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

003249

SEP 16 1983

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE:

TO: T.A. Gardner, PM#17
Registration Division (TS-767)

THRU: William L. Burnam, Branch Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: PP #2F2623 and FAP #2H5334 concerning cypermethrin on cotton and related tolerances.

TOX Chem. No. 271DD

The ICI Americas Inc. is requesting to establish permanent tolerances for their insecticide cypermethrin, a synthetic pyrethroid, as follows:

PROPOSED TOLERANCES (PP #2F2623)

It is proposed that a tolerance be established for residues of (±) α-cyano-(3-phenoxyphenyl)methyl (±) cis,trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate (cypermethrin) in or on the following raw agricultural commodities.

0.5 ppm in or on cottonseed.

0.05 ppm in the meat, fat and meat byproducts of cattle, goats, hogs, horses and sheep.

0.05 ppm in milk.

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PROPOSED FOOD ADDITIVE TOLERANCE (FAP #2H5334).

It is proposed that 21 CFR be amended by the establishment of a food additive tolerance for residues of (±) - cyano-(3-phenoxyphenyl)methyl (±)cis, trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate (cypermethrin) in or on the following agricultural commodity:

5 ppm in cottonseed oil.

This review contains data sent to TOX Branch on September 3, 1982 and May 4, 1983.

Recommendations:

Toxicology Branch (TB) cannot recommend in favor of establishing permanent tolerances for cypermethrin at this time.

Comments:

1. The mouse oncogenicity study with cypermethrin presented data indicating an increased incidence of benign alveologenic tumors in the lungs of females particularly in the high dose test group. In order to fully assess this possible oncogenic finding in lung tissue the registrant is requested to submit the following additional data regarding the mouse study:

   a. The lung and liver tissue from the mice in the interim sacrifice groups (9-10 mice/sex/group at 52 weeks) should be examined as follows:

      i. gross pathological examination including palpation of the lung tissue.

      ii. 5 sections from each lung (cut in an identical manner from all animals) should be prepared for microscopy and read.

      iii. 1 section from the liver of each of these mice should also be prepared and assessed microscopically.

   b. Historical control data for malignant lymphoreticular tumors should be submitted for the strain of mouse used for this study. Ideally, these data should be on mice from the same supplier which were studied in the same laboratory as were the mice in this study. Recent or concurrent data is most preferable.

TB is requiring information on the development of malignant lymphoreticular tumors in this strain of mouse because it was noted that as many as 55 to 75% of the female mice and 20 to 48% of the male mice in this study with cypermethrin developed this type of tumor. Although there was no indication that the presence of cypermethrin in the diets increased the incidence of this type of tumor, TB feels it necessary to assess further the general health status of the animals used in this study and the potential influence of these tumors on the development of the lung tumors observed in this study.

After the requested data (above) is received by TB, all pertinent data relating to the question of potential oncogenicity in the lungs of female mice will be re-evaluated and a request will be made to the TB statistician for statistical analysis. A future memorandum will then address the issue of potential oncogenicity of cypermethrin in more detail.

Note to PM: The mouse oncogenicity study has been retained by TB for future reference.

2. For the dog 1-year oral dosing study, the results showed that at 5 and 15 mg/kg/day there were highly increased incidences of "liquid stools." It is uncertain as to whether or not this effect was due to the method of treatment (test chemical administered orally in a corn oil solution in a gelatin capsule) or due to the test chemical actually stimulating the central nervous system or peripheral nervous system to promote a gastrointestinal disturbance.

   Certain other synthetic pyrethrins are known or suspected to cause similar gastrointestinal disturbances via direct action on the nervous system. The answer to this question will influence the assignment of a NOEL to this study--and, consequently, calculation of the ADI and MPI since the dog is more senstiive than the rat to cypermethrin in chronic studies.

   In order to resolve the problem of the source for the stimulus responsible for the production of the liquid stools, the registrant is requested to conduct the following study:

   Beagle dogs should be dosed intravenously (i.v.) with cypermethrin in a suitable vehicle and their reactions noted including the frequency and condition of the bowel movements for the following 72 hour period. At least two groups of 3 dogs should be used: a control group dosed i.v. with vehicle and the test group dosed i.v. with cypermethrin in vehicle. The group dosed with cypermethrin should show some signs of nervous system stimulation but the dose should not be so high as to

kill the dogs within the 72 hour testing period. The dogs used in this study should be on their normal diets (and not fasted prior to dosing) and should have access to water at all times. Dosing should be made a short time after the dogs have been fed. The data should be tabulated to show the signs of response to cypermethrin (or vehicle) in parallel with any signs of bowel movements.

The results of this experiment will assist TB in determining a NOEL for the dog 1-year oral toxicity study. More specifically, demonstration that the effect (liquid stools) may be caused by intravenous injection would imply that cypermethrin acts directly on the central nervous system or on the peripheral system. In this case, the NOEL may be assigned as 1.0 mg/kg/day. On the other hand, failure of the intraveneous injection to affect the dogs' gastrointestinal system would imply that the effect is due to some other (presumably local) action. In this case, the NOEL may be assigned as 5.0 mg/kg/day.

3. The rat chronic feeding/oncogenicity study and the rat three generation reproduction study were reviewed and found to be CORE GUIDELINES. No oncogenic effect was noted in rats at doses up to and including 1500 ppm. The NOEL for this study was determined to be 150 ppm (7.5 mg/kg/day). Although a NOEL was established for this study, TB must use the NOEL eventually established for the dog 1 year oral dosing study to calculate an ADI and MPI for cypermethrin because the dog has been shown to be the more sensitive species.

