1-19-82

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

002119

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Mr. W.H. Nelson (17)
Registration Division (TS-767)

SUBJECT: EPA#11273-2, 3, 4, 5, 6, 7, 8, 9, 11, 20, and 23 Bacillus thuringiensis Berliner var. Kurstaki and israelensis; human safety data. CASWELL#66

Registrant: Sandoz, Inc.
Crop Protection
480 Camino Del Rio South
San Diego, CA 92108

Action Requested:

Sandoz, Inc. submitted additional human safety data in support of #11273, 4, 5, 6, 7, 8, 9, 11, 18, 20, and 23.

Recommendations:

The data submitted are acceptable:

1) Single Oral Dose Toxicity/Infectivity Study of Thuricide 32B, Rats. Oral LD_{50} Thuricide 32B > 4.7×10^{11} spores/kg body weight (Bacillus thuriengiensis v. Kurstaki). Not toxic. Not infective.

Toxicity Category - IV
Classification - Core-Minimum Data

2) Acute Dermal Toxicity/Infectivity Study of Thuricide 32B, Rat. Dermal LD_{50} Thuricide 32B > 3.4×10^{11} spores/kg body weight (Bacillus thuringiensis v. Kurstaki). Not toxic. Not infective.

Toxicity Category - IV
Classification - Core-Minimum Data

3) Acute Inhalation Toxicity/Infectivity of Thuricide 32B, Rats. No toxicity/infectivity. Inhalation LC_{50} Thuricide 32B > 5.4 mg spores/L.

Toxicity Category - III
Classification: Core-Minimum Data

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4) Thirteen-Week Oral (Gavage) Toxicity and Infectivity Study with Thuricide 32B in the Rat.

No toxicity/infectivity.

90-day B. thuringiensis v. Kurstaki NOEL administered by the oral route, rats > $1\text{-}3 \times 10^9$ spores/kg.

Classification: Core-Minimum Data

5) Review of published studies submitted:

a. Maximal Challenge Infectivity and Eye Irritation Study. Dr. J. Shadduck, University of Texas, Dallas.

Injection of Bacillus thuringiensis israelensis H-14 into mice is not harmful unless > 10^6 are injected directly into the brain.

No evidence of B.t.i. H-14 replication in mice, rats, or guinea pigs was found.

Dry B.t.i. H-14 powder dusted into rabbit eyes is slightly irritating; the bacteria can persist at least one month after end of exposure period. When placed directly into the conjunctival sac, the B.t.i. H-14 powder is much more irritating.

Classification (infectivity): Core-Minimum Data

Classification (eye irritation): Supplementary Data (No Draize scores, exact doses; Toxicology Branch is unable to assign a toxicity category for eye irritation).

b. Dr. H. de Barjac report the WHO:

B.t.i. H-14 is similar to other B.t. strains; it does not produce B exotoxin, and does produce endotoxin that is distinct from other endotoxins produced by other B.t. strains.

Classification: Supplementary Data (Summary status report without full documentation).

Review of Data:

1) Single Oral Dose Toxicity/Infectivity Study of Thuricide 32B, Rats. Sponsor: Sandoz, Inc. Corp Protection. Tester: Bio/dynamics, Project#80-2523. February 23, 1981.

Test Material: Thuricide 32B (containing spores of Bacillus thuringiensis variety Kurstaki).

Forty Charles River rats were divided into two groups of 10 male and 10 female rats each:

Group	Test Substance	Dose Level (Spores/kg)	Number of Animals Necropsy Initial M	Initial F	Day 15 M	Day 15 F
1	Distilled water	0	10	10	10	10
11	Thuricide 32B	4.7×10^{11}	10	10	10	10

The test substance was administered by gavage at a dose volume of 18 ml/kg; 8 ml in the morning and 10 ml in the afternoon of day 1, due to the large dose volume required.

Body weights were recorded twice pretest, weekly during the study, and terminally (15 days).

At termination tissue samples of the stomach, entire intestine (small and large), mesenteric lymph nodes, lung, kidneys, liver and spleen were fixed for microscopic examination.

Results: No mortality. Most of the animals on test showed no observable abnormalities. The few remaining animals exhibited rales, alopecia and nasal discharge.

All animals on test showed normal body weight gains during the test; body weights were comparable.

Gross pathology

Group 1 - 1 Male - pink/red lungs
2 Male - dilated renal pelvis
1 Male - thickened ureter
1 Male - slight alopecia of the skin

Group 2 - 1 Male - pale brown kidneys
1 Male - renal cysts containing clear fluid
1 Male - dilated renal pelvis (unilaternal)
1 Female - dilated renal pelvis (bilateral)

Microscopic Examination:

Gram positive bacteria were found only in the stomachs and intestines of all animals on test; this represents part of the normal bacterial flora. Small numbers of gram + rods were detected in the stomachs of treated animals only that were similar in morphology to Bacillus thuringiensis. Small numbers of B.t. were still present in the intestinal tract of treated animals after 14 days; none of which were embedded in the intestinal wall.

