(2-6-73) DB-843
ANT TXR-1510

a.

Dr Eaton

001510

ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D. C. 20460

Date: February 6, 1973

Reply to Attn of:

Subject: Request for temporary negligible residue tolerances for the insecticide S-(tert-butylthio)methyl 0,0-diethyl phosphorodithioate and its cholinesterase inhibiting metabolites in or on corn grain, forage and fodder at 0.05 ppm.

TO: Mr. Lee TerBush, Acting Chief
Coordination Branch
Registration Division

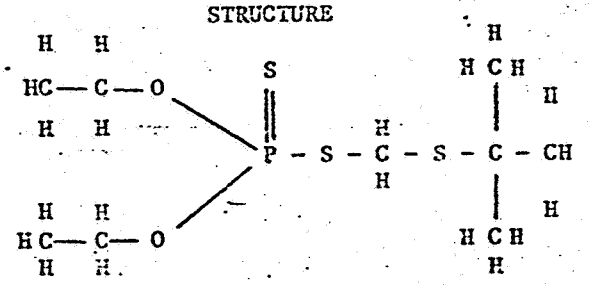
Pesticide Petition No. 3G1340

American Cyanamid Co.
Princeton, New Jersey 08540

Related Petitions: None, new chemical

Synonyms: ST-100, AC 92100, ENT-27920

STRUCTURE



S-(tert - butylthio)methyl 0,0-diethyl phosphorodithioate

FORMULATION

I. AC 92100 Technical	% w/w Typical
S-(tert-butylthio)methyl 0,0-diethyl phosphorodithioate	86.5
[redacted]	

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1/4

INFORMATION WHICH MAY REVEAL THE IDENTITY OF AN INERT INGREDIENT IS NOT INCLUDED

INFORMATION WHICH MAY REVEAL THE IDENTITY OF A PRODUCT IMPURITY OR INERT INGREDIENT IS NOT INCLUDED

[redacted]

II. AC 92100 15G

Active Ingredients	% w/w
AC 92100 Technical	18.5(16.0 real)

Inert Ingredients

[redacted]

USE

Under the proposed experimental program 42,868 pounds of ST-100 15G Soil Insecticide will be applied to 6596 acres of corn during the 1973 growing season in a 7-inch band at planting time at the rate of 6-8 ounces/1000 feet of row to control the larvae of the Northern, Western, and Southern corn root worm.

TOXICOLOGICAL EVALUATION

I. Acute Toxicity

A. Single Oral Dose

Species	Strain	Sex	Formulation	Vehicle	LD_{50}(mg/kg)
Rat	RH Wistar	M	Technical	Corn oil	4.5(2.6-7.7)
		F	"	"	9.0(5.2-15.3)
(fasted)		M	"	"	1.6(1.2-1.9)
(fasted)		F	" (96.7%)	"	1.3±0.2
(fasted)		M	15G	"	11.7(9.0-15.3)
Mouse	CF1	M	Technical	"	3.5(1.9-6.6)
		F	"	"	9.2(6.0-14.0)
		F	"	"	5.0(4.0-6.3)
Dog	Beagle	M	"	Gelatin capsule	4.5(2.2-9.0)
		F	"	"	6.3

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All affected animals exhibited signs of cholinesterase inhibition.

B. Single Dermal Dose

The material was held under an impervious cuff in continuous 24-hour contact with the shaved skin of male albino rabbits. Signs of inhibition of cholinesterase were observed.

Technical	LD_{50} 1.1(0.82-1.4) mg/kg
Technical	LD_{50} 1.0(0.67-1.3) mg/kg
15G	LD_{50} 10.2(7.7-13.4) mg/kg

C. Single Inhalation

RH Wistar albino rats were exposed to air saturated (1.99 mg/L) with vapor of AC 92100 Technical for 7 hours at 25° C. Transient irritation and discomfort were observed. The LC_{50} is in excess of 1.99 mg/L.

D. Skin Irritation

0.5 ml of either the Technical product or AC 92100 15G was held in continuous 24-hour contact with the shaved intact or abraded skin of rabbits. All rabbits died within 24 hours after dosing. Prior to their death, the rabbits showed signs of inhibition of cholinesterase.

E. Eye Irritation

0.1 ml of either the Technical product or AC 92100 15G was placed in the conjunctival sac of each of 6 rabbits/test. All rabbits died within 2-24 hours of administration of the Technical product and exhibited signs of cholinesterase inhibition prior to death. The rabbits receiving 0.1 ml of AC 92100 15G in the eye died within 72 hours.

