2-14-73 AR-1057
TXR-851

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ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Date: February 14, 1973

Reply to Attn of:

Subject: Methoprene; Altosid™, isopropyl (E,E)-11-methoxy-3,7,11-trimethyl-2, 4-dodecadienoate, request for temporary negligible residue tolerances

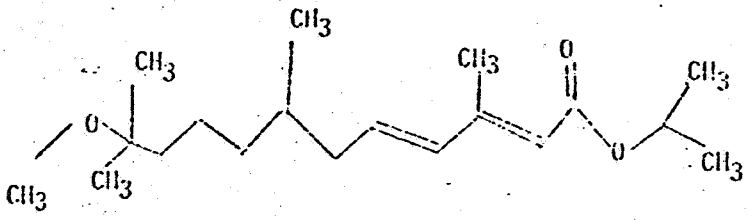
To: of 0.1 ppm in or on forage grasses and forage legumes and of 0.01 ppm in or on rice resulting from use in controlling floodwater mosquitoes.

Mr. Lee E. TerBush, Acting Chief
Coordination Branch
Registration Division

Pesticide Petition No. 3G1343

Zoecon Corporation
Palo Alto, California

Related Petitions
None, new chemical



Methoprene

TOXICOLOGICAL EVALUATION

A. Composition

1. Technical Altosid™

[redacted]

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INFORMATION WHICH MAY REVEAL A PRODUCT MANUFACTURING PROCESS IS NOT INCLUDED

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2. Altosid™ SR-10*

[redacted]

Methoprene (MP) is an insect growth regulator which is effective against several species of economically important mosquitoes especially Diptera (mosquitoes). The rate which is to be applied at 1/20 lb. active ingredient per acre and one application per flooding.

[redacted]

We understand that the use pattern for Altosid™ SR-10 reflects pre-harvest application only.

B. Toxicology

1. Acute toxicity (technical product; 93.7% pure)

	Species	Route	Effect Level	Symptoms
	Rat	PO	LD_{50} >10 Gm/kg	none ✓
	Rat	PO	LD_{50} >10 Gm/kg	none ✓
	Dog	PO	LD_{50} >5 Gm/kg	none ✓
5 < LD50 < 10gm	Dog	PO	LD_{50} >10 Gm/kg	CNS derangement, 3/4 died within 3 hours.
?	Rat	14 day diet	LD_{50} >40,000 ppm	Decreased growth
?	Dog	14 day diet	LD_{50} >20,000 ppm	Inanition; weight loss.
	Rat	21 day diet	0.1, 0.2%, 0.5, 1.0, 5.0 & 10.0%	Died ejection @ 5.0 and 10.0%
	Rabbit	Eye irritation	0.1 ml undiluted	Score of "0"; non-irritating
	Rabbit	Primary dermal	0.5 ml for 24 hours intacted and abraded	Score of "0"; non-irritating
	Rabbit	Acute Dermal	LD_{50} >3.000 Gm/kg	Acanthosis hyperkeratosis eschar formation
	Rabbit	Acute Dermal	LD_{50} >9.0 Gm/kg	Blanching mild erythema desquamation
	Rabbit	21 day Dermal	102 & 400 mg/kg/day in dimethyl phthalate	some local reddening; no systemic effects
	Rat	Acute Inhalation	LD_{50} >210 mg/L air	none
	Guinea Pig	Acute Inhalation	LD_{50} >210 mg/L air	1/10 deaths
	Rat	21 day subacute inhalation	2.0 and 20.0 mg/L air	without effect

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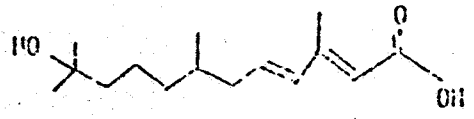
7. Acute toxicity of analogs and metabolites of Altosid[1]

Material	Species	Test	Effect Level	Symptoms
Altosid[1/] [redacted]	Rat	Acute oral	LD_{50} 5 G/kg	Impenetrivity diuresis
ZR 724[2/]	Rat	Acute oral	LD_{50} 6.81 mg/kg	none
ZR 725[3/]	Rat	Acute oral	LD_{50} Male 6.81 Female = 4.87	Salivation convulsions

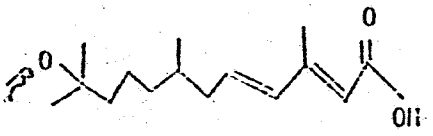
[redacted]

2/



Metabolite of Altosid

3/



metabolite of Altosid

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[redacted]

3. Subacute toxicity

Ninety Day Rat Feeding Study

Methods:

Groups of 15 male and 15 female Sprague-Dawley rats were fed diets containing 0, 250, 500, 1000 or 5000 ppm technical HP for ninety days. Blood samples were collected initially and at 4, 8, and 13 weeks for hematology and clinical and serum chemistry and consisted of measurement of WBC, RBC, Hb, Hct, Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), differentials and lymphocyte patterns. Sera were analyzed for Ca, P_i*, glucose, BUN, uric acid, cholesterol, total protein, albumin, total bilirubin, Alk P., LDH and SGOT.