4. EPA Acc. No. 071069 contains a cover letter, dated 9/1/82, from Dr. R. E. Ridsdale of ICI which contains comments on a previous review by TB concerning the use of cypermethrin on cotton (see J. Doherty review dated Oct. 28, 1981). The following comments are in response to the issues raised by Dr. Ridsdale.

4a. Neurotoxicity. TB has determined that cypermethrin does not present a practical neurotoxicity hazard to man as indicated by review of the recently submitted chronic feeding and oncogenesis rat and mouse studies.

4b. TB concedes that the product studied for the acute inhalation LC_{50} determination was GFU-061. Because the procedures used in this study did not generate a uniform spray mist the actual LC_{50} of the product when used as a spray mist could not be assessed and the study is CORE SUPPLEMENTARY.

Based on the overall toxicity of cypermethrin and because the spray mists generated will not be a respirable size, an additional inhalation LC_{50} study with this product is not required.

4c. A TB review of the products 10182-AL and 10182-AU was sent to RD on January 10, 1983. In that review, TB required that the acute oral LD_{50} stuides on both these formulations be repeated (or additional information on the available studies be submitted); proposed a label change on 10182-AL; and noted that one inert in 10182-AL was not cleared.

4d. TB acknowledges receipt of the information that the cis:trans ratio of the technical cypermethrin used for the rat and rabbit teratology studies was 50:50.

STUDIES REVIEWED

Study	Result	CORE Classification
3 Generation Reproduction-rats CTL/P/683 July 9, 1982.	NOEL for adverse reproductive effects = 750 ppm (HDT). NOEL for systemic effects = 50 ppm. LEL = 150 ppm, decreased body weight gain in maturing pups.	GUIDELINES
1 year oral dosing (gelatin capsule) - dogs CTL/P/703 July 6, 1982	Tentative conclusion: NOEL = 1.0 or 5.0 mg/kg/day. G-I tract disturbances at 5.0 mg/kg/day. Definite nervous system effects at 15 mg/kg/day (HDT). (An additional study has been requested to help determine the toxicological significance of the G-I tract disturbances.)	GUIDELINES
2 year chronic feeding/ oncogenicity - rats CTL/P/669 June 1982	NOEL = 150 ppm. LEL = 1500 ppm, weight loss, general changes in blood elements and cholesterol levels. Not oncogenic up to and including 1500 ppm (HDT).	GUIDELINES
Lifetime oncogenicity - mice CTL/P/687 June 1982	Potentially positive oncogenic response in lung tissue. [Increased incidence of benign adenomas in females (only), statistically significant at 1600 ppm (HDT) (only).]	RESERVED

REVIEW OF STUDIES

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A. Cypermethrin: Three Generation Reproduction Study in the Rat.

Central Toxicology Laboratory, ICI, #CTL/P/683, July 9, 1982. EPA Acc. No. 071074 and 071075, TAB 52C.

B. Substance tested. The test material was cypermethrin: (RS) α-cyano-3-phenoxybenzyl (IRS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate. Analysis of the test material reported that the purity was 90.6-93.1% and the cis:trans ratio was 53.4 to 46.6 (other lots of test material gave slightly different percentages). Three batches (P19, P24 and P26) of cypermethrin were used for this study.

C. The test rats used for this study were Wistar derived Specific Pathogen Free albino rats. They were obtained from the Alderley Park supplier.

The study comprised four groups each containing 15 males and 30 females which were dosed as either 0, 50, 150 or 750 ppm of cypermethrin in their diets. The high dose test group received 1000 ppm for the first twelve weeks of the study but this level was reduced to 750 ppm because of obvious signs of neurological effects.

D. Dietary analysis for the test substance. Data were presented which showed that the desired doses were achieved (within 10%) and that cypermethrin was stable for at least six weeks and the diet preparations were homogeneous with respect to distribution of the cypermethrin.

E. Three successive generations were produced, each consisting of two breedings. The pups from the second breeding were selected to be the parents for the succeeding generations.

F. Mortality and clinical signs. The death (sacrificed in extremis) of a single rat (high dose group male at 1000 ppm) was attributed to the test chemical. Other rats dosed with 1000 ppm showed signs of neurological disturbance characterized by increased sensitivity to sound, ataxia and high stepping gait (during first 3 weeks of study). The report states that no other "treatment related signs were seen in any other F_0 treatment group or in subsequent generations at any dose." Thus, although no tables regarding behavior (other than for

the F_0 generation) were presented a NOEL for nervous system effects is set at 750 ppm.

G. Body weight gain (mature rats).

A NOEL is set at 50 ppm. At 150 ppm there were many cases of decreased weight gain in both males and females. More pronounced effects were noted in the 1000/750 ppm test dose groups.

Food consumption was also reported to be less in the mid (occasionally) and high dose test groups.

H. Reproductive performance. For each of the six breedings, assessments were made regarding length of gestation, live born index, survival index, maternal neglect index, male fertility, and viable litter size.

Other than there being some "slightly reduced" actual pregnancy weight gains (to day 14) in the high dose test groups there were no other effects reported.

I. Offspring body weight gain was determined at days 0, 4, 10, 21 and 28 post partum. A NOEL is set at 150 ppm. At 1000/750 ppm, there were many instances of statistically significant decreases (of about -13%). There were no signs of behavioral changes in the offspring reported.

