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UNITED ST . ES ENVIRONMENTAL PROTECTIO GENCY

SUBJECT: Terbacil and its hydroxylated metabolites calculated as terbacil in or on alfalfa (hay and forage), and birdsfoot trefoil (hay and forage) at 5 ppm and in or on milk, meat and meat by-products of cattle, goats, hogs, horses and sheep at 0.1 ppm.

DATE: December 20, 1973

FROM:

TO: Mr. Lee TerBush
Acting Chief
Coordination Branch

821 A

Pesticide Petition No.: 4F1428 - duPont
Wilmington, Delaware

Related Petitions: 6F0510, 7F0549

Tolerances have been established at 0.1 ppm on citrus fruits and pineapple CFR 180.209 for terbacil per se.

TOXICOLOGICAL REVIEW

The toxicity of terbacil (3-tert-butyl-5-chloro-6-methyl uracil) has been defined for the purpose of establishing tolerances in RAC's in connection with PP Nos. 6F0510 and 7F0549 (reviews of 10/5/66 and 1/16/67, O.G. Fitzhugh). Final reports of long term rat and dog feeding studies had been submitted but not evaluated before the tolerances were set. Therefore, since finite residues in milk are proposed, we herewith submit our evaluation of those data and our recommendations will be based in part on them.

Two Year Rat Feeding Study (#125-010)

Methods:

Charles-River CD rats were randomized into two control and three test groups of 36 males and 36 females each and were given 0, 50, 250 or 2500 ppm AI* in the diet for two years. The 2500 ppm group received increments of 500 ppm or 1000 ppm every two weeks until a level of 10,000 ppm was attained in the 46th week.

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* As Herbicide 732; 80% WP.

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Daily observations were made for mortality, appearance, behavior and/or gross signs of toxicity.

Body weight and feed consumption were measured at appropriate intervals, and food efficiency values were calculated.

Clinical observations:

Hematology consisted of total and differential leucocytes, RBC, Hct., Hb., performed on six male and six females per group at 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 months.

Biochemistry examinations consisted of SGOT, SGPT and Alk. P. at similar intervals but on different rats in each group.

Urinalyses were performed at similar intervals and consisted of detection of glucose, albumin, bilirubin, occult blood, Sp. Gr., pH and formed elements.

Pathological examination was done at one year and at termination. The following tissues were prepared and examined histologically (*) organ weights obtained:

Brain*	Peripheral N.	Pituitary*
Adrenal*	Heart*	Spleen*
Thymus*	Salivary gland	Sm. Intestine
Pancreas	Kidney*	Gonads*
Spinal Cord	Eye	Thyroid*
Lung*	Aorta	Lymph Node
Bone Marrow	Stomach	Lge. Intestine
Liver*	Bladder	Prostate or Uterus
	Skel. M.	

Results:

No effects on appearance or behavior were noted at any time during the study.

Body weights of the low and middle dose rats increased normally but those of the high dose group leveled off at about 1 year into the study. Feed consumption and utilization were not affected by terbacil intake.

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A respiratory epizootic occurred in all groups during the study which required tetracycline, penicillin and streptomycin therapy. The problem subsequently abated but did not disappear entirely.

Hematological, urological and biochemical parameters were not appreciably altered during the study.

Mortality from incidental causes, principally pneumonia, was high in all groups, with 100% dying in the 250 ppm female group.

Histopathological examination of representative tissues failed to demonstrate any clear-cut dose-related adverse effects at any level of terbacil administration.

Conclusions:

Few toxic effects, if any, can be attributed to terbacil in the diet of rats for two years. Therefore, we conclude that the NEL is 250 ppm based on retarded weight gain in the high dose group (2500 to 10,000 ppm).

Two Year Dog Feeding Study (#125-011)

Methods:

16 male and 16 female purebred young adult beagles were randomized into four groups, each containing four males and four females. Each group received terbacil at 0, 50, 250 or 2500 - 10,000 ppm in the diet for two years*, following a three week control period.

Food consumption and body weights were measured weekly and periodic detailed physical examinations were done; in addition, daily observations for signs of toxicity or pharmacologic action were made.

12 months into the study, one male and one female from each group were killed and necropsied.

Laboratory tests during the control period and at 1, 2, 3, 6, 12, 18 and 24 months included:

Hematology: total and differential WBC's, Hct., Hb., sed rates and RBC's.

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* As the Formulation, Herbicide 732, 80% WP in terms of AI.