II. Six Month Feeding Study in Dogs on 92100 (FDRL; 1193)

A. Procedure

Four beagle dogs/sex/dosage level were fed diets containing 0, 2.5, 10, or 40 mcg/kg BW of AC 92100 for 6 months (6 days/week). The dogs were observed daily for drug effects. Body weights and food consumption were recorded pretest and weekly thereafter. Physical examinations were conducted pretest and weekly thereafter. Ophthalmoscopic examinations were conducted pretest and after 3 and 6 months of treatment.

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Hematological, biochemical, and urinary examinations were performed twice prior to initiation of the study and after 1, 3, and 6 months of treatment.

Hematology - hemoglobin, hematocrit, RBC, total and differential WBC, coagulation time, sedimentation rate, prothrombin time, and reticulocyte count.

Clinical Chemistry - SAP, BUN, fasting blood sugar, SGPT, SGOT, serum ChE, RBC ChE, and brain ChE (6 months) (pH/hr.).

Urinalysis - appearance, specific gravity, occult blood, protein, pH, bilirubin, ketone, and glucose.

At the conclusion of the study period, all animals were examined at gross necropsy. The weights of liver, kidneys, heart, adrenals, and gonads (with adnexa) were recorded. The following tissues were examined histologically (H&E stain):

adrenals	kidney	stomach
aorta	lens	pyloric
bone (rib junction)	liver	fundus
bone marrow	lungs	thymus
brain	lumph node (mesenteric)	thyroid
cholecyst	mammary glands	urocyst
epididymis	nerve (with muscle)	uterus
eye	pancreas	all gross lesions
gonad	pituitary	
heart (with coronary vessels)	prostate	
intestine	salivary gland	
colon	skeletal muscle (with nerve)	
duodenum	spinal cord	
ileum	spleen	
jejunum		

B. Results

One control male died of a pulmonary infection. No overt signs of intoxication were observed. No ocular lesions were observed during the ophthalmoscopic examinations. The plasma ChE was significantly ($p = 0.01$) depressed for both the 10 and 40 mcg/kg groups. The RBC and brain ChE was not significantly depressed. The remaining results

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from this study will be submitted when the histopathology is completed and complete tabulations of all pre-mortem findings become available.

C. Conclusion

The data submitted is inadequate for the determination of a systemic NEL. ChE NEL are not determined on the basis of plasma ChE inhibition. Based upon RBC ChE inhibition, the NEL $\geq$ 40 mcg/kg.

III. A 3 and 24 Month Oral Toxicity and Carcinogenicity Study of AC 92100 in Rats - Three Month Report (Bio/dynamics Inc.; 71 R-725)

A. Procedure

Groups of 60 Long-Evans rats/sex were fed diets containing 0, 0, 0.25, 1.0, and 2.0 mg of AC 92100 Technical/kg of diet (ppm) for the duration of the study. The high dosage group was increased to 4.0 ppm on day 35 and to 8.0 ppm on day 77.

The rats were observed daily for physical appearance, signs of local or systemic toxicity, pharmacologic effects, or mortality. Body weights and food consumption were recorded weekly.

Three rats/sex/control group and 6 rats/sex/dose group were tested at 3 months (at sacrifice) for:

Hematology - clotting time, hemoglobin, hematocrit, RBC, total and differential WBC, and RBC morphology.

Clinical Chemistry - SGPT, SAP, fasting blood sugar, BUN, and ChE (whole blood (also measured at 1 month), plasma, and brain), (Δ pH/hr).

Urinalysis - gross appearance, protein, glucose, pH, refractive index, ketones, bilirubin, and occult blood.