J. Pathology (mature rats) was conducted on rats which died or showed signs of reproductive impairment. Later F_0 and F_2 parents were subjected to a gross post partum examination and selected tissues (including the testes) were examined microscopically. The F_1 parents (25 females and 10 males) were subjected to a full post mortem examination including histopathology of 17 or more tissue types.

No pathological findings were attributable to the test chemical.

K. Pathology (pups). "Full" post mortem examinations were conducted on any grossly abnormal pups and five males and five females from the F_1B and F_2B litters and 10 males and 10 females per group from the F_3B litters. Selected rat pups (less than 18 days of age) were preserved for teratological examination.

No evidence of test chemical induced pathology or terata were presented in this report.

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Conclusion.

This study is CORE GUIDELINES. The NOEL for adverse effects on reproductive parameters is 750 ppm (HDT). The NOEL for systemic effects is 50 ppm. At 150 ppm (LEL), body weight decreases in maturing rats were noted and at 750 ppm pup weight was also decreased.

A. Cypermethrin: One Year Oral Dosing (Gelatin Capsule) Study in Dogs

Central Toxicology Laboratory, ICI, # CTL/P/703, July 6, 1982, EPA Acc. No. 071069, TAB 49C.

B. Substance tested: RS-α-cyano-3-phenoxybenzyl (IRS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcycloproanecarboxylate or cypermethrin. The material was identified as being from batch No. P26 Ref. No. C4921/187 or Y00334/017/005. The purity was stated as being 90.6% (w/w) and as being 53.9% cis: 46.1% trans. The impurities (9.4%) were not identified.

C. 4 groups of 6 beagle dogs (Alderly Park strain, 16-20 weeks old) of each sex were dosed with 0, 1, 5, or 15 mg/kg/day of cypermethrin in corn oil for 52 weeks. The test chemical was administered by gelatin capsule and the amount administered was based on the current weight of the dog. The dose level was adjusted for the 90.6% purity of the test material. Water was available ad libitum.

D. Ten preparations of cypermethrin in corn oil were made and each of these were analyzed for their content. The analysis revealed that the actual level was similar (within 10%) to the desired level.

E. Survival and reactions to the test chemical. There were no mortalities.

Males and females in the high dose (15 mg/kg) test group (only) displayed signs of nervous system stimulation in the form of body tremors, gait abnormalities and uncoordination, disorientation, and hypersensitivity to noise. These symptoms would be expected in test animals dosed with high levels of synthetic pyrethroids.

Detailed neurological examination was said to have been conducted which evaluated some 21 parameters involving reflexes, cranial nerve function, postural reactions, attitudinal reactions and assessment of temperment. The report states that these studies did not reveal additional information (about the neurotoxicity of cypermethrin) but there were no tables or other indication showing the data or extent of investigations.

The dogs dosed with cypermethrin showed increases in vomiting during the first week and the passing of liquid feces throughout the study, symptoms which have been associated with synthetic pyrethroids in other dog studies especially when gelatin capsules and corn oil are used. The incidences of the passing of liquid stools over the 52 week period is given below:

	Males	Females	
Control	28	25	
Low (1.0 mg/kg)	19	36	
Mid (5.0 mg/kg)	133	254	LEL
High (15.0 mg/kg)	875	767	

There is noted an increase of about five-fold for males and about ten fold for females in the groups dosed with 5 mg/kg/day. There is about a 30-fold increase in incidences for the groups dosed with 15 mg/kg/day. A true NOEL is 1.0 mg/kg/day for this effect.

F. Body Weight and food consumption. A NOEL is set at 5 mg/kg/day for males and females. It is noted that a trend toward lower weight gain was evident in the mid (5 mg/kg/day) male group. Loss of appetite was noted only in the high dose test group.

NOTE: For sections G, H and I below, analyses were made at pretest and in weeks 4, 8, 12, 16, 20, 26, 39 and 52. Jugular vein blood was used for hematology and clinical biochemistry. Urinalysis was performed at pretest and at weeks 8, 16, 24, 39 and 50.

G. Hematology included determinations on hemoglobin, hematocrit, RBC, MCV, mean cell hemoglobin, mean cell hemoglobin concentration, total and differential white blood cell count, platelet count and prothrombin times. No consistent dose related changes in these parameters were noted.

H. Clinical biochemistry determinations were made on BUN, glucose, triglycerides, albumin and total protein, cholesterol, Ca^{++}, K^{+}, alkaline phosphatase, alanine transaminase, aspartate transaminase, and creatine kinase. Occasional deviations from the control values were noted but there were no consistent dose related changes reported.

I. Urinalysis determinations were made on glucose, ketones, urobilinogen, pH, specific gravity and protein. No consistent dose related effects were reported.

J. Gross Pathology - The gross necropsy observations are included on the individual animal pathology sheets but the data are not tabulated in a summary table. Inspection of the individual dog pathology sheets did not reveal the presence of a specific type of grossly observable lesion associated with the treated dogs.

K. Organ weights- The liver, heart, adrenal, thyroid, brain, pituitary, lung, thymus, kidney, spleen and gonad weights were determined at sacrifice. The following changes in organ weight were noted:

a. The liver was slightly higher in weight but statistical significance was not attained. For example, the high dose group females were 6.4% higher. No definite dose related toxic chemical effect is noted. (Note: liver weight increases are known to be associated with higher doses of synthetic pyrethroids).

b. The heart weight of the high dose group males was statistically significantly lower (-6%).

c. The adrenal weight in males was 19% higher or 11% higher depending upon exclusion of a single dog from the control group with a small adrenal.