Conclusions:

Thuricide 32B (Bacillus thuringiensis) did not produce any toxic effects, or exhibit any infective properties, when 4.7×10^{11} spores were introduced by the oral route to rats.

1) Acute Oral LD_{50} of Thuricide 32B > 4.7×10^{11} spores/kg body weight Bacillus thuringiensis v. Kurstaki.

Toxicity Category - IV
Classification: Core-Minimum Data

2) Acute Dermal Toxicity/Infectivity Study of Thuricide 32B in Rats. Sponsor: Sandoz, Inc. Crop. Protection. Tester: Bio/dynamics. Project#80-2531. March 11, 1981.

Material Tested - Thuricide 32B (Bacillus thuringiensis var. Kurstaki)

Forty Charles River rats were treated dermally as follows:

			Number of Animals			
			Necropsy			
	Test	Dose Level	Initial		Day 15	
Group	Substance	(Spores/kg)	M	F	M	F
1	Distilled water	0	10	10	10	10
11	Thuricide 32B	3.4×10^{11}	9	11	9	11

An area equal to approximately 10% of the total body surface was clipped on all animals 1 day prior to testing.

The dose volume/animal was 13.2 ml/kg body weight.

The skin of 5 animals/sex/group was abraded. Appropriate quantities of Thuricide 32B were applied to intact and abraded skin sites of treated animals with syringe and glass stirring rods, while control animals similarly received distilled water.

Elizabethan collars were placed on all animals to prevent oral ingestion of test material; however, the tester did not state whether the treatment sites were protected by gauze and occlusive dressings.

Physical observations for mortality and gross signs of toxicologic or pharmacologic effects were recorded pretest and daily for 14 days - Dermal observations were recorded at 24, 48 and 72 hours, and at 7 and 14 days. Body weights were measured twice pretest, week 1 and at termination. On day 15 survivors (19 males and 21 females) were necropsied and tissues were fixed for histopathological examination.

Sections of skin taken from the treated and control areas were saved and fixed microscopic evaluation.

Results: No mortality. Physical observations in both sexes occurring in approximately the same incidence in both control and treated groups included alopecia, swollen cervical area, excessive lacrimation, nasal discharge, and ano-genital staining. These observations were sporadic in time of appearance; and it may be concluded that application of Thuricide 32B did not affect the physical condition of either test or control animals.

All animals on test showed normal body weight gains; there were no significant differences between body weights for control and test animals.

The only significant effect of Thuricide B dermal treatment was to elicit slight edema in both sexes 48 hours after treatment; which persisted another 24 hours, and in the males had diminished by 96 hours. By 7 days all treated animals were free of any dermal effects.

No significant macroscopic observations were found. Of 80 (2/animal) skin sections examined microscopically, 7 contained Gram + bacteria - in both control and treated rats. Gram + bacteria were not found in the epithelial layer, dermis or the subcutis of skin. No blood smears at necropsy contained Gram + bacteria.

Conclusions:

Thuricide 32B (Bacillus thuringiensis var. Kurstaki did not cause any adverse effects or infect intact or abraded skin of rats treated dermally.

LD_{50} of Thuricide 32B > 3.4×10^{11} spores/kg body weight

Toxicity Category - IV

Classification: Core-Minimum Data

3) Acute Inhalation Toxicity/Infectivity Study of Thuricide 32B, Rats. Sponsor: Sandoz, Inc. Crop Protection. Tester: Bio/dynamics. Project No. 80-7472. March 16, 1981.

Test Material: Thuricide 32B

Two groups of 10 male and 10 female Sprague-Dawley rats served as control (Group 1), or test (Group II) animals.

The control rats (Group I) were exposed to dry air at a back pressure of 20 p.s.i. and flow rate of 20 L/min. in a 100 liter plexiglass exposure chamber containing individually housed animals for 4 hours.

Group II test animals were similarly exposed for 4 hours to undiluted Thuricide 32B containing a stated 2.6×10^{10} spores/ml aerosolized by an air atomizing nozzle. Dry air, at a back pressure of 20 p.s.i. was used to generate the aerosol. During the test gravimetric samples were collected at 30 min. intervals at 3.6 L/min. on filter paper.
The actual cloud concentration in the test chamber was calculated by dividing the sample weights collected by the amount of air passed through the collecting filter. Particle size distribution was determined by using cascade impactors to collect aerosol samples twice/hour.

The difference in weight loss of the aerosol generation flask divided by the total volume of air delivered into the test chamber represented the nominal exposure concentration.

Test animals were observed every 15 min. during the first hour of exposure, hourly for the remainder of the exposure period, hourly for 4 hours post exposure and twice daily thereafter for 14 days. Individual weights were recorded on days 0, 2, 3, 4, 7, 13 and 14; at sacrifice.

Fourteen days after exposure, all animals were sacrificed and gross necropsy examinations were performed; the following tissues were fixed for histopathological examination: nasal cavity, trachea, mesenteric lymph nodes, lungs, kidneys, liver, and spleen. Blood smears (2/animal) were taken at necropsy and fixed in methanol for Gram staining.

