7-12-79

UNITED [redacted] AGENCY

Caswell 427 AA

DATE: July 12, 1979

SUBJECT: 11273-EUP-15. PP# 9G2176. SAN 326 Insecticide for Control of Rootworm in Field Corn.

FROM: Robert J. Taylor, D.V.M., Toxicology Branch
Leonard A. Nessitt 7/13/79

003863

TO: William Miller, Product Manager # 16

THRU: Dr. Adrian Gross, Chief, Toxicology Branch

Applicant seeks an Experimental Use Permit and temporary residue tolerance of 0.2 ppm for SAN 326 Insecticide [O-(6-ethoxy-2-isopropyl-4-pyrimidinyl)-O-O-diethyl-phosphorothioate] for control of Northern, Southern, and Western Corn Rootworm in Corn (except sweetcorn).

Sandoz, Inc., 480 Camino del Rio So., Suite 204, San Diego, California 92108 desires both an EUP and a temporary tolerance. However, if a temporary tolerance cannot be established promptly, the applicant then requests an EUP with a "crop destruct" provision in order that the 1979 crop year will not be lost for purposes of experimental use.

CONCLUSIONS AND RECOMMENDATIONS:

1. The Experimental Use Permit may be granted with crop destruct provisi.

2. In the absence of a teratology study, a 0.2 ppm temporary tolerance may not be granted.

3. Undoubtedly the applicant recognizes that, in addition to a teratology study, the usual long term and reproduction studies will be required for registration.

4. For a future tolerance, residue studies also should be conducted in meat, milk, and eggs of livestock consuming 0.2 ppm of the chemical.

Toxicity studies were as follows: Unless otherwise indicated, all studies were conducted by Sandoz Agrochemical Research Department, Basle, Switzerland.

ACUTE ORAL LD_{50} in CHICKENS: LD_{50} = 47 mg/kg
Technical grade material of 91.5% purity was used. Five dose levels (36, 45, 56, 72, & 90 mg/kg) were used in 4 female birds per group. Toxic signs were typical of an organophosphate: exophthalmos, salivation, dyspnea, ataxia, and tremors. Study is classified CORE MINIMUM.
Toxicity Category I.

ACUTE ORAL LD_{50} in MALE AND FEMALE RATS: LD_{50} = 137 mg/kg, female; 114 mg/kg in males. Ten rats per sex per dose group were used (56, 72, 90, 112, 140, 179, 224, and 289 mg/kg). Technical chemical was administered in polyethylene glycol, 5 ml/kg, via stomach tube. A CORE GUIDELINES STUDY. Toxicity Category II.

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ACUTE DERMAL TOXICITY OF SAN 326 in MALE & FEMALE RABBITS: LD_{50} = 1100 mg/kg.

Two male & 2 female rabbits per dose. Five dose groups (560, 896, 1120, 1400, and 1792 mg/kg). Gauze pads (5 cm x 5 cm) were soaked with the desired amount of test material and fixed onto the clipped backs of the animals by means of plastic sleeves for 24 hours. The treated skin areas were then washed and the animals were observed for 14 days. Study is CORE MINIMUM. Toxicity Category II.

ACUTE DERMAL TOXICITY OF 10% GRANULE FORMULATION IN MALE AND FEMALE RABBITS:

LD50 is greater than 8 g/kg. Three male & 3 female rabbits per dose per group. Desired dose was put on gauze pads (15 x 12 cm) which were moistened with 20 ml water and then fixed onto the clipped backs of the animals by means of plastic sleeves for 24 hours. The treated skin areas were then washed with water and the animals observed for 14 days. The maximum applicable dose of 8 grams granules/kg body weight was well tolerated by all animals without any symptoms of intoxication or skin irritation. Because no toxicity was seen with the maximum applicable dose, no other doses were probed. A CORE MINIMUM Study. Toxicity Category III.

PRIMARY EYE IRRITATION OF TECHNICAL CHEMICAL IN RABBITS: Three male & 3 female rabbits were used. 0.1 ml. of test material was instilled into the conjunctival sac of the left and the right eye of each rabbit. The right eye-lids were gently held together for 2-3 seconds, and half a minute later only the right eyes were washed gently with 20 ml lukewarm water. The eyes were examined after 24, 48, 72 hours, and 7 days, using the Illustrated Guide for Grading Eye Irritation by Hazardous Substances."

No irritation was observed on the rinsed eyes. On unwashed eyes 1 to 2 rabbits showed slightly more injected blood vessels up to the third day. The overall primary eye irritation score is 0.14 (of 3.0) up to 72 hours, which classifies the test substance as a non eye irritant. A CORE MINIMUM study. Toxicity Cat.