Plasma Biochemistry: glucose, total protein, total albumin, BUN, Alk. P., prothrombin times, SGOT, SGPT and serum Cholesterol, as well as BSP retention times.

Urinalyses: pH, Sp. Gr., bilirubin total N, sediments, formed elements and albumin.

Pathological Examination:

Gross Examination:

At the completion of one year of compound feeding, one male and one female dog from the control and each dietary level were sacrificed by exsanguination while anesthetized with sodium pentobarbital and subjected to necropsy examination. Major organs were weighed and representative tissues were collected into 10 per cent neutral formalin for subsequent histologic processing and microscopic examination. Specimens of brain, liver, kidney, spleen, fat, muscle, testes, blood, urine and feces were collected at necropsy from each dog, frozen and forwarded to the sponsor.

After two years, all remaining dogs were sacrificed and necropsied as described above.

Microscopic Examination:

The following tissues from each control and 2500-10,000 ppm dietary level dog sacrificed after one or two years of compound feeding were paraffin embedded, sectioned, stained with hematoxylin and eosin and examined microscopically:

brain	spleen	liver
spinal cord	lymph node	gall bladder
peripheral nerve	thymus	kidney
pituitary	bone marrow	urinary bladder
thyroid	salivary gland	testis or ovary
parathyroid	stomach	prostate or uterus
adrenal	small intestine	skeletal muscle
lung	large intestine	bone
heart	pancreas	

Specimens of liver and kidneys from the 250 ppm dietary level dogs sacrificed after one year were also prepared and examined.

Results:

No adverse effects on any parameter were noted except that a dose-related increase on thyroid gland/body weight ratios were noted at termination; a marginal increase was seen at 250 ppm and a definite increase in this parameter was seen at the high dose level.

Conclusions:

Terbacil did not induce any overt signs of toxicity in dogs apart from a mild increase in thyroid weights at the 250 ppm levels and above. Accordingly, we conclude that the NEL for this study is 50 ppm terbacil in the diet of dogs for two years.

Three Generation Rat Reproduction Study (125-012)

Methods:

Terbacil was fed through three generations, two litters per generation, to Charles River CD rats at 0, 50 and 250 ppm. 10 males and 20 females per group were used. Rats from the second litter of each generation served as parents for the succeeding generation. All animals were maintained on diet for 100 days prior to mating, then 2 females were placed with one male for three weeks. Three weeks later all litters were weaned and studied. Five days following weaning of the first litter, females were again bred to different males and a second litter was thus produced.

All animals were observed for appearance and behavior and body weight and food consumption was recorded at weekly intervals.

The first litters in each generation were examined grossly and destroyed. Rats from second litters served as parents for the next generation and were placed on diet for 100 days prior to mating, as before.

Special observations on all offspring were made and included: fertility, embryo development, abortion, casting of litters, live births, litter size, viability and pup survival (see Table I).

All pups were examined for possible teratogenic effect.

At weaning, 10 male and 10 female pups from each control and treated group from the F_{3b} litter were sacrificed with chloroform and subjected to necropsy examination. Major organs were weighed and representative tissues from each rat were collected into 10 per cent neutral buffered formalin for subsequent histologic processing and microscopic examination.

Microscopic Examination:

The following tissues from each of 10 male and 10 female rats from the control and each treated group were paraffin embedded, sectioned, stained with hematoxylin and eosin and examined microscopically (*organ weights obtained):

brain*	heart*	pancreas
spinal cord	spleen*	liver*
peripheral nerve	thymus*	kidney*
pituitary*	bone marrow	urinary bladder
thyroid*	stomach	testis or ovary*
adrenal*	small intestine	skeletal muscle
lung*	large intestine	bone

Results:

No abnormalities that could be attributed to treatment were seen in any of the parameters measured. Table I summarizes the Indices of Reproduction. A decrease in the Fertility Index (Pregnancies/matings) was noted for the females bearing the 250 ppm F_{2b} litter. There was a decrease in the rate of body weight gain in the males servicing these dams. Other indices of reproduction were not appreciably altered by treatment.

Histological examination of representative tissues failed to reveal compound-related effects.

Conclusions:

Although there was a significant reduction of Fertility Index in the females casting the F_{2b} litter, this could be attributed to a secondary effect on the servicing males, since these failed to thrive as evidenced by failure of normal body weight gain. Unfortunately, daily vaginal smears of mating females were not taken; thus there is no direct evidence that mating in fact occured, although all the other groups demonstrated satisfactory fertility indices. Therefore, since the aberrent values are confined to the one group, we conclude that they do not represent a compound effect, and we thus find that the NEL for this study is 250 ppm.

