoplasms.

Organ/tissue (number examined) neoplasm (number observed)	Group and Sex: Males control	Males low	Males mid	Males high	Females control	Females low	Females mid	Females high
Adrenals	119	93	79	120	119	40	43	120
phaeochromocytoma and adenoma	7	1	3	2	1	1	2	1
Mammary gland	59	47	39	58	59	22	21	60
benign fibro-epithelial adenoma	6	0	3	1	29	34	34	32
malignant fibro-epithelial adenoma	0	0	0	0	0	0	0	2
carcinoma	0	0	0	0	3	0	0	0
Pancreas	59	47?	38?	60	59	29?	17?	60
adenoma	4	3	8	2	1	1	0	1
Parathyroid	107	78	69	110	111	38	36	117
adenoma	1	1	3	1	0	0	0	0
Pituitary	54	45	40	58	43	31	34	30
adenoma	14	14	17	9	25	23	25	23
carcinoma	0	0	0	1	1	0	0	0
Skin	**	**	**	**	**	**	**	**
(miscellaneous)	6	10	10	7	4	6	3	3
Systemic	**	**	**	**	**	**	**	**
(malignant lymphoma)	0	0	4	3	2	2	1	1
Thyroid	115	118	114	115	114	116	117	118
adenomas	6	3	12	4	4	4	2	8
carcinomas	1	0	0	0	0	1	0	0
Testis	120	94	78	120	-	-	-	-
interstitial cell tumor	1(3)*	2	1	6	-	-	-	-
Other neoplasms	9	8	10	3	4	7	7	1
Total neoplasms	55 (57)*	42	71	39	74	79	74	72

This information is based on data obtained from the "Addendum to Final Report" EPA Accession Number 07037.
*The addendum identifies 1 rat with interstitial cell tumors but the final report and individual animal pathology sheets indicate 3 rats were affected.
** It is assumed that all available animals were evaluated for skin neoplasms and malignant lymphoma.

The testing laboratory asserts that there was no evidence of any neoplastic response to treatment.

In females: Most of the tumors were in the mammary or pituitary glands and no dose dependent effect was evident. There was no evidence that either the mammary gland or pituitary gland tumors in the treated animals developed at an earlier stage for the treated groups than for the control groups.

In males: The mid dose group had a higher total incidences of neoplastic lesions than the other groups. Breakdown of the tumor pattern indicated that the mid dose group had higher frequencies of tumors in the pituitary, thyroid, pancreas, parathyroid, GI tract, bone and skin and there were more malignant lymphomas.

The non-neoplastic and neoplastic characteristics of certain selected tissues/organs are outlined below.

1. Livers of both sexes were shown to have a dose dependent increase in a lesion described as "periacinar hepatocyte hypertrophy". The frequency of occurrence for this lesion is as follows:

Dose Group	Males	Females
Control	3 (5%)*	3 (5%)
Low	4 (7%)	2 (3%)
Mid	10 (17%)	11 (18%)
High	21 (35%)	38 (63%)

* Number of lesions (as % of starters except for 59 in mid dose group males).

This lesion (periacinar hepatocyte hypertrophy) was noted to be associated with a decrease in panacinar hepatocytic (reflecting decreased glycogen content) pallor and an increase in periacinar hepatocytic large fatty vacuolation in females. The NOEL for this lesion is 10 mg/kg.

Other nonneoplastic lesions found in the liver included bile duct hyperplasia that was associated with hyaline degeneration and lymphocytic infiltration. The males were more severely affected but no true dose relationship was evident in either the frequency or the intensity.

There were a total of two hepatocellular adenomas and one hepatocellular carcinoma reported. Toxicology Branch reviewer could not find the animal in the low dose male group reported in Table II D as having hepatocellular adenoma by inspecting both the individual animal pathology reports and the addendum to the final report. The report did not include animal number 150 as developing a oncogenic effect in the liver although this animal had "nodular hyperplasia" which is often considered oncogenic in the rat (JNCI: 64:180-190 [1980]). A single rat (#139) had hepatocellular carcinoma.

2. There were no primary lung tumors. Neoplastic tissue reported in the lungs consisted of sarcomal metastases (animal number 73), granulocytic leukemia (number 156), and malignant lymphoma cells (number 168). These did not show evidence of being related to ingestion of the test chemical.

   Gross necropsy of the lung tissue revealed areas of "foci" which when followed up by histological examination were usually diagnosed as accumulations of alveolar macrophages (see table below). There were some nodules observed at gross necropsy that were found to be related to pleuritis (see for example animal number 143). No evidence that the lungs were adversely affected by the test chemical was presented.