PRIMARY EYE IRRITATION STUDY OF 10% GRANULE FORMULATION IN MALE & FEMALE RABBITS:

Six rabbits (3 male & 3 female) were used. 100 mg of the granules were applied to each conjunctival sac of both eyes and the eyelids were held together for approximately 2 seconds. The right eyes were then flushed with a gentle flow of 20 ml lukewarm water. The eyes were examined after 24, 48, and 72 hours. At no time were any signs of irritation observed except in one rabbit which showed slight erythema in both eyes at 24 hours. Despite its granular nature, the test material is no irritant. A CORE MINIMUM STUDY. Toxicity Cat.III.

ACUTE INHALATION STUDY OF TECHNICAL CHEMICAL IN MALE & FEMALE RATS: LC_{50} is greater than 94 mg SAN 326 per liter of air. The test material was diluted to 25% active ingredient with PEG 200 and blown into air to yield concentrations of 0, 0.83, 9.4, and 94 mg SAN per liter of air. Actual chamber concentration was determined by sampling chamber air for 10 minutes twice during one hour in the proximity of the rats' breathing zone. Each dose group consisted of 5 male and 5 female rats exposed for one hour and observed for 14 days subsequent to observation. They were observed for mortality and toxic symptoms daily, for body weight on days 1, 2, 3, 4, 7, 10, & 14, and for blood cholinesterase activit at one hour and on day 14. They then were killed with pentobarbital sodium anesthetic solution.(1 ml/kg i.p.), necropsied, the lungs examined, and tissues

taken for histopathological examination. 003863

No toxic symptoms were observed in the rats at any level. Plasma cholinesterase activity was inhibited in rats at the 2 highest levels, while red blood cell cholinesterase activity in all groups remained within tolerance limits.

On necropsy the relative lung weights in the treated groups did not differ significantly from the controls.

The acute LC_{50} is greater than 94 mg SAN 326 per liter of air. The NEL based on inhibition of cholinesterase activity in blood plasma is greater than 0.83 mg per liter of air, yet less than 9.4 mg/liter. Study is CORE GUIDELINES. Toxicity Category III.

ACUTE INHALATION STUDY OF 10% GRANULE FORMULATION: Five male and 5 female rats were exposed to the highest airborne dust concentration attainable of the formulation for 2½ hours. (The supply of dust formulation ran out at this time.) Daily observations were made for mortality and toxic symptoms. Observations for body weight were made on days 1, 2, 3, 4, 7, 10, and 15. And cholinesterase activity was measured 1 and 24 hours after inhalation and on day 15.

The chemically measured actual concentration of SAN 326 in the air was 19 ug active ingredient per liter, or 0.22 mg formulation per liter.

The inhalation of the dust from 1.2 kg SAN 10% granules by rats during 2.5 hours produced no mortality, but produced a transient inhibition of the cholinesterase in the plasma only. A CORE MINIMUM Study. Toxicity Category II - IV.

NEUROTOXICITY OF TECHNICAL SAN 326 in CHICKENS. 20 White Leghorn chickens were treated orally with 64 mg/kg technical SAN 326 via capsules and protected with atropine and obidoxime. Another 10 chickens were treated with 80 mg TOCP via capsules. The birds were observed daily for 3 to 4 weeks.

Moribund birds were killed and necropsied; dead birds were necropsied. Surviving birds treated with SAN 326 were killed and necropsied. Multiple sections of the spinal cord and the sciatic nerve were prepared into slides. In the case of TOCP, the sciatic nerves were inspected.

For SAN 326, 12 of the 20 birds survived the observation time, during which the average weight gain was 436 g. For TOCP, all birds survived but lost weight to an average of 62g.

In the SAN 326 birds, during the first 30 hours typical toxic symptoms were observed in all birds. Trembling, ataxia, muscle twitches, and slight signs of limpness were the main symptoms.

In contrast, the TOCP birds showed no obvious toxic symptoms during the first 12 days. Thereafter the birds began to show restlessness and instability, and during the subsequent days were no longer able to roost. Ataxia developed and they no longer could stand upright. Eventually all 10 birds showed obvious paralysis.

Necropsy showed no gross lesions in either the SAN 326 birds, nor in the TOCP-treated chickens.

Histopathological examination of the sciatic nerves and spinal cords of chickens treated with SAN 326 showed no changes which could be correlated with the compou The sciatic nerves of the TOCP-treated controls showed remarkable changes typica of TOCP poisoning.

SAN 326 does not cause delayed neurotoxicity in the hen. Study is CORE GUIDELIN

MUTAGENICITY:

Ames/Salmonella Microsome Plate Test on SAN 326. Conducted at IITRI and at Litton Bionetics, Inc.