Five rats/sex/control group and 10 rats/sex/dose group were killed after three months and examined histopathologically. The following tissues were examiued with H&E stain:

adrenals
aorta
bone marrow (sternum)
brain (2 sections)
gonad
mammary gland
pancreas
pituitary
salivary gland
prostate

(continued)

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heart (with coronary vessels)*°	spinal cord (thoracic)
eye (with optic nerve)	skin
intestine	spleen
colon	stomach (fundic, pyloric)
duodenum	thyroid*
ileum	urinary bladder (with neck)
kidneys*°	uterus
liver*°	skeletal muscle with nerve
lung°	any unusual tissue or lesion
lymph node (mesenteric)	

*organs weighed
°organs examined in 0.25 and 1.0 mg/kg groups

B. Results

Compound induced signs were observed in all high-dose females during the period of feeding a diet containing 8.0 ppm of AC 92100. Muscle tremors, hypernea, tachycardia, hyperactivity, and excessive salivation were attributed to the cholinomimetic properties of AC 92100. Over half the rats in this group exhibited exophthalmos and decreased lacrimation and the formation of a cloudy film over the eye were sequelae. One mid-dose female exhibited exophthalmos, followed by the formation of a cloudy film over the eye, and subsequent rupture of the eye.

Statistically significant ($p < 0.01$) depressions in mean body weights were observed in the high-dose males at weeks 5 and 13 and these females at week 13. The actual mean food consumption in the high-dose males and females at week 13 were statistically significantly less ($p < 0.01$).

A statistically significant increase ($p < 0.05$) in total WBC was observed in high-dose females which was not considered to be dose related. The blood sugar level of the high-dose females was significantly lower ($p < 0.01$) while the BUN was significantly elevated ($p < 0.01$). The SAP of high-dose males was statistically significantly depressed ($p < 0.05$). With the exceptions of the plasma and 1 month RBC ChE activity in the low-dose males, and the plasma ChE activity in the mid-dose males, a depression of all ChE measurements at both one and 3 month determinations was observed in all groups treated with AC 92100. The RBC ChE was statistically significantly depressed in the 1.0 mg/kg F and the high-dose males at $p < 0.05$ and the high-dose females at $p < 0.01$. The brain ChE of the high-dose rats was significantly depressed at $p < 0.01$ while the plasma ChE was depressed for the high-dose females ($p < 0.01$). Urinalysis values, for all parameters were comparable for both sexes.

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The actual mean thyroid weight and the mean thyroid/body weight ratio was depressed in the high-dose males. When the high-dose females were compared to the combined controls, a depression of the mean terminal body weight and an elevation of the organ/body weight ratios in the liver, kidney, and heart were observed. The macroscopic lesions and histologic changes are considered to represent spontaneous changes which were unrelated to the administration of AC 92100.

C. Conclusions

A systemic no-effect level can not be described for this study. Treatment-related changes were confined to the high-dose groups after this group was fed AC 92100 at 8.0 ppm for 2 weeks. The rats were fed AC 92100 at 2.0 ppm for 5 weeks and at 4.0 ppm for 7 weeks. If the rats had been fed at these levels for the entire 91 day period, perhaps effects would have been produced at these levels. Based upon RBC ChE inhibition by the 1.0 ppm females, the NEL for AC 92100 is 0.25 ppm.

IV. Status Report - A Three and Twenty-Four Month Oral Toxicity and Carcinogenicity Study of AC 92100 in Rats (Bio/dynamics Inc.; 71R - 725)

A. Procedure

The protocol for this study has been given in the preceeding study. This is an extension of this same format to 24 months at a 6 month interim period. The dosage of the high level group was reduced from 8.0 ppm to 4.0 ppm at day 105.

B. Results

Nine animals (3 males, 6 females) have died in the 4.0 ppm group compared to 2 rats in all other groups (both control males). The histopathologic evaluation is not yet completed on these animals. The body weights of both male and female 4.0 ppm rats is statistically significantly depressed ($p < 0.01$).

The occurrence of symptoms noted in the high-dose females (muscle tremors, hypernea, tachycardia, hyperactivity, exophthalmos, excessive salivation) in the 13th week have continued to be observed with the same frequency through the 32nd week of test. In addition, the occurrence of these symptoms was observed, although not as frequently, in the low and mid-dose female groups as early as the 14th week of test. Muscle tremors observed in 2 high-dose males beginning at the 29th week of test were the only significant observations noted in the male groups.

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With the exception of exophthalmos, which was observed in all female treatment groups, all of the symptoms observed were attributed directly to the cholinomimetic properties of AC92100.

The RBC ChE of the 1.0 and 4.0 ppm male rats was significantly depressed at $p < 0.01$ while the low-dose male rats were depressed at $p < 0.05$. The RBC and plasma ChE of the 4.0 ppm females were significantly depressed at $p<0.01$.

C. Conclusion

This interim report indicates that the systemic and ChE NEL may be less than 0.25 ppm in rats.