TABLE - Frequently Occurring Non-neoplastic Lesions in Lung Tissue

	MALES				FEMALES			
	Control	Low	Mid	High	Control	Low	Mid	High
Peribronchiolar lymphoid hyperplasia	26	14	20	22	31	13	21	28
Perivascular lymphocytic infiltration	31	14	16	19	24	10	15	23
Focal alveolar macrophages	29	16	15	28	35	19	41	43
Tissues Examined	60	48	42	60	58	31	47	60

Incidences reported

3. In the thyroid tissue there was a dose related increase in a lesion described as "focal disturbance in growth pattern of follicular cells". The frequency of occurrence for this non-neoplastic lesion is as follows:

Group	Males	Females
Control	3*	0
Low	3	0
Mid	8	1
High	10	4

* Number of animals in each group with lesion, all thyroids were reported examined, p. 18 sec. 5,3,4. (See also EPA Acc. No. 070307).

Most of the affected animals died prior to terminal sacrifice .

4. Analysis of the data related to the development of adenomas in the pituitary gland in males indicated that there may be a test chemical related effect on the time of onset for these tumors. The following table shows that there are more adenomas in the test animals dosed with 21Z which died during the experiment than among the survivors. In contrast most of the adenomas in the controls are in the surviving animals.

Pituitary Adenomas

	Control	LOW	MID	HIGH
Animals dying first year	0/1	1/4	0/1	1/8
Animals dying second year	5/31	12/40	14/36	7/38
Survivors	10/22	1/1	3/3	1/12
Total	15	14	17	9
/ Number examined	54	45	40	58

The average time to development for each of the groups for the pituitary adenomas in males was 97.9, 89.8, 94.7 and 85.0 weeks for the controls, low, mid and high dose groups respectively.

Toxicology Branch notes the presence of the apparent (and marginally statistically significant) earlier onset but does not consider this earlier onset to be related to the presence of the test chemical. There is no dose relationship evident with respect to the total number of pituitary adenomas which occur commonly in aged rats. There is only a small difference in the time to development between the control and mid dose groups which had the most survivors.

5. The sciatic nerve, other peripheral nerves and the spinal cord were not reported as having unusual lesions nor was there evidence for a dose dependent increase in nerve tissue lesions which commonly occur in the Wistar rat (see Table). This comment is included because some synthetic pyrethrins have been implicated as causing a characteristic type of neuropathy.

	MALES				FEMALES			
	Control	Low	Mid	High	Control	Low	Mid	High
Nerve Fiber atrophy and degeneration	2	7	3	3	0	1	0	1

There were a total of six tumors in nerve tissue (including brain) reported. One instance of ependymoma (number 98, low dose group male), one instance of oligodendroglioma (number 124, mid dose group male), one instance of astrocytoma (number 52, control group male), two instances of heart neurinoma (number 243 control and number 336 low dose group female), and one instance of meningioma (number 351 low dose group female).

6. A variety of gross necropsy observations in kidneys which commonly occur in rats were noted. Hydronephrosis was higher in the high dose group males (13 animals) than in controls (8 animals) but this was not considered to be related to ingestion of the test chemical.

Histopathology revealed that a decrease in the severity of a lesion described as "geriatric nephropathy" as shown in the following table.

ANIMALS WITH LESION

	MALES				FEMALES			
Geriatric Nephropathy	CONTROL	LOW	MID	HIGH	CONTROL	LOW	MID	HIGH
Slight	5	0	7	10	14	6	7	34
Moderate	27	22	23	26	33	10	6	18
Marked	27	21	9	12	4	1	0	1
Total animals affected	59	43	39	48	51	17	13	53
Number of Kidneys examined	120	95	79	120	116	36	37	120

The toxicological significance (if any) of this decrease in the nonneoplastic lesion of geriatric nephropathy noted in the mid and high dose groups is not known nor is this problem considered to be conclusively related to Permethrin at this time.

7. In males, malignant lymphoma was present in the mid and high dose groups only. There were 4 affected animals in the mid dose group (numbers 122, 168, 178 and 134 which survived 53, 95, 77 weeks and to termination respectively). There were 3 affected animals in the high dose group (numbers 203, 205, and 191 which survived 103, 49 weeks and to termination respectively). In females, there were 2, 2, 1, 1 animals affected with malignant lymphoma in the control, low, mid and high dose groups respectively. The development of malignant lymphoma in males is not considered to be related to ingestion of the test chemical.

8. Many of the rats in all of the male test groups showed "myocarditis" in the heart but a dose response in frequency or intensity was not observed. This type of lesion is common in aged rats.

Conclusions and Discussion:

This study demonstrates that dosing Wistar rats with Permethrin (25% cis, 75% trans isomers) results in a no observed effect level (NOEL) of 10 mg/kg/day for any effect. At the next highest dose level tested, 50 mg/kg/day, both male and female rats developed "periacinar hepatocyte hypertrophy". Another possible lesion occurring at the mid and high dose levels was described as "focal disturbance in growth pattern of follicular cells" in thyroid. At the highest dose level (250 mg/kg/day) there is also an increase in liver weight in the males.

Other then there being some signs of body tremors in the high dose groups, other findings were considered to be incidental and not conclusively related to ingestion of Permethrin.

Based upon the data as submitted, no definite oncogenic effect for Permethrin (25% cis, and 75% trans isomers) was evident in this experiment.