All other organs did not show statistically significant differences in weight. The overall conclusion is that a NOEL of 15 mg/kg is supported for organ weight changes. It should be noted that at 15 mg/kg the effects noted on the heart and the adrenal are not definitely related to the test material in the opinion of this reviewer.

L. Microscopic pathology. The protocol provided that all dogs were to be evaluated for 37 tissue types. The pathologist responsible for evaluation of the tissue was S.F. Moreland (Pathologist/Veterinarian). The following individual tissue types are discussed as follows.

a. No dose related changes in the structure of the liver were reported. The lesions reported were fibrosis, bile duct proliferation, increased golden pigment accumulation, necrosis and inflammatory cell infiltration, but these were in all dogs in the study.

b. The lungs of the male dogs had 1, 1, 1 and 3 incidences of granuloma (out of six dogs), but this nonneoplastic lesion is not considered to be definitely related to the presence of the test chemical. A similar increase in females was not evident.

c. The spleen had increased incidences of "siderofibrotic nodules or fibrous capsular thickening" in the high dose test groups of both males and females. In males there were 2,3,3 and 4 incidences and 2,3,3, and 5 incidences among the females for the control, low, mid and high dose test groups (out of six dogs). In the absence of changes in hematology parameters, this effect in the spleen is not considered to be a definite test chemical effect by this reviewer.

d. There were reported 0, 1, 2 and 3 incidences (of 6 dogs per group) of "focal interstitial lymphocytic infiltration" among the control, low, mid and high dose test groups in the epididymis to indicate a possible chronic inflammatory process. In other organs (e.g., the salivary gland) a similiar lesion was noted as occurring at higher incidences in the control groups.

e. There was a single tumor noted in one dog of all of the dogs on the study at termination. This was a hamartoma in a high dose test group male. One female had a benign papilloma surgically removed from its lip. Thus, cypermethrin did not give indications of being oncogenic in this dog study.

Conclusion. This study is classified as Core Guidelines. TB is unable at this time to assign a toxicological NOEL for this study. The dogs dosed with 5 mg/kg/day (the level suggested by the registrant as the NOEL) had a clearly increased incidence of liquid stools. This effect could be a result of stimulation of the central nervous system (or the peripheral nervous system) by the test material or could be the result of some other (presumably local) action.

In order to resolve this issue a study in which dogs are treated intravenously with cypermethrin and their bowel movements monitored should be conducted and submitted. For more details and a rationale for this requirement, see 2. under Comments (page 3 of this review).

A. Cypermethrin: 2 year feeding study in rats

ICI Central Toxicology Labs., #CTL/P/669, June, 1982. EPA Acc. No. 071070 and 071071, TAB 50C.

B. Substance tested: Cypermethrin, RS-α-cyano-3-phenoxybenzyl (IRS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, cis-trans ratio of 55:45 (nominal). The substance identification number was Y00334/017. Analysis of the test substance indicated that the test material was 88 to 93% pure. The chemical nature of the impurities was not defined.

C. The test animals used were Wistar derived albino rats obtained from the Specific Pathogen Free (SPF) colony maintained at the Alderly Park facility in Cheshire, England. 5 groups of 52 male and 52 female rats were dosed with diets containing 0(two groups), 20, 150, or 1500 ppm of cypermethrin. Satellite groups of 12 male and 12 female rats were also maintained and designated for an interim sacrifice at 52 weeks. For a brief period (first six weeks) the animals requiring the highest dosage level were dosed with 1000 ppm rather than 1500 ppm. The rats were approximately 36 days old when they were initiated on their test diets.

D. Dietary analysis indicated that the test diets were usually within $\pm$ 10% of the desired levels. The cypermethrin was found to be stable for up to six weeks. Usually fresh diets were mixed at 2-3 week intervals.

E. Survival at 104 weeks was considered acceptable and is shown in the following table.

Dose Level (ppm)	Males	Females
0	27 (52%)*	24 (46%)
0	28 (54%)	22 (42%)
20	26 (50%)	23 (44%)
150	21 (40%)	24 (46%)
1500	27 (52%)	27 (52%)

* Number of survivors (as percent of 52 starters). No test chemical effect on survival is noted.

There were some initial behavioral signs of reaction (first six days) to the test chemical (frequent face washing, increased sound sensitivity and lack of co-ordination in the hind limbs) in six high dose males. Some signs of reaction which consisted of thin appearance and hair loss were later reported as being evident in the high dose test group.

A NOEL of 1500 ppm is set for these effects. The effects reported at this level are too indefinite and/or of transient nature to assign this level as a LEL.

F. Body weight gain was definitely considered to be decreased for the high dose male and female test groups. The mid dose test groups were also occasionally affected (statistically significant) and were on some occasions as much as 4-5% lower for females. The high dose groups were -18% (females) and -12% (males) lower at termination. Food consumption was reported to be decreased in the high dose test groups.

NOEL for body weight gain = 150 ppm. The slight effect noted in females at 150 ppm is not considered to be of sufficient magnitude or consistency to offset this assignment of a NOEL.

For sections G, H and I (below) samples were taken at pretest, week 4, week 13 and at each 13 weeks afterward until termination. 12 rats from each sex from each group were selected.

G. Hematology. The following parameters were measured: Hb, total white cell count, total red cell count, mean cell volume, mean cell hemoglobin and cell hemogloblin concentration, hematocrit, differential white cell count. At selected intervals prothrombin and kaolin/cephalin time tests were determined. Bone marrow smears were also sampled.