V. Status Report - An 18-Month Carcinogenicity Study of AC 92100 in Mice (Bio/dynamics Inc.; 71R-723)

A. Procedure

Charles River CD-1 COBS (ICR derived) mice were divided into 5 groups composed of 75 mice/sex and fed diets containing 0(2 groups), 0.5, 2.0, or 8.0 ppm of AC 92100 for 18 months.

The test has been conducted for 6 months. The mice have been observed daily for pharmacologic and toxicologic effects. Body weights of 5 animals/group were recorded weekly through the first 3 months and bi-weekly for the next 3 months. Gross necropsies were performed on all animals found dead and on moribund mice.

B. Results

The pattern and frequency of deaths and macroscopic examinations did not suggest any correlation between treatment and findings. At week 36 the males in the 8.0 ppm group were exhibiting a slight decrease in body weight. Exophthalmos, adjudged to be a compound-induced effect, was observed in one mid-dose and 4 high-dose males. This observation was first seen at week 22. In a few male mice in both mid and high-dose groups rupturing of the eyes, usually but not always following exophthalmos occurred.

C. Conclusions

No evidence of carcinogenicity has been presented yet but systemic toxicity is evident in the 8.0 ppm males and probably in the 2.0 ppm males also.

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VI. Teratogenicity Study with AC 92100 Technical in Albino Rats (Ind Bio-Test Lab., Inc.; B1374-(B))

A. Procedure

Groups of approximately 20 bred Charles River albino female rats were orally intubated with 0, 0.075, or 0.150 mg/kg/day of AC 92100 Technical in corn oil from the 6th day of gestation through the 15th day. Daily records of mortality and reactions were maintained throughout the investigation. Mean group body weights were recorded at day 6 (initial dosing), 9, 12, 15 (final dosing), and 20 (at sacrifice).

At sacrifice, the fetal swellings, implantation sites, resorption sites, corpora lutea and viable fetuses were recorded. All viable fetuses were weighed.

An external examination of the fetuses was conducted and all fetuses were examined for either skeletal development (Hurley's method of Alizarin staining) or internal development (free-hand razor blade section technique of Wilson and Warkamy).

B. Results

There were no treatment-related effects among either the maternal rats or the fetuses by any of the criteria evaluated.

C. Conclusion

AC 92100 Technical produced no teratogenic effects in rats at doses as high as 0.150 mg/kg/day.

VII. A Neurotoxicity Study of AC 92100, An Organic Phosphate Cholinesterase Inhibitor, in Hens (Bio/dynamics Inc.; 72S-788)

A. Procedure

Sex-linked hens, approximately 1 year of age, were orally intubated and tested for AC 92100 neurotoxicity by the following protocol:

Group	Compound	Volume (mg/kg)	Concentration (mg/ml)	Volume (ml/kg)	No. animals
I	Corn oil	-	-	1	4
II	AC 92100	40.0	40.0	1	10
III	TOCP	500	1162	0.43	10

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All birds were observed daily for signs of toxic or pharmacologic effects or mortality. Birds were weighed at time of dosing. The birds were observed for their ability to maintain a position on a perch, stand on solid floor, and walk on 1, 3, 7, 11, 14, 17, and 21 days after drug administration.

All hens demonstrating signs of neurotoxicity 21 days after dosing were scheduled for sacrifice on day 22. However, all surviving hens (inadvertently including those demonstrating neurotoxicity) were again dosed and observed for 21 days. The hens receiving a second dose of AC 92100 were pretreated with Atropine (10 mg/kg) in 10-15 minutes prior to treatment with AC 92100.

All hens showing signs of neurotoxicity which were surviving 29 days after the first dosing were sacrificed at that time. Those hens not demonstrating neurotoxicity before the second dose were sacrificed 21 days after the second dose. The spinal cord (lumbosacral section) of the controls and hens showing neurotoxicity were examined histologically (H&E stain).

B. Results

Four of 10 hens treated with AC 92100 in a dose of 40 mg/kg exhibited signs of locomotor toxicity characterized by a slightly wobbly stance or gait, in all cases except one, if the animal survived the first 24 hours, these signs were no longer evident after 72 hours, and the animal remained normal for the remainder of the study.

All hens treated with TOCP behaved normally for at least 7 days. On day 11, signs of locomotor impairment, characterized by a wobbling gait or shaky stance, were noted in all TOCP-treated hens. In 6 cases locomotor ability deteriorated further to a point where the hens walked in a squatted position or were unable to walk or stand by day 21. In no case did any hen treated with TOCP recover from these symptoms.