This study is assigned a Core Guidelines classification. The high rate of spontaneous deaths in the male low and high dose test groups detracts from, but does not offset the Core Guidelines classification.

OPP:HED:TOX: J.DOHERTY:sb 6/10/81 RE:12/4/81 X73711 Rm. 814 CM 2 #ml

reading

008163

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE: MAR 3 1982

SUBJECT: Permethrin. EPA Reg. No. 59-ROU and PP 0F2324. Review of Burroughs-Wellcome 92-Week Oncogenic Study in Mice.

TOX Chem. No. 652BB

FROM: Edwin R. Budd, Section Head
Section II
Toxicology Branch/HED (TS-769) 3/1/82 OEP 3/3/82

TO: F. D. R. Gee, PM #17
Registration Division (TS-767)

Summary of Study

Permethrin (cis:trans::25:75) was administered in the diet to female and male mice (CFLP strain) for 92 weeks at dosage levels of 0 (control), 10, 50 and 250 mg/kg/day. Observations for clinical signs of toxicity were made daily. Food consumption and body weights were determined weekly. Complete gross necropsies and histopathological examinations of all major tissues and organs were conducted on all animals that died or were sacrificed in extremis during the study and on all animals sacrificed at the end of the study.

For males, there were no apparent differences between control and test animals in the following parameters: clinical signs of toxicity, food consumption, water consumption, mortality, body weights, gross necropsies (except for slightly increased liver weights in high-dosage level males), and incidence of non-neoplastic or neoplastic lesions as determined by histopathological examination. Treated males may have had smaller stores of liver glycogen than controls.

For females, there were no apparent differences between control and test animals in the following parameters: clinical signs of toxicity, food consumption (except for a slight decrease during the last 13 weeks of the study in high-dosage level females), water consumption, mortality (except for a slightly increased survival rate for mid- and high-dosage level females after 65 weeks), body weights, gross necropsies (except

for slightly increased kidney weights in high-dosage level females), and incidence of non-neoplastic or neoplastice lesions (except as described below for lung tumors) as determined by histopathological examination. Treated females may also have had smaller stores of liver glycogen than controls.

For females (but not males), a dose-related increased incidence of primary adenomatous tumors in the lungs was observed. The overall incidence for this neoplastic lesion (for all animals in each female group) was 3%, 7%, 9% and 20% for control, low-, mid- and high-dosage level groups respectively. Statistical analysis of this finding revealed the dose response rate to be statistically significant ($p < .01$) when adjusted for time of death (or diagnosis) using Peto's Prevalence Method and also to be statistically significant ($p < .02$) using the Cox-Armitage Test for trend analysis. In addition, direct comparison of the rate of occurrence of these tumors between high dosage level and control females showed statistical significance at $p < .01$. (These statistical calculations were performed and/or reported by Bertram Litt, statistician, Toxicology Branch.)

In high- and mid-dosage level females, three adenocarcinomas of the lungs were reported whereas none were reported for the control or low-dosage level females. Also, in high- and mid-dosage level females, six animals were reported to have cuboidal/columnar metaplasia of the alveolar epithelia whereas none were reported to have this lesion in the control or low-dosage level female groups.

Correlations of findings between "in-life" observations, gross necropsies and histopathological examinations were satisfactory.

The study was well planned and conducted and adequately reported. It is classified as Core-Guideline. No questions or issues (of particular concern) are raised in this review or remain outstanding except that there is some concern that the "maximum tolerated dose" of permethrin may not have been achieved in this study. With the exception of the increased incidence of lung tumors in females, other effects observed in high-dosage level animals were generally minimal and are considered to be largely inconsequential.

Detailed Review of Study

Study Title and Description:

Carcinogenicity Study in Mice with Permethrin (21Z73). No final study date but received by EPA on December 17, 1980. (In life phase of study was from April 21, 1977 to January 25, 1979.) Two volumes (EPA Accession numbers 243975 and 243976). Laboratory number HEFG 80-29.

Sponsor:

Burroughs Wellcome Company
Research Triangle Park, N.C.

Testing Laboratory:

The Wellcome Foundation Ltd.
London and Berkhamsted
England

Test Material:

21Z73, Permethrin
dl cis:trans 25:75
batch ZJ, Lot No. C8165-106

Study Conduct

Test Animals:

Grade 2 male and female mice, CFLP strain, from Anglia Animal Laboratories. Aged 28-33 days at beginning of test diet administration.

Study Design:

Male and female mice were randomly divided into groups and fed Permethrin continuously in the diet at the following nominal concentrations for the entire duration of the study.

Group Number	Sex	Number of Animals	Permethrin Dosage
1	F	100	Untreated Diet (Control)
2	M	100	Untreated Diet (Control)
3	F	75	10 mg/kg/day
4	M	75	10 mg/kg/day
5	F	75	50 mg/kg/day
6	M	75	50 mg/kg/day
7	F	75	250 mg/kg/day
8	M	75	250 mg/kg/day

Animal Maintenance:

All mice were group housed with five males or five females per cage except that sick animals were placed in separate cages when discovered to prevent cannabalism. Standard animal husbandry techniques were utilized (including a daily cycle of 12 hours of light and 12 hours of darkness). Food and tap water were available to all animals ad libitum. Water consumption was estimated by visual inspection of water bottles (i.e. not directly measured).