The high dose test group was associated with slight adverse effects on several hematological parameters. These included reduced mean cell volume (and related changes in Hb and hematocrit); slightly increased white cell count (increase in lymphocyte count with a decrease in neutrophil count). There was also a slight increase in prothrombin time.

A NOEL for hematological parameters is 150 ppm. The LEL for generalized changes is 1500 ppm (HDT).

H. Clinical Biochemistry. The following parameters were investigated: alkaline phosphatase, alanine transaminase, aspartate transaminase, plasma cholesterol, albumin, total protein, urea, glucose, and triglycerides.

Plasma cholesterol levels were decreased for the high dose test groups (both male and female) about 25%. The mid dose group was decreased occasionally but the

decrease did not reach statistical significance. Plasma triglycerides were also apparently decreased in males at 1500 ppm (there was a large variation in triglyceride data which hindered a more definite conclusion). Other parameters showing effects at 1500 ppm were urea (increased) and glucose (decreased). Other parameters were occasionally higher or lower than the controls.

NOEL for blood biochemistries = 150 ppm. The LEL for generalized changes is 1500 ppm (HDT).

I. Urine analysis. Parameters investigated included volume, pH, specific gravity, glucose, protein, bilirubin and analysis for cypermethrin metabolites.

Effects reported for the groups dosed with 1500 ppm included reduction in volume, decreased pH and an increase in the specific gravity. A slight decrease in protein was also reported. Dose related amounts of the cypermethrin metabolite, 3-(4'-hydroxyphenoxy)-benzoic acid, were assayed in the urine.

A NOEL for this aspect of the study is 150 ppm.

J. Organ weights. The adrenals, brain, heart, kidneys, liver, lung, gonads, pituitary gland and spleen were weighted at 52 weeks and at termination. The thymus was weighted at 52 weeks.

The only organ the testing laboratory considered definitely affected by treatment was the liver (females in the high dose test group only) and this effect was only evident at 52 weeks and was evident by a 21% increase in relative liver/body weight.

At 104 weeks, the female kidney weight for the high dose test group was decreased in weight - 17% absolute and relative. Spleen weight also appeared to be decreased 13% absolute and relative for the high dose test group males.

NOEL for organ weight changes = 150 ppm, LEL = 1500 ppm, liver and possibly also kidney weight changes.

K. Gross necropsy (on all rats). No table summarizing or tabulating the gross necropsy observations was presented. The gross necropsy findings are reported on the individual animal pathology reports. Using these reports it can readily be determined if gross necropsy observations were followed up histologically. Inspection of these individual animal pathology reports indicates that

followup of the gross necropsy observations by histopathological descriptions of lesions was acceptable.

The testing laboratory reported no gross lesions considered to be attributable to the test material.

L. Histopathology - A comprehensive list of some 42 tissue types were prepared for histological examination. All rats were scheduled for complete microscopic analysis.

No single tissue type was indicated in the study to be a neoplastic or nonneoplastic target organ for cypermethrin at any dose level. The following organs are commented on for various reasons as given.

1. The liver showed some increases in weight gain (in high dose females at 52 weeks). The liver has been shown to be a target organ for toxicity for other pyrethroids.

There were a total of three neoplasms reported in the liver - two incidences of hepatocellular carcinoma (one control male and 1 high dose female) and one incidence of hepatocellular adenoma (low dose group male). There were no "nodular hyperplasia" or "hyperplastic nodules" reported. Light microscopic analysis of the liver did not reveal dose related increases in commonly occurring non-neoplastic liver lesions.

Some special studies were conducted to assess for possible liver effects of cypermethrin. These were induction of hepatic aminopyrine-N-demethylase (APDM) activity and electron microscopy of the smooth endoplasmic reticulum (SER). Increased enzyme activity (up to 64%) was noted in the high dose test group (1500 ppm) especially in the females. The high dose test group was also associated with increases in the content of the smooth endoplasmic reticulum. The mid dose group females also showed a statistically significant increase (~ 20.5% increase) in SER.

Slight increases in APDM activity and in smooth endoplasmic reticulum is considered by TB to be an adaptation response to the test chemical, rather than a true toxic response.

2. The testis developed a slightly higher incidence of interstitial cell adenomas in the high dose test group among animals dying before termination.

Interstitial Cell Adenomas

Dose Group	52 weeks to termination	Termination	Total
Control - 1	2/25	5/27	7/52*
Control - 2	0/22	8/28	8/50
20 ppm	0/26	7/26	7/52
150 ppm	2/28	5/21	7/49
1500 ppm	6/24^{xx}	7/27	13/51^{NS1}

* incidences/number examined (does not include rats dying in first year).

^{xx} P=.007) Fisher's one-tailed P
^{NS1} P=.098) statistic by TB computer.

Only the rats which died prior to termination of the study showed a statistically significant increase in this tumor type (at the high dose level only).

Nonneoplastic pathology of the testis was unremarkable in that only non-dose-related lesions were present. The report indicated that there were slight increases in incidences of tubular atrophy and calcification of the testes. Testes weight changes did not show statistically significant increases. An 18% apparent increase in relative weight is reduced to 7% when one extreme value is eliminated from the high dose test group.

TB notes that at least one other synthetic pyrethroid has been demonstrated to induce testicular interstitial neoplasms in rats. However, due to the failure of the data with cypermethrin in the above table to reach consistent statistical significance, TB cannot conclude from these data that the testis is a neoplastic target organ for cypermethrin.