Urine samples were collected at 13 weeks and examined for color, Sp. Gr., protein, pH, glucose and formed elements.

At termination, all animals were examined for gross lesions. Microscopic examination on ten of each sex in the control and 5000 ppm groups was done on the following tissues (*) weights obtained:

Thyroid	Heart*
Thymus	Lung
Bone marrow	Diaphragm
Liver*	Kidney*
Spleen*	Pancreas
Stomach	Lge. & Small Intestine
Bladder	Gonads*
Lymph nodes	Adrenal Gl.
Brain	Pituitary Gl.
Prostate Gl.	Uterus

Salivary Gl.

In addition, livers and kidneys of the remaining 5000 ppm animals and from the 1000 ppm animals were examined microscopically.

*P_i - Inorganic Phosphorus

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Results:

Body weights, food consumption, hematology, serum chemistry and urinalyses were not adversely affected in the test groups. Mortality was confined to those rats which were inadvertently killed by the blood-collecting procedure.

Male and female liver ratios and male kidney ratios at 5000 ppm were significantly higher than those of the controls, otherwise, organ ratios of other treatment groups were similar to the control values.

Microscopic examination of representative tissues failed to reveal any clear-cut lesions that could be attributed to treatment, although several instances of renal tubular necrosis were noted at the 5000 ppm level.

Conclusions:

MP has a low order of toxicity in rats. A no-effect level for systemic toxicity of MP is 1000 ppm in the diet for three months based on increased organ-body weight ratios and renal pathological changes at 5000 ppm.

Ninety Day Dog Feeding Study

Methods:

Groups of four male and female beagle dogs were fed 0, 250, 500 and 5000 ppm technical MP in the diet for ninety days (13 weeks). Animals were observed daily for appearance, behavior, and response; body weights and food consumption were measured weekly. In addition, the eyes of all dogs were examined initially and at termination by ophthalmoscope. Urine and blood samples were obtained initially and at 4, 8 and 13 weeks. Hematological examination included WBC, RBC, Hb, Hct, MCV, MCH and MCHC. Differential cell counts were also made. Sera were analyzed for Ca, P_i, glucose, BUN, uric acid, cholesterol, total protein, albumin, total bilirubin, Alk P., LDH and SGOT.

Urinalysis included determination of color, Sp. Gr., pH, protein, glucose, occult blood and formed elements.

Animals were necropsied at termination and samples of the following tissues and organs were examined microscopically (*) organ weight obtained:

Thyroid	Heart*
Thymus	Lung
Bone marrow	Diaphragm
Liver*	Kidney*
Spleen*	Pancreas
Stomach	Lge. & Small intestine
Bladder	Gonads*
Lymph nodes	Adrenal Gl.
Brain	Pituitary Gl.
Prostate Gl.	Uterus

Salivary Gl.

Tissue sections were examined only from the animals on the 5000 ppm diet and the control diet. It is TB's policy to require that all tissues from all dogs be examined. Since no macroscopic pathology was noted at any level tested, TB will accept the microscopic pathology presented for a temporary negligible residue tolerance but for a permanent tolerance the rest of the dog tissues should be examined. The data should also be reported more completely as was done with the rat pathology data.

Results:

Activity, appearance, food intake, body weight gain and behavior did not vary appreciably between the groups. Hematological values were within normal limits in all groups. Serum chemistry determinations revealed no untoward effect of feeding the material. Serum enzymes were unremarkable except that Alkaline Phosphatase values were elevated in the 5000 ppm males at 4, 8, and 13 weeks and in the 5000 ppm females at 8 weeks. 250 and 500 ppm animals had no real differences in activity of this enzyme.

Urinalyses and ophthalmic examination failed to reveal any adverse effect of MP in these dogs.

Liver weight ratios for male and female dogs were increased at 5000 ppm but not at lower levels.

No dose-related lesions were demonstrated either grossly or microscopically that could be attributed to treatment.

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A. Rodents

As in the rat, this species seems to have a low order of toxicity to dogs. A no-effect level is judged to be 500 ppm based on the finding of increased liver ratios and increased Alkaline Phosphatase activity in the 5000 ppm males and females.

Rat Teratology Study

Methods:

Charles River Porcite rats were mated on day 0 with pregnancy risk being confirmed by the presence of vaginal sperm. Fifteen, 22 and 21 dams were then gavaged once daily with HP at 0, 500 or 1000 mg/kg HP body weight respectively from day six through day 15 of gestation (ten doses in all). Body weights, appearance and reactions were recorded during the test period.