Preparation of Test Diet and Administration:

A complete rodent diet in powdered form (modified 41B diet ex Heygates) was the basal diet to which appropriate amounts of 21Z73 were added. Fresh diets were prepared weekly. Based on body weight and food consumption data, dietary levels of test material were adjusted weekly for each dosage group so as to provide each group with the prescribed mg/kg/day intake of test material.

Food consumption was determined weekly for each cage of mice. Weekly calculations of achieved dosages, expressed as mg/kg/day, were calculated. Feed was analyzed periodically (at weeks 1, 4, 13, 26, 52, 59, 78, and 92) for content of 21Z73.

Duration of Study:

Test material was continuously available to test animals for 92 weeks (or 21 1/2 months)—from April 21, 1977 to January 25, 1979.

Clinical Observations:

Observations for clinical signs of toxicity were made daily on all animals. Individual animals were palpated weekly for presence and size of masses. Individual body weights were determined weekly.

Gross Necropsy:

Palpation and gross necropsies were performed on all animals dying or sacrificed in extremis during the study and on all surviving animals at termination of the study at 92 weeks. Organ weights for the following organs were determined unless precluded by autolysis: adrenals, brain, heart, kidney, liver, lungs, ovaries, pituitary, spleen, testes and uterus.

Unless precluded by autolysis, samples of the following organs and tissues were preserved (and subsequently examined histopathologically) from each mouse.

Standard Set of Organs and Tissues

Adrenal gland	Lymph nodes
Bone marrow	Mammary gland (posterior)
Brain	Ovary
Caecum	Pituitary gland
Cervix/vagina	Pancreas
Duodenum	Prostate
Eye and optic nerve	Skin
Heart	Spleen
Ileum	Stomach
Jejunum	Testes
Kidney	Thymus
Liver	Thyroids
Lungs	Urinary bladder
Sciatic nerve	Uterus
	Blood Film

The following organs and tissues were also preserved from each mouse for future reference--but were not examined histopathologically unless specifically indicated by observations made during the gross necropsy.

Additional Set of Organs and Tissues

Aorta	Second Eye
Bone	Skeletal muscle
Colon	Spinal cord
Mammary gland (anterior)	Trachea
Esophagus	Tongue
Salivary gland	Rectum
Seminal vesicles	

Histopathological Examination:

All tissue sections were stained with H and E. The standard set of organs and tissues (listed above) was microscopically examined from all mice from all dosage groups. Additional organs and tissues were examined when indicated.

Pathology Personnel:

Pathological operations were apparently under the direction of Mr. (?) Beckenham with assistance by Mr. B. Trefty. Dr. F. J. C. Roe was the consulting pathologist. The precise responsibilities of each of these three persons is not clear from the report.

Study Results

Feed Analysis:

Chemically analyzed concentrations of 21Z73 in test diets were consistently found to be within 10% of the target levels (with only a few sporadic exceptions). Control diets were apparently not analyzed for content of 21Z73.

Food and Water Consumption:

Food consumption for high dose female mice decreased slightly (roughly 15%) during the last 13 weeks of the study compared to other female groups. Food consumption for male mice was comparable in all male groups throughout the study.

Visual observation of water bottles for water consumption revealed no evidence of differences between groups.

Achieved Dosages:

Throughout the duration of the study, the achieved dosages (calculated from food consumption and body weight data) were consistently within 10% of the nominal dosages (with only a few scattered exceptions).

Mortality:

For female mice, the percentages surviving the 92 week study period for 0, 10, 50 and 250 mg/kg/day groups were 44%, 43%, 51% and 57% respectively. The actual number of surviving animals were 44, 32, 38 and 43 respectively. For the first 65 weeks, mortality rates were comparable for all female groups. After 65 weeks, survival tended to be higher in the 2 highest dosage groups.

For male mice, the percentages surviving the 92 week study period for 0, 10, 50 and 250 mg/kg/day groups were 70%, 65%, 63% and 64% respectively. The actual number of surviving animals were 70, 49, 47 and 48 respectively. Mortality rates were comparable for all male groups during the entire study.

Body Weights:

For both female and male mice, there were no appreciable differences between control and any test group during the entire duration of the study.

Clinical Observations:

No apparent contagious diseases occurred during the study. Clinical observations were negative for apparent effects that may have been due to the test material. Ocular abnormalities in females and males, swollen abdomens in females and anogenital swellings in males were observed but occurred with about the same incidence in control and test groups and are not considered to be related to ingestion of the test material.