A Salmonella/microsome plate test was conducted on concentrations of SAN 326 in DMSO at 0.1 to 1000 ug at IITRI and at 0.01 to 20.0 ul at Litton Bionetics in Salmonella typhimurium strains TA-1535, 1537, 1538, 98, and 100. At Litton Bionetics SAN 326 was also tested in Saccharomyces D4 at concentrations of 0.01 to 20 ul. SAN 326 was tested directly and in the presence of liver microsomal enzyme preparations obtained from animals induced with AROCHLOR 1254 in triplicate plates and for respective positive controls in each strain tested.

The results of both laboratories indicated that SAN 326 tested directly in the indicated strains was negative. However, IITRI, which tested the compound initially, showed that SAN 326 gave positive results in the activated phase of the study in the TA-98 strain at the 100 and 1000 ug SAN 326 concentrations. A dose related pattern was also observed. IITRI then retested SAN 326 in both the activated and non-activated test systems again, under the same conditions. The repeat test gave similar positive results in the TA-98 strain with the addition of the ug concentration being positive as well. Litton Bionetics then tested SAN 326 in similar fashion as IITRI and the results were negative, except that Litton tested on a ul basis instead of ug basis of SAN 326. Litton then was advised of the positive findings of an Ames/Salmonella test at IITRI, and the TA-98 activated phase was retested 3 different days, giving negative results.

Based on these results, SAN 326 was shown to be weakly potentially mutagenic in the TA-98 activated strain at IITRI and not mutagenic in a similar test system used at Litton Bionetics, Inc. STUDIES ARE CORE MINIMUM.

IN VITRO MALIGNANT CELL TRANSFORMATION OF BALB/3T3 CELLS ASSAY on SAN 326. Conducted at Litton Bionetics, Inc.

A preliminary test was performed to assess the cytotoxicity of SAN 326 on the BALB/3T3 cell line. SAN 326 was dissolved in acetone and diluted with culture medium. In order to assess the cloning efficiency of the BALB/3T3 cells, dose levels of SAN 326 ranging from 0.06 to 1000 nl/ml were added to the cell cultures and efficiency determined 72 hours post treatment. Based on these preliminary results, a final assay was conducted on SAN 326 where concentrations ranging from 0.078 to 40 nl/ml were applied to 17 flasks, each flask containing approximately 10,000 cells. In addition, 15 flasks were treated with acetone serving as a negative control and 20 flasks were treated with 10 ug/ml of Benzo(a)pyrene which served as a positive control. Each flask was incubated for a three-day exposure period; the cells then were washed and incubation was continued for four weeks

with refeeding twice a week. The assay was terminated by fixing the cell monolayers with methanol and staining with Giemsa. Stained flasks were examined by eye and by microscope to determine the number of foci of transformed cells.

The final assay results were as follows: The negative control yielded a total o 4 foci among 13 culture flasks for an average of 0.31 foci per flask. This number of transformed foci is significantly different at the 95% confidence leve in comparison to the historical negative controls obtained previously, which consisted of 142 flasks containing a total of 20 foci. Therefore, the current negative control was utilized in determining the statistical significance of foc in the treated cultures. The positive control compound, Benzo(a)pyrene, induce a high frequency of transformed foci: Total number of 76 foci among 14 flasks was significantly different when compared to the negative control. A positive significant dose related response obtained in transforming activity was clearly demonstrated in the 10 and 40 nl/ml dose ranges (40 nl/ml produced 26 foci among 14 flasks; 10 nl/ml produced 13 foci among 10 flasks). These results are statis tically significant and considered biologically significant in comparison with the negative control at P less than 0.01. In addition, the next two lower dose levels of SAN 326, 0.312 and 2.5 nl/ml were approaching significance. If one mo focus per these respective dose levels were observed, then these dose levels woul have been considered statistically significant from the negative control as well. The lowest dose level tested in the final assay which was 0.07 nl/ml, was not statistically significant from the negative control.

Based on the results of this study, SAN 326 caused a significant increase in the number of transformed foci at two dose levels of 10 and 40 nl/ml. SAN 326 and Benzo(a)pyrene seemingly appear to have comparable transforming efficiencies and equivalent lethality based on these data. SAN 326 therefore is positive in the BALB/3T3 transformation assay. This study meets CORE GUIDELINES for a mammalian somatic cell in-culture test for detecting gene mutations.

SUBCHRONIC (13-week) FEEDING STUDY IN RATS: A 3-months feeding study with SAN 32 in rats was initiated with the dosages 0, 3, 10, and 30 ppm respectively. Because the rbc-Cholinesterase activity clearly was inhibited also in the low dose (3 ppm) females, this dose was reduced to 1 ppm after 3 weeks.