3. Pathology of the nervous system. At least some synthetic pyrethroids have given indications that a particular type of axonal lesion results from exposure to high doses. The sciatic nerves were routinely fixed in formol saline and examined in this study. In addition, some special examination of the sciatic and posterior tibial nerves was conducted by fixing the tissues in formol glutaraldehyde and embedding in glycol methacrylate. The nerves were cut and stained in H&E and in addition were stained by Palmgrins silver impregnation techniques for axons and the solochrome cyanin technique for myelin. Histological findings did not reveal a test chemical effect in the structure or integrity of

the nerves from rats dosed with cypermethrin. The overall incidence of lesions in nerve tissue is shown in the following table.

Sciatic Nerve-Neuropathy

	Males				Females			
	Combined Control	20 ppm	150 ppm	1500 ppm	Combined Control	20 ppm	150 ppm	150 ppm
Number examined	102	49	47	51	102	48	50	51
minimal/slight	31	13	14	13	40	18	26	21
moderate/marked	64	32	27	33	55	23	23	28
severe	5	1	3	3	0	2	1	1

4. Examination of the pituitary revealed frequent occurrences of adenomas and occasional carcinomas but there was no evidence of a dose response. There were 66 incidences of adenomas among the male groups and 213 incidences among the females. These were distributed as 22/61, 12/64, 15/55, 10/59 and 7/57 among the males and 46/62, 46/63, 38/59, 42/62 and 41/61 among the females for the controls, low, mid and high dose test groups.

There were 2 incidences of carcinomas in the pituitary for the males (a control and low dose group) and 13 incidences among the females. There were 4, 2, 1, 2 and 4 in the control groups, low, mid and high dose groups respectively (see above for denominators.)

There was no indication that the pituitary neoplasms developed earlier in the rats dosed with cypermethrin.

Other Oncogenic Aspects

The following table indicates the total number of neoplasms in each group (not including testicular interstitial adenomas, pituitary adenomas or generalized lymphosarcomas):

	Incidences of Neoplasms*	
	Males	Females
Control-group #1	35	32
Control-group #2	30	47
20 ppm	34	28
150 ppm	27	32
1500 ppm	35	37

* Total of 64 rats in each group.

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The various neoplastic types which developed did not show evidence of being related to increasing the dose of cypermethrin in the diet.

Conclusion: This study is CORE GUIDELINES. A NOEL of 150 ppm is assigned. Some minor developments (slight weight loss, increased SER and blood effects) are not considered sufficient to determine that 150 ppm is a LEL. The LEL is 1500 ppm, at this level there is weight loss, general changes in blood elements and cholesterol levels and evidence of liver weight increases.

No evidence that cypermethrin induced an oncogenic response at up to and including 1500 ppm was presented.

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A. Cypermethrin: Lifetime Feeding Study in Mice

Central Toxicology Laboratory, ICI, #CTL/P/687, June 1982. EPA Acc. No. 071072 and 071073(TAB 51C) and 071570.

B. Substance tested: The test material was cypermethrin technical (PP383; WL43467) or RS-α-cyano-3-phenoxybenzyl (IRS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate. The test material was obtained from the ICI Company (reference no. P19 with a purity of 91.5% w/w and cis:trans ratio of 53:47) or from the Shell Company (reference no. ACD/79/134 or Batch 57, with a purity of 94.2 or 94% and a cis:trans ratio of 54:46).

C. The test mice were obtained from the Alderley Park stock of Specific Pathogen Free Swiss derived strain. Five groups of 70 male and 70 female mice were selected and dosed as either control (2 groups) or 100 ppm, 400 ppm or 1600 ppm of cypermethrin in their diets. Of these, 9-10 males and 9-10 females per group were selected at 52 weeks for an interim sacrifice. Mice were delivered at 19 days of age and were 4-5 weeks old at the start of the study. They were housed 5/cage by sex.

D. Diet analysis. Periodic dietary analyses were made on some 23-24 occasions. Usually the achieved concentration of cypermethrin in the diet was within 10% of the expected level. Tests for homogeneity showed uniformity within the diet batch and cypermethrin was shown to be stable in the diet for over a three month period.

E. Survival and clinical responses to the test chemical. No obvious test chemical effects were reported in the behavior of the test mice. Signs of neurological effects which might be expected in animals treated with a pyrethroid were not reported as developing.

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No test chemical effect on survival was noted. The males were sacrificed at week 97 when there was 72.7 to 84.7% mortality in the five groups. The females were sacrificed at week 101 when there was 76.7 to 88.9% mortality among the groups. For both sexes about 50% mortality was reached at about week 80-84. There were 13, 15, 16, 9 and 13 males and 14, 10, 9, 12 and 7 females which survived through weeks 96 or 100 respectively.

F. Body weight gain was decreased in the high dose test groups (males and females) only. Statistical significance in the difference for the high dose test groups was evident only in the first year, but a weight difference was still evident in the latest weeks. For example, at week 6 the high dose males were about 10% lower in weight than the controls, at week 80 they were 13.5% lower. The females were 9% lower at week 6 and 7% lower at week 80. The low and mid dose groups were essentially similiar to the control groups.

Food consumption and utilization data were collected and periods of lower consumption (although not consistent) for the dosed mice were noted.