Absolute Organ Weights and Relative Organ/Body Weight Ratios:

For animals killed at termination of the study, statistically significant increases ($p \leq 0.05$) in kidney weights of females and in liver weights of males were reported as follows:

Dose	Measure	Females, Kidneys % of Cont.	p	Males, Livers % of Cont.	p
Low	Absol.	-	-	-	-
Low	Rel.	-	-	-	-
Mid	Absol.	-	-	-	-
Mid	Rel.	108	0.02	-	-
High	Absol.	110	0.04	110	0.04
High	Rel.	110	0.04	112	0.01

Weights for pituitary glands for females and males were not provided, although the protocol states they were determined.

Gross Necropsy:

No consistent dose-related findings attributable to ingestion of the test material were observed for either female or male animals in this study. Enlargement of lymph nodes and/or lymphoid tissues, blood stained fluid (especially in the abdomen) and dilated and fluid filled uteri (in females) were reported with about equal frequency at all dose levels.

Histopathological Examination, Non-neoplastic Lesions:

Sporadic non-neoplastic changes were observed in the following organs of females and males but were of low frequency and were not considered to be related to treatment: brain, sciatic nerve, eye, heart, duodenum, jejunum, ileum, caecum, pancreas, thymus, bone, bone marrow, cartilage, skin, pituitary gland, thyroids, parathyroids, adrenals.

In the lungs of both females and males, congestion, interstitial pneumonitis and/or round cell infiltration (around main airways) were observed with about equal frequency in all groups. Focal consolidation was decreased in mid and high dose male groups.

In salivary glands, focal round cell infiltration was increased in high dose females sacrificed at termination but was decreased in treated males. These changes are not considered to be biologically significant.

There was a slight increase in the incidence of focal round cell infiltration of the mucosa of the glandular part of the stomach in high dose females sacrificed at termination but these changes are not considered to be biologically significant. In males, no treatment related effects were observed in the stomach.

In the livers of both females and males, the following changes were observed with about equal frequency in all groups: round cell infiltration, areas of coagulative necrosis, and extramedullary hemopoiesis. Decreased margination of cytoplasm was observed in liver cells of both female and male treated animals (compared to controls) suggesting treated animals had smaller stores of glycogen. Decreased fatty degeneration was also observed in treated males.

In the kidneys of both females and males, focal round cell infiltration, tubular cast formation and focal tubular hyperplasia were found with an approximately equal incidence in all test groups.

In the urinary bladder of males, control animals had a higher incidence of subepithelial inflammatory infiltration than did high dose males.

In the spleens of control females, there was a higher incidence of enlargement and/or abnormal architecture (which was associated with an increased incidence of lymphoid hyperplasia and malignant lymphoma in these same animals).

Other than a higher incidence of malignant lymphoma in control females, changes observed in lymph nodes were similar in all groups for both females and males.

In females, no changes attributable to the test material were observed in the mammary glands or ovaries. There was a slightly increased incidence of cystic hyperplasia of the uterine horns in the high dose group, but it is considered to be biologically insignificant.

In males, testes were observed to have varying degrees of atrophy which were similar in all test groups. No treatment related changes were observed in the prostate.

In blood films, most abnormal counts were associated with malignant lymphoma. Other observed changes were sporadic and not related to treatment.

Histopathological Examination - Neoplastic Lesions:

The frequent occurrence of malignant lymphoma in many animals in all groups dominated the neoplastic findings in this study. In both females and males, these lesions were generally widespread and infiltrated numerous organs. Lymphoid hyperplasia was also frequently observed and in some cases was reported to be difficult to distinguish from early malignant lymphoma. A few reticulum cell sarcomas were also diagnosed--especially in females. The frequency of occurrence of these tumor types, together with the overall incidence of animals with one or more tumors of any kind and animals with one or more malignant tumors is presented below. Since nearly all animals in all groups were examined histopathologically at least to some degree (with only a very few exceptions scattered across all groups), this data set is presented in relation to the total numbers of animals in all groups.

ANIMALS WITH ONE OR MORE TUMORS OF ANY KIND

				D		T		TOTAL	
GROUP	D	T	TOTAL*	no.	%	no.	%	no.	%
Females									
Control	56	44	100	33/56	59	18/44	41	51/100	51
Low	43	32	75	23/43	53	13/32	41	36/75	48
Mid	37	38	75	21/37	57	19/38	50	40/75	53
High	32	43	75	22/32	69	19/43	44	41/75	55
Males									
Control	30	70	100	24/30	80	45/70	64	69/100	69
Low	26	49	75	16/26	62	26/49	53	42/75	56
Mid	28	47	75	19/28	68	24/47	51	43/75	57
High	27	48	75	19/27	70	25/48	52	44/75	59

D = died or sacrificed during study.

T = sacrificed at termination of study.

* based on total numbers of animals in study.

ANIMALS WITH ONE OR MORE MALIGNANT TUMORS

GROUP	D	T	TOTAL*	D		T		TOTAL	
				no.	%	no.	%	no.	%
Females									
Control	56	44	100	32/56	57	14/44	32	46/100	46
Low	43	32	75	21/43	49	10/32	31	31/75	41
Mid	37	38	75	21/37	57	11/38	29	32/75	43
High	32	43	75	17/32	53	16/43	37	33/75	44
Males									
Control	30	70	100	17/30	57	23/70	33	40/100	40
Low	26	49	75	12/26	46	11/49	22	23/75	31
Mid	28	47	75	13/28	46	14/47	30	27/75	36
High	27	48	75	16/27	59	11/48	23	27/75	36

* based on total numbers of animals in study.