No dose-related deviations were observed in the hematology nor urinalysis. The plasma cholinesterase clearly was inhibited in the middle and high group of the females, but only slightly inhibited in the high dose group of the males. The PBC-cholinesterase showed a significant inhibition in the high dose groups of males and females, whereas in the middle dose in the males the activity was within the normal range. The brain cholinesterase was inhibited in the females of the high dose group only. No gross or microscopic lesions attributable to the chemical were observed in any of the groups.

Based on the cholinesterase inhibition, the no-effect level of SAN 326 was 1 ppm. Study is CORE MINIMUM.

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[redacted] in Dogs (3-Month Interim Report): A 6-month feeding study in Beagle dogs is currently underway with the insecticidal active ingredient SAN 326 at the dosage levels of 0 (control), 1, 3, and 30 ppm. Submitted was an interim report after 3 months on test.

The food consumption, and therefore the weight gain, was dose-dependently reduced in the female animals, but not in the males. The actual doses of pesticide consumed in the food corresponded to the theoretical dosages: 1.4 ppm (low); 4.0 ppm (mid); and 39.5 ppm (high). One male in the high dose group appeared to suffer from an unidentified illness; all other control and treated dogs remain in a good state of health.

The hematological investigations and urinalyses revealed no dose-dependent effects of biological significance. In the blood plasma the cholinesterase activity was clearly depressed at the high dosage, somewhat depressed in the mid dose group, but remained within the normal range in the low dose group. The red blood cell cholinesterase was strongly inhibited at the highest dosage; yet no inhibition was observed in the other groups.

According to the results after 13 weeks, a no-effect level of 1 ppm of SAN 326 has been determined, based on plasma cholinesterase inhibition. Study is CORE MINIMUM, to date.

INERT INGREDIENTS in the formulation are all cleared under 180.1001. They are [redacted]

RESIDUES: The predicted major plant metabolite of SAN 326 is [redacted] (0-6-ethoxy-2-isopropyl-4-pyrimidinol). A method sensitive to 0.1 ppm failed to demonstrate any residues of [redacted] in corn harvested 86 to 155 days following application of SAN 326.

A method sensitive to 0.01 ppm detected SAN 326 residues of 0.01 to 0.11 ppm in 2 silage samples from Virginia, one stover sample from Nebraska, and 2 silage samples and 2 stalk samples from Ohio harvested 86 to 155 days following application of 2 pounds SAN 326 active ingredient per acre. No residues were found in any of nine other samples assayed.

CALCULATIONS:

$$\text{NEL} = 1 \text{ ppm} = 0.1 \text{ mg/kg/day} \quad \text{(for rats)}$$

$$\text{ADI} = \frac{\text{rbc cholinesterase inhibition NEL}}{200} = \frac{0.1}{200} = 0.0005 \text{ mg/kg/day}$$

$$\text{MPI} = \text{ADI} \times 60 = 0.03 \text{ mg/day}$$

$$\text{TMRC} = 0.2 \times 1.5 \text{ kg} \times 0.02524 = 0.0075 \text{ mg furnished in all of corn at residue level of 0.2 ppm}$$

Applicant has a temporary residue tolerance of 0.2 ppm. Being a new chemical, no tolerances currently exist for SAN 326. As can be seen by the calculations, using a factor of 2.524% for corn (chiefly corn grain), residues of 0.2 ppm would yield a TMRC of 0.0075 mg. Applying a safety factor of 200 to the cholinesterase NEL of 1 ppm, we derive a MPI of 0.03 mg residue for a 60 kg person. Therefore, if corn (particularly the grain) were to contain 0.2 ppm residue, the MPI would not be exceeded.

The 200-fold safety factor is applied (to cholinesterase inhibition) since we are basing a temporary tolerance consideration on NELs derived from 90-day studies.

For purposes of registration and obtaining a permanent tolerance, the registrant should conduct studies to determine secondary residue levels in meat, milk, or eggs of livestock or chickens, respectively, fed 0.2 ppm of SAN 326.

The requested temporary tolerance of 0.2 ppm can be toxicologically supported. The TMRC utilizes 10% of the ADI. (See computer printout).

ACCEPTABLE DAILY INTAKE DATA

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RAT,Ulcer	NOEL	S.F.	PADI	MPI
mg/kg	ppm		mg/kg/day	mg/day/60kg
0.100	2.00	200	0.0005	0.0300

Current Action 11273-EUP-15/9G2176

CROP	Tolerance	Food Factor	mg/day/1.5kg
Corn,grain(68)	0.200	1.00	0.00300

MPI	TMRC	% ADI
0.0300 mg/day/60kg	0.0030 mg/day/1.5kg	10.00

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