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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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SUBJECT: PP# 6G1705/6H5111 - Bay NTN 9306, proposal for residue tolerances for the insecticide O-ethyl O-[4-(methylthio) phenyl]S-propyl phosphorodithioate and its cholinesterase-inhibiting metabolites in cottonseed at 0.5 ppm and in cottonseed oil and cottonseed hulls at 1 ppm.

DATE: 6 JAN 1976

File Symbol 3125-EUP

Chemagro/Baychem Chemical Co.
Kansas City, Mo.

Related Petitions
None

FROM: Toxicology Branch

TO: Libby Zink, SRS/RD
and Chemistry Branch

Toxicological Review

Summary of Acute Toxicity

1. Technical Material

Species	Test	Result	Signs of Toxicity
Rat	Acute Oral LD_{50}	Male - 304 mg/kg Female - 176 mg/kg	"Signs of ChE Inhibition"
Mouse	Acute Oral LD_{50}	Male -1831 mg/kg Female -1617 mg/kg	
Rat	Acute IP LD_{50}	Male - 355 mg/kg Female - 225 mg/kg	
Rat	Acute Dermal LD_{50}	> 1 ml/kg	
Rat	Acute Inhalation LC_{50}	> 0.661 mg/L	
Mouse	Acute Inhalation LD_{50}	> 0.490 mg/L	
Hamster	Acute Inhalation LD_{50}	> 0.490 mg/L	
Rabbit	Dermal Irritation	Single application score 0.6/8.0; not a dermal irritant	
Rabbit	Eye Irritation	5 min. exposure - no irritation at 7 days 24 hr. exposure - do	

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1. Technical Material (cont'd)

Species	Test	Result	Signs of Toxicity
Rat	4 week Oral	NEL of 0.1 mg/kg based on RBC & Brain ChE depression.	
Rat	3 week Inhalation	NEL = 14 ug/L	
Rat	Inhalation LC_{50}	> 3,840 ug/L @ 60 min. exposure > 4,130 ug/L @ 240 min. exposure	
Rat	Oral LD_{50}	Male - 107 mg/kg Female - 65 mg/kg	Tremors, lacrimarion salivation, diarrhea
Rat	Oral LD_{50}	Female - 88 mg/kg Male - 178 mg/kg	do
Rabbit	Acute Dermal LD_{50}	Female - 994 mg/kg Male - 820 mg/kg	
		Ataxia, tremors, diarrhea; rupture of the pyloric duodenum with GI lesions.	

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2. Formulation

Rat	Oral LD_{50}	Male - 154 mg/kg Female - 90 mg/kg	GI Irritation
Rat	Inhalation LC_{50}	Male -2050 ug/L Female -2050 ug/L	None
Rabbit	Dermal LD_{50}	Male - 745 mg/kg Female - 874 mg/kg	
Rabbit	21 day Dermal Irritation	Mild erythema and edema, control & treated (100 mg formulation) Depressed RBC, Plasma & Brain ChE Activity - not a dermal irritant but do get systemic toxicity.	
Rabbit	single dose Dermal Irritation	Score of 0.0/8.0 not a dermal irritant (intact or abraded).	
Rabbit	Eye Irritation	5 min. and 24 hr. exposure produced conjunctival ulceration @ seven days - Corrosive to the eyes.	

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Summary of Subacute Toxicity

1. Male mouse dominant lethal study for mutagenic effects (Bayer AG # 5627).

   Male mice were dosed once with 200 mg/kg PO of NTN 9306 or with inert vehicle only. Matings at weekly intervals for eight weeks thereafter with untreated females were permitted. These females were isolated and the uterine contents were examined for pre- and post-implantation losses and dead and living implantations.

   There were no statistically significant differences between treated and control groups.

2. Rabbit teratology study (Bayer AG # 5590).

Methods

   Mated does were gavaged orally with 0, 3, 10 or 30 mg/kg NTN 9306 during days 6 through 18 of gestation. Fetuses were examined grossly for external abnormalities, sex, weight and number of stunted fetuses. Viscera were examined for amalies and skeletons were cleared and stained for bone definition.

Results

   No visceral or somatic abnormalities attributable to treatment were seen. Signs of toxicity in the does were those of strong ChE inhibition; i.e., drowsiness, salivation, lethargy, anorexia and diarrhea were noted in the 10 and 30 mg/kg does with 5 of the 13 30 mg/kg does dying between days 15 and 20.

Conclusions

   NTN 9306 is not a teratogen in rabbits at doses up to 30 mg/kg given orally from days 6 through 18 of gestation.

3. Rat ninety day feeding study (Chemagro # 45389).

Methods

   Groups of 20 males and 20 females each were offered diets containing 0, 10, 30, 100 or 300 ppm NTN 9306 for ninety days. Feed consumption and weight gains were recorded weekly and observations of behavior and mortality were made daily.

Blood and urine samples were obtained on days 28, 63, and 84 and were examined for RBC and plasma ChE inhibition, RBC's, platelets, WBC's, Hct., and Hb.; Alk PT, BUN, SGPT and SGOT activities. Ten male and ten females were fasted and fasting blood sugars were determined. Urinalysis included examinations for pH, protein, glucose, ketones, bilirubin, blood, urobilinogen and Sp. Gr. Pathological examinations.