ANIMALS WITH ONE OR MORE MALIGNANT LYMPHOMAS

GROUP	D	T	TOTAL*	D		T		TOTAL	
				no.	%	no.	%	no.	%
Females									
Control	56	44	100	28/56	50	13/44	30	41/100	41
Low	43	32	75	18/43	42	10/32	31	28/75	37
Mid	37	38	75	19/37	51	7/38	18	26/75	35
High	32	43	75	13/32	41	11/43	26	24/75	32
Males									
Control	30	70	100	11/30	37	20/70	29	31/100	31
Low	26	49	75	11/26	42	10/49	20	21/75	28
Mid	28	47	75	10/28	36	7/47	15	17/75	23
High	27	48	75	13/27	48	9/48	19	22/75	29

* based on total numbers of animals in study.

ANIMALS WITH ONE OR MORE RETICULUM CELL SARCOMAS

				D		T		TOTAL	
GROUP	D	T	TOTAL*	no.	%	no.	%	no.	%
Females									
Control	56	44	100	5/56	9	4/44	9	9/100	9
Low	43	32	75	3/43	7	0/32	0	3/75	4
Mid	37	38	75	1/37	3	0/38	0	1/75	1
High	32	43	75	1/32	3	2/43	5	3/75	4
Males									
Control	30	70	100	1/30	3	0/70	0	1/100	1
Low	26	49	75	0/26	0	0/49	0	0/75	0
Mid	28	47	75	0/28	0	0/47	0	0/75	0
High	27	48	75	0/27	0	1/48	2	1/75	1

* based on total numbers of animals in study.

Examination of the data in the preceding tables does not suggest an increased overall incidence in test animals of (1) tumors of any kind, (2) malignant tumors, (3) malignant lymphomas or (4) reticulum cell sarcomas in either females or males.

Data on the incidence of primary adenomatous tumors and metaplasia in the lungs is presented in the following three tables. Incidence data in these tables are based on the total numbers of lungs examined histopathologically.

ANIMALS WITH ONE OR MORE PRIMARY ADENOMATOUS TUMORS IN THE LUNGS

				D		T		TOTAL	
GROUP	D	T	TOTAL**	no.	%	no.	%	no.	%
Females									
Control	53	43	96	0/53	0	3/43	7	3/96	3
Low	41	30	71	0/41	0	5/30	17	5/71	7
Mid	36	38	74	2/36	6	5/38	13	7/74	9
High	31	43	74	8/31	26	7/43	16	15/74	20
Males									
Control	30	69	99	9/30	30	17/69	25	26/99	26
Low	26	49	75	1/26	4	13/49	27	14/75	19
Mid	27	46	73	5/27	19	12/46	26	17/73	23
High	27	47	74	5/27	19	11/47	23	16/74	22

D = died or sacrificed during study.
T = sacrificed at termination of study.
** based on total numbers of tissues examined (lungs).

GRADING OF PRIMARY ADENOMATOUS TUMORS IN THE LUNGS (HIGHEST GRADE IN EACH ANIMAL)

Group	Number of Animals with Tumors/Number of Tissues Examined	Number of Animals			
		Grade 1	Grade 2	Grade 3	Grade 4
Females					
Control	3/96	0	3	0	0
Low	5/71	3	2	0	0
Mid	7/74	5	1	1	0
High	15/74	7	6	2	0
Males					
Control	26/99	8	15	2	1
Low	14/75	7	6	1	0
Mid	17/73	7	8	2	0
High	16/74	10	5	1	0

Grade 1 = non-invasive adenoma.
Grade 2 = adenoma extending into airway or surrounding lung tissue.
Grade 3 = adenocarcinoma metastasizing in lobe of origin or replacing whole lobe.
Grade 4 = adenocarcinoma metastasizing to other lobes of lung but not outside thorax.

ANIMALS WITH CUBOIDAL/COLUMNAR METAPLASIA OF ALVEOLAR EPITHELIUM IN THE LUNGS

GROUP	D	T	TOTAL**	D		T		TOTAL	
				no.	%	no.	%	no.	%
Females									
Control	53	43	96	0/53	0	0/43	0	0/96	0
Low	41	30	71	0/41	0	0/30	0	0/71	0
Mid	36	38	74	0/36	0	1/38	3	1/74	1
High	31	43	74	1/31	3	4/43	9	5/74	7
Males									
Control	30	69	99	0/30	0	0/69	0	0/99	0
Low	26	49	75	0/26	0	1/49	2	1/75	1
Mid	27	46	73	1/27	4	0/46	0	1/73	1
High	27	47	74	1/27	0	3/47	6	3/74	4

** based on total numbers of tissues examined (lungs).

An increased incidence of primary adenomatous tumors in the lungs of females is observed to occur in a dose-related manner for females which died or were sacrificed during the study and for the total numbers of females in the study. For those females sacrificed at termination of the study, there is an increased incidence in all test groups above the control incidence. For males, there is no difference in the incidence of primary adenomatous lung tumors between control and test animals although the overall incidence rate for males is higher than for females.