G. Hematology - Blood samples were taken from 10 male and 10 female mice at weeks 52 and at termination (where possible). The parameters investigated included determination of hemoglobin, hematocrit, total white cell count, red cell count, mean cell volume, mean cell hemoglobin and concentration, platelet count and examination of peripheral blood films. Several deviations possibly due to the test chemical were noted.

a. Reduction in hemoglobin, hemotocrit and RBC count were noted for males in the 1600 ppm test group at the interim sacrifice but not at the terminal sacrifice.

b. Mean cell volume and hemoglobin were significantly reduced in the females in the high dose test group at the interim sacrifice but not at the terminal sacrifice.

c. Platelets counts were increased for males at both the interim and terminal sacrifice for the high dose group; they were also slightly elevated for the high dose group females at the terminal sacrifice.

d. Neutrophil counts were significantly increased for the males and suggested for the females at interim kill but not at the terminal sacrifice.

e. Eosinophils were statistically significantly reduced at the interim and terminal sacfifice for both the high dose group males and females.

The overall conclusion of the hematology determinations is that a NOEL is set at 400 ppm. At 1600 ppm there are generalized changes in the blood elements. Note a somewhat similiar conclusion was made for the rat chronic feeding study.

H. Clinical Biochemistry - no determinations were made.

I. Urinalysis - No determinations were made.

J. Organ weights. At weeks 52 and at termination the liver, spleen, testes, kidney, lung, heart, and brain weights were determined.

The liver weight was affected at both the interim and terminal sacrifices as shown in the following table:

	Males		Females	
Group	Interim	Terminal	Interim	Terminal
Control - 1	2.83/2.79[1]	2.74/2.89	2.36/2.38	3.08/3.14
Control - 2	2.79/2.81	3.49/3.41	2.46/2.42	2.34/2.35
100 ppm	2.69/2.72	2.90/2.91	2.47/2.59	2.71/2.61
400 ppm	3.37*/3.18	2.81/2.86	2.67/2.59	2.69/2.60
1600 ppm	3.39*/3.56*	3.96/3.88	2.79/2.77*	2.47/2.67

*Statistically significant
[1] absolute weight/relative weight (as adjusted for bodyweight).

At 400 ppm the liver absolute weight is increased by 20% (at the interim sacrifice only). Increases in liver weight are an expected result of ingestion of synthetic pyrethroids.

The testis weight was decreased for the high dose test group (-18% absolute and relative) at the interim sacrifice only.

The other organs did not show consistent evidence of a compound related change in weight.

A NOEL for changes in organ weights is set at 400 ppm. At 1600 ppm there is noted an increase in liver weight. The changes in liver weight at 400 ppm (interim sacrifice only) and testis weight at the highest dose level (interim sacrifice only) are not considered consistent and definite responses to the test material.

K. Gross Pathology - All mice were reported to be necropsied as soon as possible after death or sacrifice. No tables tabulating the incidences of the various gross pathology findings were presented. The gross pathology for each mouse is described on the individual mouse pathology data sheets. Using these sheets, the extent of followup of the gross pathology by microscopic analysis can be readily determined. In the opinion of this reviewer a satisfactory follow up of gross necropsy lesions with microscopic findings was presented.

L. Microscopic Evaluation:

A series of approximately 45 tissue types and organs from each mouse were taken and examined histologically following fixation. Microscopic examination was performed on all mice dying during the study and on the survivors.

9-10 mice from each group for each sex were sacrificed at 52 weeks for an interim kill; except for the sciatic nerves for selected control and high dose test group mice, no other histopathological analysis of the tissues was made. The tissues from the mice in the interim sacrifice groups were preserved for possible future analysis.

a. Non-oncogenic aspects

A table was prepared at the request of TB which lists and tabulates the nonneoplastic findings for this study. Inspection of this table did not indicate the presence of any dose or compound related increases of nonneoplastic lesions. Major lesions observed were typical of Alderly Park mice and occurred in all groups with similar frequencies. NOTE: This table can be found in EPA Acc. No. 071570. In particular, no evidence of changes in liver pathology were noted at any dose level.

b. Oncogenic aspects.

The following table summarizes the overall neoplastic responses for the mice in this study.

	Males				Females			
	n	Total Incidences (1)	Malignant Incidences (1)	Mice Affected (2)	n	Total Incidences (1)	Malignant Incidences (1)	Mice Affected (2)
ontrol-1	61	66	43	44	61	90	55	54
ontrol-2	60	80	40	40	60	82	47	46
00 ppm	60	93	49	49	61	69	40	44
00 ppm	60	60	31	41	60	74	43	45
600 ppm	61	68	44	47	60	74	45	48

(1) as summarized in Table 55 of the study report.

(2) as indicated in Table 56 of the study report.

n = number of mice (not including those sacrificed at the 1 year interim kill). Inspection of the above table does not indicate an overt or obvious increased incidence of neoplasms or increase in malignancy in treated mice. Certain types of neoplastic findings and selected organs are discussed below. In particular, most of the malignant tumors were in the lymphoreticular system.

1. Lungs: A statistically significant increased incidence of benign alveologenic neoplasms in the lungs of female mice was observed in the high dose test group as shown in the following table:

Alveologenic Tumors

(Total incidences in all observed mice)

	Males[1/]				Females				
Group	n	Benign	%	Malignant	%	n	Benign	%	Malignant	%
Control - 1	61	4	6.6	1	1.6	61	4	6.6	0	0
Control - 2	60	10	16.7	1	1.7	60	4	6.7	2	3.3
100 ppm	60	10	16.7	1	1.7	61	6	9.8	0	0
400 ppm	60	6	10.0	0	0	60	7	11.7	1	1.7
1600 ppm	61	7	11.5	3	4.9	60	13**	21.6	0	0

**Statistically significant increase P = 0.016 by TB computer using Fisher's one-tailed P statistic.

n = number of mice available for examination.

1/ In addition, one mouse in the low dose test group (males) was reported as having "secondary carcinoma (occult primary)."