Gross autopsies were conducted on animals which died during the experiment and on the survivors after 90 days of feeding. At the end of feeding the survivors were narcotized with chloroform and sacrificed by exsanguination. The heart, liver, lungs, adrenals, kidneys, thyroid, brain, spleen and gonads were weighed at the time of sacrifice.

The following tissues were fixed in 10% buffered formalin for histopathological examinations: lung, heart, liver, pancreas, lymph node, cervical and mesenteric, stomach (cardia, fundus and pylosus), small intestine (duodenum, jejunem and ileum), colon, caecum, kidney, adrenal gland, urinary bladder, testes, ovaries, prostate, thyroid, subcarillary salivary, parathyroid, uterus, brain (cerebrum, cerebellum and pons), femur (bone marrow and bone), nerve (optic, sciatic), eye, aorta, esophagus, spinal cord, trachea and sternum. Tissues from all animals in the control and 300 ppm level were examined histologically while tissues from ten animals of each sex were examined for levels of 10, 30 and 100 ppm.

Results

Animals in the 0, 10, 30 and 100 ppm groups showed no differences in feed consumption, body weights or appearance and behavior. 300 ppm females consumed more food but gained less weight than the other females. These females were hyperactive and had pellage that was in poor condition. 300 ppm males were unaffected.

Clinical chemistry - apart from a modest decrease in Hb in the 300 ppm females no differences were noted between control and test animals at any dose level tested.

Histopathology - No changes attributable to treatment were noted at any level tested.

ChE inhibition - Plasma - NEL of 10 ppm
RBC - NEL of 30 ppm
Brain - NEL of 30 ppm.

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Conclusions

Cholinesterase inhibition is the principle mode of action of this material in mammals, and other signs of toxicity are secondary to it.

We therefore conclude that the ChE NEL is 30 ppm based on RBC and Brain inhibition.

4. Ninety Day Dog Feeding Study (Chemagro # 45392).

Methods

Groups of four female and four male beagle dogs each were offered diets containing 0, 10, 20 or 200 ppm NTN 9306 for ninety days. Feed consumption, behavior and appearance were noted daily while body weights were recorded weekly.

Blood and urine samples were obtained at -7, 29, 56 and 90 days and plasma and RBC ChE inhibition, rbcs, WBCs, differential counts, platelets, Hct., and Hb. were determined. AlkP., BUN, SGPT and SGOT were also determined. Urinalysis included tests for pH, protein, glucose, ketones, blood, urobilinogen and Sp. Gr. Brain ChE was determined at termination.

Histopathology

A gross autopsy was conducted on the one animal which died during the experiment and on the survivors after 90 days of feeding. At the end of feeding, the survivors were anesthetized with phenobarbital sodium and sacrificed by exsanguination. The heart, liver, lungs, adrenals, kidneys, thyroid, brain, spleen, pituitary and gonads were weighed at the time of sacrifice.

The following tissues were fixed in 10% buffered formalin for histopathological examinations: lung, heart, liver, pancreas, lymph node, cervical and mesenteric, stomach (cardia, fundus and pylosus), small intestine (duodenum, jejunem and ileum), colon, caecum, kidney, adrenal gland, urinary bladder, testes, ovaries, prostate, thyroid, submarillary salivary, parathyroid, uterus, brain (cerebrum, cerebellum and pons), femur (bone marrow and bone), nerve (optic, sciatic), eye, aorta, esophagus, spinal cord, trachea and sternum. Tissues from all animals in the control and treatment groups were examined histologically.

Results

One 200 ppm male died on day 84 of the test; cause was not attributed to treatment. Male and female dogs receiving 10 and 20 ppm NTN 9306 were not affected in any way by treatment. 200 ppm males exhibited some degree of anorexia which was reflected in their failure to gain weight. 200 ppm females had less weight gain but did not have reduced food intake.

200 ppm animals suffered from diarrhea and regurgitation; females in this group had some hind legg paralysis during the final two weeks of the study.

There were no compound-related differences of importance in blood chemistry or in urinalysis between treated and control groups.

ChE Inhibition

Plasma - NEL of 10 ppm
RBC - NEL of 20 ppm
Brain - NEL of 20 ppm

Histopathologic lesions attributable were said to be minor and were confined to the 200 ppm groups.

Conclusions:

On the bases of these findings, we conclude that toxic NEL based on RBC and Brain ChE inhibition is 20 ppm.

Overall Conclusions

Cholinesterase inhibition is the principle mode of action of this phosphorodithioate insecticide in mammals. We can reasonably conclude that its action is cummulative from the fact that there was hind limb paralysis in the final two weeks in the high-dose female dogs, and longer periods of exposure should show similar effects at lower doses. Since tolerances of 1 ppm are proposed in cottonseed oil, a human food item and 0.5 ppm in cottonseed per se also a potential human food item (see CB memo of telecon with FDA, J. G. Cummings, 11/12/75), we will need additional toxicity data:

For the permanent tolerances;

Two year feeding studies in rats and mice; Two Carcinogenicity studies in mice and rats (may be combined with the above);

A multi-generation rat reproduction study

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For the temporary tolerances ~~will~~ we will need either interim reports as to the progress of the above studies or assurances that such studies are in fact being done.

We defer to CB the question of potential residues in meat, eggs, poultry and milk.

Comments on the Label:

The signal word must be upgraded to "Danger" due to the findings of corrosive eye damage using the formulation. The precautionary paragraph must be brought into conformance with Enclosure S76-A.

David L Ritter 1/5/76

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Toxicology Branch
Registration Division (WH-567)

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