With regard to grading of these tumors, one mid- and two high-dosage level females were reported as having grade 3 tumors (adenocarcinomas) whereas no control or low-dosage level females had adenocarcinomas. For males, three adenocarcinomas ~~tumors~~ were reported for controls, one for low-, two for mid- and one for high-dosage level groups.

In addition, one mid- and five high-dosage level females were reported as having cuboidal/columnar metaplasia of the alveolar epithelium. For males, one low-, one mid- and three high-dosage level males were reported as having this lesion.

Statistical analysis of the lung tumors in females revealed the dose response rate to be statistically significant ($p < .01$) when adjusted for time of death (or diagnosis) using Peto's Prevalence Method and also to be statistically significant ($p < .02$) using the Cox-Armitage Test for trend analysis. In addition, direct comparison of the rate of occurrence of these tumors between high dosage level and control females showed statistical significance at $p < .01$. (These statistical calculations were performed and/or reported by Bertram Litt, statistician, Toxicology Branch.)

Incidence data for primary parenchymal cell tumors, hemangiomas and hemangiosarcomas in the liver and for tumors in the pituitary gland are presented in the following three tables. There is no increased incidence for any of these tumor types in test animals above the incidence in control animals that merits concern.

ANIMALS WITH ONE OR MORE PRIMARY PARENCHYMAL CELL TUMORS IN THE LIVER

				D		T		TOTAL	
GROUP	D	T	TOTAL**	no.	%	no.	%	no.	%
Females									
Control	53	44	97	1/53	2	0/44	0	1/97	1
Low	42	32	74	0/42	0	0/32	0	0/74	0
Mid	35	38	73	0/35	0	0/38	0	0/73	0
High	29	43	72	0/29	0	0/43	0	0/72	0
Males									
Control	30	70	100	4/30	13	12/70	17	16/100	16
Low	26	49	75	3/26	12	8/49	16	11/75	1[illegible]
Mid	28	47	75	4/28	14	1/47	2	5/75	7
High	31	43	74	4/31	13	8/43	19	12/74	16

** based on total numbers of tissues examined (livers).

ANIMALS WITH ONE OR MORE HEMANGIOMAS AND/OR HEMANGIOSARCOMAS IN THE LIVER

				D		T		TOTAL	
GROUP	D	T	TOTAL**	no.	%	no.	%	no.	%
Females									
Control	53	44	97	0/53	0	0/44	0	0/97	0
Low	42	32	74	2/42	5	0/32	0	2/74	3
Mid	35	38	73	1/35	3	2/38	5	3/73	4
High	29	43	72	0/29	0	0/43	0	0/72	0
Males									
Control	30	70	100	3/30	10	3/70	4	6/100	6
Low	26	49	75	0/26	0	2/49	4	2/75	3
Mid	28	47	75	1/28	4	3/47	6	4/75	5
High	31	43	74	1/31	3	3/43	7	4/74	5

** based on total numbers of tissues examined (livers).

ANIMALS WITH ONE OR MORE TUMORS IN THE PITUITARY GLAND

				D		T		TOTAL	
GROUP	D	T	TOTAL**	no.	%	no.	%	no.	%
Females									
Control	31	37	68	1/31	3	1/37	3	2/68	3
Low	31	30	61	2/31	6	1/30	3	3/61	5
Mid	24	34	58	0/24	0	3/34	9	3/58	5
High	23	38	61	1/23	4	2/38	5	3/61	5
Males									
Control	20	58	78	0/20	0	1/58	2	1/78	1
Low	17	50	67	1/17	6	0/50	0	1/67	1
Mid	19	43	62	0/19	0	0/43	0	0/62	0
High	17	47	64	0/17	0	1/47	2	1/64	2

** based on total numbers of tissues examined (pituitary glands).

Tumor types and incidence, other than those already described in this review, did occur but were sporadic across groups, were of low frequency and did not appear to be related to ingestion of the test material.

Study Conclusions

Except as noted below, differences between control and test animals were considered to be spurious and not of biological importance. Differences that may be of biological significance are presented below.

Females:

1. Food consumption for high-dosage level females was slightly decreased during the last 13 weeks of the study.

2. Survival for mid- and high-dosage level females tended to be higher than for control and low-dosage level females after 65 weeks.

3. Increased kidney weights (110% of control values) were observed in high-dosage level females killed at termination of the study but histopathologic examination of these kidneys revealed no differences attributable to the test material.

4. Treated females may have had smaller stores of liver glycogen than controls (suggested by decreased margination of cytoplasm in liver cells of treated females).

5. A statistically significant dose-related increased incidence of primary adenomatous tumors in the lungs of treated females was observed. In addition, direct comparison of high dose females with control females revealed statistical significance at $p < .01$ for these neoplasms.