Inspection of the summary Table (EPA Acc. No. 071570) for nonneoplastic lesions in the lungs did not indicate increased incidences of lesions which could be interpreted to be pre-neoplastic conditions. There were no dose dependent increases in any of the nonneoplastic lesion types in the lungs reported. Nonneoplastic lesion types were of low frequency (~ 8-9%) with regard to the number of mice affected. Thus, the lung tissue needs evaluation in terms of possible induction of a neoplastic response in females.

TB does not consider that the lungs of the male mice in this study are affected by cypermethrin.

The development of malignant tumors in the three male mice in the high dose test group (3 incidences vs only 1 in each of the controls and low dose group) is considered to be spontaneous and not conclusively linked to the presence of cypermethrin in the diet. Moreover, chemical induction of lung tumors would be expected to be expressed by both increased incidences of benign neoplasms and malignant types. In the case of male mice in this study there is no increase in benign neoplasms.

Malignant neoplasms among the females is not an issue with regards to increased malignancy being related to the presence of the test material in the diet. For example, of the three mice with malignant neoplasms in the lungs, two of these mice were in the control group - 2.

The following table shows the lack of a decreased time for onset of development of lung tumors with the presence of cypermethrin in the diet.

Group		n[1]	Average Week of Death			Week of Earliest Tumors
C-1	0	4	86.50	±	17.08	63
C-2	0	6	85.17	±	17.62	53
Mid	100 ppm	6	90.33	±	10.91	73
Low	400 ppm	8	80.25	±	16.55	46
High	1600 ppm	13	90.92	±	11.10	66

[1] n = mice with benign or malignant neoplasms

Based on these data, it appears that cypermethrin induces an increase in benign alveologenic neoplasms in female mice in the later months of the study.

The registrant/petitioner for the use of cypermethrin (The ICI Corp.) presented historical control data regarding the incidences of benign alveologenic neoplasms. These data indicated that the % of mice affected after 96-99 weeks was 6.8 - 15.7. This information is useful but does not in itself eliminate the possibility that the frequency in the high dose female group (21.6%) was due to chance alone. It should be noted that the two control groups in the study with cypermethrin had 6.6% and 6.7% of the females affected with benign alveologenic neoplasms.

2. Liver: The following table indicates the neoplastic findings in liver in this study with cypermethrin.

Liver Tumors
(Incidences of neoplasms in all observed mice,

	MALES					FEMALES				
Group	n*	Benign	%	Malignant	%	n	Benign	%	Malignant	%
Control - 1	61	11	18.0	11	18.0	61	1	1.6	2	3.
Control - 2	60	13	21.7	9	15.0	60	2	3.3	5	8.
100 ppm	60	10	16.7	16	26.7	61	3	4.9	2	3.
400 ppm	60	10	16.7	12	20.0	60	4	6.7	4	6.
1600 ppm	61	4	6.6	13	21.3	60	1	1.7	2	3.

* Number of mice examined.

The above data do not indicate an oncogenic response to cypermethrin in the diet. Inspection of the nonneoplastic findings in the liver also did not indicate evidence of a test chemical effect.

3. Pituitary: The pituitary in females had many instances of adenomas. There were 69 incidences reported but there was no indication of a dose related effect. There were 23/59, 17/57, 6/59, 11/52, and 12/59 instances for the two control groups, the low, mid and high dose test groups respectively. The incidences among the controls were almost twice that of the dose group female animals.

4. Harderian gland: The Harderian gland developed adenomas in both males (total 31) and females (total 21) but there was no evidence of a test chemical related effect.

5. Thyroid: There were a total of 2 tumors in the thyroid reported. Both incidences were follicular carcinomas and both occurred in high dose group males. TB considers this finding not to be related to ingestion of cypermethrin.

6. Haemopoietic and lymphoreticular systems: Many mice in both the male and female test groups developed malignant lymphoreticular tumors. The distribution of the mice affected was reported to be as follows: 28, 24, 29, 17 and 24 among the male groups and 44, 35, 35, 33 and 35 among the female groups for the control-1, control-2, low, mid and high dose test groups. (60-61 mice per group were available for analysis and it is assumed that all of or nearly all of the mice were evaluated for lymphoreticular tumors). Although many mice in this study developed lymphosarcoma, these was no indication presented in the report which indicated that the lymphosarcoma present was influenced by the presence of the test material. In particular, there was no indication that the mice dosed with cypermethrin developed the lymphosarcomas at an earlier time than did the control mice.

The high rate of lymphoreticular tumors suggests that the mice may have been in poor health, particularly in the later weeks of the study. Although this strain of mice is apparently susceptible to this type of tumor, the frequency which was displayed in this study is much greater than at least one other study submitted by the ICI company with a closely related chemical (see the ICI mouse study with permethrin). It is noteworthy that most but not all of the mice with lung tumors also had malignant lymphoreticular tumors. See also 1.b. under Comments (page 2 of this review).

Conclusion: CORE Classification of this study is RESERVED pending submission and review of the histopathology data from the mice sacrificed at the interim kill. See 1.a under Comments (page 2 of this review).

Sufficient data have thus far been presented to indicate that this study shows an apparent oncogenic effect in that there is noted an increased incidence of benign alveologenic neoplasms in the female mice particularly in the high dose test group.

The NOEL for nononcogenic aspects of this study based on the limited observations made is 400 ppm. At 1600 ppm there is noted an increase in liver weight and generalized changes in the blood.

John D. Doherty, Ph.D.
Toxicology Branch
Hazard Evaluation Division
TS769

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