TABLE I

INDICES OF REPRODUCTION IN THE RAT

TERBACIL

Litter	Diet ppm	Fertility (a)	Gestation (b)	Viability (c)	Lactation (d)	Litter size
F_{1a}	0	85%	100%	95%	95%	11.8
	50	90%	100%	96%	89%	10.9
	250	90%	100%	92%	87%	13.4
F_{1b}	0	85%	100%	94%	82%	12.5
	50	61%	100%	94%	63%	10.2
	250	80%	100%	93%	71%	12.1
F_{2a}	0	70%	93%	95%	99%	11.9
	50	90%	100%	97%	96%	10.2
	250	60%	100%	92%	99%	10.0
F_{2b}	0	70%	100%	98%	89%	10.1
	50	85%	100%	96%	93%	11.1
	250	45%	100%	89%	91%	11.6
F_{3a}	0	90%	100%	98%	96%	11.1
	50	90%	100%	100%	96%	11.9
	250	90%	100%	97%	95%	11.7
F_{3b}	0	76%	100%	96%	92%	10.4
	50	72%	100%	97%	91%	12.1
	250	100%	95%	93%	84%	12.2

(a) Pregnancies/Matings (b) Litters born/Pregnancies (c) Pups surviving 4 days/pups born
(d) Pups weaned/Pups at 4 days

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Metabolites of Terbacil

A metabolism study of terbacil in the dog revealed that the major metabolites of terbacil are 3-tert-butyl-5-chloro-6-hydroxymethyluracil (compound A) and 6-chloro-2,3-dihydro-7-(hydroxymethyl)-3,3-dimethyl-5H-oxazolo-[3,2a]-pyrimidin-5-one (compound B). Compound A is present in the urine at a concentration of 100-fold that of the parent, while Compound B is present at 10 times that of the parent. These are excreted in the urine and feces and are almost entirely eliminated within 48 hours following single dose ingestion of 2-C^{14}-radiolabeled terbacil.

There is good evidence that 5-chlorouracil is not a metabolite of terbacil in the dog.

Compounds A and B are said to be the principle metabolites in alfalfa (Sec. D, appendix 5, Exhibit 1).

Discussion:

Since the principle metabolites (A and B) in plants are presumably also the principle metabolites in dogs, and since the toxicity of the parent compound reflects also the toxicity of the metabolites, we are not now inclined to ask for more toxicity data on the metabolites (A and B); however, should CB find that these metabolites are present in meat and milk in amounts greater than the proposed 0.1 ppm combined tolerance we would have to reconsider this position.

Toxicity to cattle:

In cow feeding study levels of 2 ppm and 50 ppm caused no adverse effects.

Recommendations:

These findings are in basic agreement with Dr. Fitzhugh's estimate of the toxicity of this compound as detailed in his reviews noted above. Our principle concern is for the tolerance in whole milk at 0.1 ppm combined residues. A 50 ppm NEL based on two year dog systemic toxicity and an NEL for reproductive capacity represents a relatively safe level, and the calculated ADI is 0.75 mg/day (50 ppm/100-fold systemic toxicity safety factor times 1.5 kg), in excess of the 0.15 mg/day to be expected from ingestion of 1.5 kg/day of milk (at 100% of the dietary).

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Chemistry Branch considerations permitting TB therefore recommends that the proposed tolerances for residues of the herbicide, terbacil (3-tert-butyl-5-chloro-6-methyluracil) and its hydroxylated metabolites calculated as terbacil be established:

Alfalfa (hay and forage); birdsfoot trefoil (hay and forage) at 5 ppm and in milk, meat, fat and meat by-products of cattle, goats, hogs, horses and sheep at 0.1 ppm.

David Ritter, 12/21/73

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cc: CB
EEB
Division File
Branch Reading File
PP# 4F1428

R/D Init:CHWilliams:12/20/73
DLRitter:sss:12/21/73
Init:CHWilliams

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Chemical:	**Terbacil**
PC Code:	**012701**
HED File Code	**13000 Tox Reviews**
Memo Date:	**12/20/73**
File ID:	**TX000706**
Accession Number:	**412-03-0116**

HED Records Reference Center
06/30/2003