[illegible] treated with a second dose of AC 92100 exhibited signs of acute toxicity during the first 72 hours after treatment. In all cases the hens had completely recovered by day 7 and remained healthy for the remainder of the study.

TOCP induced minimal to moderate demyelination of the fiber tracts of the spinal cords of all hens. Similar changes were not detected in spinal cords from negative control birds.

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C. Conclusion

AC 92100 is not neurotoxic for hens at 40 mg/kg.

VIII. Status Report - A Three Generation Reproduction Study of AC 92100 in Rats (Bio/dynamics Inc.; 71R - 727)

A. Procedure

Groups of 10 male and 20 female Long-Evans, Blue Spruce weanling rats (designated F_0 parents) will be placed on diets containing 0, 0.25, or 1.0 ppm of AC 92100 for 60 days. With dosing by diet continuing, cohabitation in units of one male and 2 females for the purpose of mating will be done. One litter/generation will be adequate provided it is judged normal based on the indices of fertility, gestation, survival, and viability. The progenies of the first mating of each generation will provide the parents (10 males, 20 females/group) designated F_1 for the next generation. All litters will be reduced to 10 animals (5 males and 5 females, if possible) at 4 days. The procedure will be repeated for 3 generations resulting in F_{1a}, F_{2a}, and F_{3a} progenies. All young of F_{3a} progenies will get gross necropsy at weaning. The study is presently at the growth period (post-weaning) of the F_{1a} generation.

B. Results

Treatment with AC 92100 has not resulted in any significant difference in the fertility, gestation, survival or viability indices. The body weights of the treatment group progeny are greater than those of the control group.

C. Conclusions

AC 92100 has exerted no adverse effect upon the reproductive capacity of the rats to date at 0.25 or at 1.0 ppm in the diet.

IX. Acute Toxicity of Phosphorous - Containing Metabolites of AC 92100 (Cyanamid; A-72 - 34 through 38)

Groups of 5 female albino CF1 mice were dosed with a corn oil dispersion of each product at a constant volume of 0.5 ml/mouse. Signs of intoxication included mainly tremors with prostration, lethargy, and ataxia occasionally noted.

[illegible]

Esters of Phosphorothioic acid	LD_{50} (mg/kg)
S-(tert. butylthio)methyl 0,0-diethyl ester	2.2
S-(tert. butylsulfinyl)methyl 0,0-diethyl ester	1.1
S (tert. butylsulfonyl)methyl 0,0-diethyl ester	3.4(2.2 - 5.2)
Esters of Phosphorodithioic acid	
S-(tert.-butylsulfinyl)methyl 0,0-diethyl ester	3.4(2.4 - 4.7)
S-(tert.-butylsulfonyl)methyl 0,0-diethyl ester	14.0(8.8 - 22)

X. Acute Toxicity of Non-Phosphorous- Containing Metabolites of AC 92100 (Cyanamid; A-72-92 through 94)

Groups of 10 female CF1 albino mice were dosed with a corn oil dispersion of each product at a constant volume of 1.0 ml/mouse. The main sign of intoxication was diuresis with prostration, convulsions, or dyspnea.

	LD_{50} mg/kg
(Tert.-butylsulfonyl)methyl methyl sulfoxide	> 5000
Tert.-butyl(methylsulfonyl)methyl sulfoxide	3450
Tert.-butyl(methylsulfonyl)methyl sulfone	4660(3560 - 6090)

XI. 8-Day Dietary LC_{50} Study with AC 92100, 96.7% Pure, in Ringneck Pheasants (Ind. Bio-Test Lab., Inc.; J 1778)

Groups of 10 ringneck pheasants (10 - 15 days old) were fed diets containing 0 ppm of test material (5 groups); 14.7, 31.6, 46.4, 68.1, or 100 ppm of Dieldrin (positive controls); or 31.6, 68.1, 147, 215, or 316 ppm of AC 92100 for the first 5 days of the 8-day dietary study period.

No abnormal behavioral reactions or other signs of toxicity were observed in birds fed AC 92100 at levels of 316 ppm or lower. Postmortem examination revealed no specific organ damage due to the compound. Signs of toxicity in birds fed Dieldrin included dyspnea, hyporeactivity, and anorexia. Postmortem examination revealed no gross tissue alterations. Autopsy of test survivors at 8 days revealed no adverse gross pathology.