Rats were killed on day 20 of gestation and numbers and location of pups, implantation sites and resorption sites as well as corpora lutea were noted. All pups were weighed and carefully examined grossly for defects of somatic architecture. Skeletal development was evaluated by the alizarin staining method and visceral examination was made using the razor section technique.

Results:

No adverse effects were noted in maternal behavior, weight gain or appearance, nor were any deaths seen. Gross uterine abnormalities were not seen. No differences were noted in numbers of implantation sites, resorption sites, viable fetuses or corpora lutea.

Adverse effects of HP on fetal body weight and appearance did not occur. One 500 ~~ppm~~ mg/kg fetus had a spiral tail; one 1000 mg/kg fetus had anophthalmia.

Skeletal defects at the 1000 mg/kg level included two incidences of sternal bifurcation; three of cleft sternum and one of asymetric sternum (these abnormalities are not common to this strain of rat according to petitioner). One 500 mg/kg fetus had complete absence of rib and vertebral development.

No other fetal abnormalities that could be attributed to NP administration were seen.

Conclusions:

The occurrence in the 1000 mg/kg group of skeletal defects in several pups is suggestive of a possible teratogenic effect at this compound. 1000 mg/kg is equivalent to 20,000 ppm of NP in the diet and thus constitutes an extremely high level of challenge; one is tempted to say an excessive level.

These defects did not occur at the lower or control levels; the evidence therefore suggests that NP is a very weak teratogen at very high levels in the rat, but a no-effect level for teratogenic effect can reasonably be set at 500 mg/kg, equivalent to 10,000 ppm in the diet.

Hormonal activity [illegible]

Methods:

1. Estrogenic activity was assayed by the immature mouse uterine weight method. Intact immature female mice received NP in sesame oil subcutaneously daily for three days at doses of 0.5 or 5 micrograms/mouse. The mice were killed on the fourth day and the uterine weights were obtained.

A group of mice received esterone as a positive control.

Results:

No estrogenic effect was demonstrated for NP, but estrone showed typically estrogenic response, characterized by increased uterine weights.

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2. Androgenic/anabolic activity was assayed in immature castrate male rats. Following treatment with either 0.2 or 2 milligrams/rat daily for seven days, the rats were killed and ventral prostate, seminal vesicle and levator ani muscle were weighed. A group of rats received testosterone as a positive control.

Results:

No increase in weight of the tissues occurred following treatment with HP; rats receiving testosterone showed increases in all tissue weights.

3. Corticoid activity was assayed in immature adrenalectomized male rats. The animals received 6 daily subcutaneous injections of either 0.4 or 4 milligrams per rat. A group of rats received hydrocortisone as a positive control. At termination the animals were killed and the thymus gland was weighed.

Results:

No reduction was seen in the thymus weights of HP-treated animals; those animals receiving hydrocortisone showed greatly reduced thymus weights.

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[illegible]:

[illegible] In [illegible] activity of [illegible] is demonstrated by [illegible].

[illegible] Studies in [illegible]

This [illegible] has not been [illegible] and will be [illegible] with the [illegible] period.

[illegible] Exposure to [illegible]

Petitioner reports twelve instances of workers accidentally exposed to [illegible]. No instances of reaction or other adverse effects were noted.

[illegible] Studies

1. Distribution and elimination of tritiated [illegible] in mice, determination by [illegible].

Methods:

Tritiated [illegible] was administered intragastrically to males, virgin female and pregnant mice. Urine and feces were collected and the amount of activity was determined by liquid scintillation. Individual animals were sacrificed at intervals up to 96 hours, frozen and sectioned and thin whole-body slices were opposed to nuclear emulsion plates for twenty days.

Results:

82 percent or virtually all of the activity had been recovered by 96 hours with 63 percent appearing in the urine and the remainder in the feces. Most, if not all the activity had appeared in the body wastes by 24 hours.

The autoradiographic analysis confirmed the previous study for twenty-four hour elimination; distribution of activity within the body was confined to the alimentary canal, liver and the kidneys.

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B. Toxicological Data

Toxicology data from various investigations support that the principle [illegible] of HP in metabolism systems is the O-demethylation product, [illegible].

C. Conclusions and Recommendations

Dithiopyr appears to have a low order of toxicity to mammals. It does not appear to be teratogenic at reasonable levels. It does not have hormonal properties in mammals.

The most sensitive no-effect level is 500 ppm based on systemic effects in the ninety day dog feeding study.

We therefore find that the requested temporary negligible residues tolerance of 0.1 ppm in or on forage grasses and forage legumes and of 0.01 ppm in or on rice is safe and will protect the public health.

Before a permanent tolerance is granted the metabolism studies on all the dogs from the 90-day feeding study should be examined and complete microscopic pathology report made.

David L. Ritter 2/15/73

David L. Ritter, Pharmacologist
Toxicology Branch
Registration Division

cc: Chemistry Branch
Ecological Effects Branch
Division Reading File
Branch Reading File
PP# 3G1343

R/D Init:JRWilliams
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