   In high- and mid-dosage level females, three adenocarcinomas of the lungs were reported whereas none were reported for the control or low-dosage level females. Also, in high- and mid-dosage level females, six animals were reported to have cuboidal/columnar metaplasia of the alveolar epithelia whereas none were reported to have this lesion in the control or low-dosage level female groups.

   The totality of lung tumors data and related findings in this study are interpreted by Toxicology Branch as presenting suggestive evidence of lung tumorigenicity in female CFLP mice at dosage levels above 250 mg/kg/day.

Males:

1. Increased liver weights (110% of control values) were observed in high-dosage level males killed at termination of the study but histopathologic examination of these livers revealed no differences attributable to the test material.

2. Treated males may have had smaller stores of liver glycogen than controls (suggested by decreased margination of cytoplasm in liver cells of treated males).

Correlations of findings between "in-life" observations, gross necropsies and histopathologic examinations were satisfactory.

In general, the study was well planned and conducted. The study is classified as Core-Guidelines.

There is some concern that the "maximum tolerated dose" of permethrin may not have been achieved in this study. With the exception of the increased incidence of lung tumors in females, other effects observed in high-dosage level animals were generally of little concern and are considered to be largely inconsequential.

OPP:HED:TOX: E.BUDD:sb 7/14/81 X77395 TS-769 Rm. 820 Cl 2 #1

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

OCT 26 1981

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg. No's. 59-EUP-R, and PP 0G2299 and PP 0F2324: 21-Day Rat Neuropathology Study with Permethrin Submitted by the Burroughs-Wellcome Company.

TOX Chem. No. 652BB

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Background:

The Burroughs-Wellcome Company has submitted a study to appraise the potential for Permethrin (25% cis and 75% trans isomers) to cause a neuropathy that is sometimes associated with synthetic pyrethrins and is characterized by swelling and disintegrations of the axons in the sciatic nerve of rats.

Conclusions:

1. No histological findings which resemble the neuropathy that is caused by some synthetic pyrethrins (characterized by swelling and disintegration of the axons in the sciatic nerve) were reported as being observed in this study. No further testing is required at this time.

Review of Study

(The data are included in EPA Acc. No. 099259).

21-day neuropathological study in the Sprague-Dawley rat of Permethrin (21Z73ZJ) administered in the diet.

The Wellcome Foundation, Beckenham, England, Report No. 393, issued December, 1980.

Four groups of Sprague-Dawley rats (10 males and 10 females per group) were dosed with diets containing permethrin (94.5%, 21Z73, batch ZJ and nominally 24.4% cis and 74.6% trans) at either 0, 4000, 6000, or 9000 ppm. The duration of the feeding was for 21 days or until the rats became moribund.

The objective of this experiment was to determine if permethrin at these doses which are near the LD50 causes neuropathy in either the peripheral or central nervous systems.

During the feeding period, observations were made on the animals for signs of neurotoxicity (behavior changes and body weight effects). After 21 days, the rats were sacrificed and the nerve tissue was fixed for analysis. Tissue fixatation was by the method of perfusion of the ventricle with Karnovsky's fluid for most of the rats. The nerve tissue from three females in the high dose group only were fixed by immersion in formalin. Histology was scheduled to be performed on:

1. Brain
2. Trigeminal ganglion (both sides) and proximal and distal nerve trunk, (also, a strip of skin from each side of the face).
3. Spinal cord and dorsal root ganglia.
4. Peripheral nerves - sciatic nerve in thigh, posterior tibial nerve, sural nerve. (These tissues were fixed flat in formal saline).
5. Thigh muscle (quadriceps) from both sides.
6. Toe pad.

The above scheduled histology was not executed for all animals because the laboratory decided it was not necessary to process all of the slides without evidence for treatment related effects. Histology was performed on all animals in the high dose group and one half of the males and one half of the females in the 6000 ppm and control groups only. No tissues were examined for the low dose (4000 ppm) group.

Results:

1. Survival - All animals in the control, low, and mid dose groups survived. All rats in the high dose male group died before day 5. Only 1 female in the high dose test group survived.
2. Behavior - All test animals (not controls) exhibited varying degrees of nerve dysfunction which included trembling, stiff limb and clumsy gait. Severity increased with dose level increase.
3. Test animals did not gain weight as well as controls.

4. Histology - - No consistant pattern of lesions at any of the sites examined in the central and peripheral nervous systems as far as could be detected by the histological techniques and light microscopy used were reported as developing.

Conclusion:

This experiment does not include a positive control to ascertain that the strain of test animal and staining techniques used are adequate to study the type of neuropathology in question. The high rate of early death in the high dose groups limited the usefulness of these groups. However, the interpretation of the test results in animals dosed for 21 days at higher than 6000 ppm would not necessarily give information useful in appraising the human health risk for this chemical. No additional test is required. Previously submitted test data have not demonstrated that permethrin induces this type of neuropathy (at most only a "very slight" positive response has been reported, see PP 7G1917, EPA Acc. No. 096005).

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