AC 92100	LC_{50} 145(96 - 219) ppm
Dieldrin	LC_{50} 31(23.7 - 40.6) ppm

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XII. 8-Day Dietary LC_{50} Study with AC 92100, 96.7% Pure, in Mallard Ducks (Ind. Bio-Test Lab., Inc.; J 1777)

Groups of 10 mallard ducks (10-15 days old) were fed diets containing 0 ppm of test material (5 groups); 14.7, 31.6, 100, 215, or 464 ppm of Dieldrin (positive controls); or 46.6, 68.1, 147, 316, or 464 ppm of AC 92100 for the first 5 days of the 8-day dietary study period.

No abnormal behavioral reactions or other signs of toxicity were observed in birds fed AC 92100 at levels of 464 ppm or lower. Postmortem examination revealed no specific organ damage due to the compound. Signs of toxicity in birds fed Dieldrin included hyporeactivity and anorexia. Postmortem examination revealed no gross tissue alterations. Autopsy of survivors at 8 days revealed no adverse gross pathology.

AC 92100	LC_{50} 185(128.5 - 266) ppm
Dieldrin	LC_{50} 62(33.3 - 100.4) ppm

XIII. The Acute Toxicity of Cycocel and Experimental Insecticide AC 92100 to Bluegill (Lepomis macrochirus) and Rainbow Trout (Salmo gairdneri) (Bionomics, Inc., June, 1972)

Ten fish were tested at each concentration, the mass/volume ratio never exceeded 1.0 gram of fish/liter of water. The 96-hour median tolerance limit (TL_{50}) was computed with the response being measured being death. AC 92100 of 86.3% purity was used.

	24 hr. TL_{50} (mg/L)	96 hr. TL_{50} (mg/L)	NEL (mg/L)
Bluegill	0.006(0.005-0.009)	0.004(0.003-0.005)	0.002
Rainbow trout	0.034(0.030-0.039)	0.010(0.008-0.013)	0.005

DISCUSSION

AC 92100 is an extremely toxic insecticide. Its phosphorous-containing metabolites exhibit an equal or slightly greater toxicity based upon the acute oral toxicity. The 3-month rat feeding study disclosed a NEL based upon ChE inhibition of 0.25 ppm. Sufficient rats were used in this study to extend this protocol over a 2-year period. An interim report at 6 months indicated that the ChE NEL had decreased to less than 0.25 ppm (the lowest level fed) and that exophthalmos (with possible rupture of the eyeball) was noted in all female treatment groups. (At 3 months exophthalmos had been limited to only the high dose females.) An interim (6 month) report of a mouse carcinogenicity study indicated that exophthalmos and ruptured eyeballs were reported in both 2 and 8 ppm males.

An incomplete 6-month dog study was submitted for which a NEL can not be determined. The plasma ChE was depressed at 10 and 40 mcg/kg BW (0.01 and 0.04 mg/kg BW) but not the RBC or brain ChE. ChE NEL's usually are based upon RBC ChE inhibition. The systemic NEL could not be determined since the histopathology had not been reported and the various other criteria-hematology, clinical chemistry, urinalysis-had not yet been tabulated.

The requested tolerance is for an animal feed crop rather than human food crop, but because of the high toxicity of both the technical material and this formulation, TB will require the following information before a temporary tolerance can be granted:

1) The completed 6-month dog study
2) A cataractogenicity study
3) A separate tabulation of eye lesion observations in rats and mice (from carcinogenicity studies - interim reports).

   a. animal number
   b. treatment group
   c. time of onset
   d. unilateral or bilateral
   e. indications of reversibility
   f. specific statement on histology

4) If available, a histopathological report on the spinal cords of the AC 92100-treated chickens (neurotoxicity study).

RECOMMENDATIONS

Toxicology Branch prefers to defer judgement on the safety of the requested temporary tolerance until the above items No. 1-4 are submitted.

William E. Parkin, D.V.M., D.P.H.
Toxicology Branch
Registration Division

cc: Chemistry Branch
Ecological Effects Branch
Division Reading File
Branch Reading File
PP #3G1340

WEParkin/km 02-07-73

R/D Init: C.H. Williams 02-06-73

Init: C.H. Williams 2/9/73

FAST ANSWER