Results: No mortality. During the exposure period, most rats exhibited decreased activity and partially closed eyes; after removal from the test chamber, two male rats showed singular incidences of facial or whole body tremors. Most animals showed lacrimation and/or mottled fur.

Small, transient body weight losses were observed; the male body weights recovered to pre exposure values by day 4, and most females recovered by day 7.

The nominal concentration was 191 mg/L, and the actual cloud concentration in the test chamber was determined to be 5.4 mg/L (2.6×10^7 spores/liter), with an average mass median diameter of 6.9 u and a mean standard deviation of 2.0. The only adverse effect seen in test animals was slight, irreversible irritation of ocular mucous membranes.

Gross observations at necropsy revealed a few minor lung lesions in animals of both control and test groups; these findings are common to this rat strain and age.

No Gram + bacteria were found in any of the tissues examined thus it was clearly indicated that Bacillus thuringiensis did not infect the rats in this study.

Conclusions:

Thuricide 32B (Bacillus thuringiensis var. Kurstaki), caused no adverse toxic or pharmacologic effects, and did not infect rats exposed by the inhalation route.

LC_{50} of Thuricide 32B > 5.4 mg spores/L
Toxicity Category - III
Classification: Core-Minimum Data

4) Thirteen-Week Oral (Gavage) Toxicity and Infectivity Study with Thuricide 32B in the Rat. Sponsor: Sandoz, Inc. Crop Protection. Tester: Bio/dynamics. Project#80-2505, February 2, 1982.

Test Material: Thuricide 32B, at an undiluted B. thuringiensis concentration of 1-3 x 10^{10} spores/ml.

Two hundred ninety six young Charles River rats were randomly divided into 4 groups of 37 males and 37 females; each received by gavage daily for 13 consecutive weeks:

Group 1 - distilled water
Group 2 - 1-3 x 10^7 spores/kg
Group 3 - 1-3 x 10^8 spores/kg
Group 4 - 1-3 x 10^9 spores/kg

All animals were dosed at a volume of 10 ml/kg.

Tissue infectivity evaluations were made for the spleen, lungs, liver, kidneys and ligated stomach and small intestine (the ligated stomach and small intestine served as positive controls). A blood smear was evaluated for Thuricide using the Gram stain.

Sacrifice schedule for each of the 4 groups of rats on test (initially 37 male and 37 female/group):

Interim Sacrifice

Systemic toxicity at 45 days; 10 males and 10 females.

Infectivity at 14, 15, 43 and 44 days; 3 males and 3 females.

Terminal Sacrifice

Systemic toxicity at 91 and 92 days; 13 males and 13 females.

Infectivity at 90, 91, 120 & 121 days; 3 males and 3 females.

Weekly body weights and food consumption were recorded throughout the study. A pretest Phase opthalmoscopic exam as well as a clinical pathologic evaluation in Test weeks 1, 4, 9 and 13 were performed on ten males and 10 females per group. A complete macroscopic and microscopic examination was performed on all systemic toxicity animals placed on study.

The Thuricide concentrate used for dosing was tested during weeks 6, 8, and 12. Also, samples of the actual dosing solutions for groups 2-4 were tested during weeks 4, 8, and 12 for spore content determination.

All animals were observed twice per day for mortality and gross signs of toxicity. Detailed physical examinations for signs of local or systemic toxicity and pharmacologic effects were conducted daily, palpation for tissue masses was performed weekly.

Ten animals/sex/group were selected for blood sampling at weeks 1, 4, 9 and 13.

Hematology parameters included:

Hemoglobin
hematocrit
erythrocytes
reticulocytes
total and differential WBC

Clinical chemistry:

serum glutamic oxaloacetic transaminase
serum glutamic pyruvic transaminase
alkaline phosphatase
fasting glucose
total protein
sodium
potassium
chloride

Urinalysis:

gross appearance
sp. gravity
pH
protein
glucose
ketones
bilirubin
occult blood
microscopic analysis

Organ/body weight ratios calculated for:

adrenals
brain
gonads
heart
kidneys
liver
pituitary
thyroids

Tissues preserved for histopathologic examination:

adrenal
aorta
costochondral junction
blood
brain
esophagus
eye
femus
heart
intestine
kidney
larnyx
liver
lungs & bronchi
lymph node(s)
thoraco-lumbar vertebrae
thymus
trachea
ureter
urinary bladder
uterus
vagina
gross lesions
tissue masses
mammary gland
nasal cavity
nerve
ovaries
pancreas
parathyroids
pituitary
prostate
salivary gland
seminal vesicles
skin
spinal cord
spleen
stomach
testes

Infectivity studies (reisolate B. thuringiensis):

lung	kidneys
spleen	ligated stomach
liver	ligated small intestine

Results:

No systemic toxicity was observed throughout the study; either by gross or microscopic examination.

A total of seven animals; four controls, two animals administered 1-3 x 10^8 spores, and one animal administered 1-3 x 10^9 spores/kg of Thuricide) died prior to scheduled termination. None of these deaths were related to Bacillus thuringiensis administration. In no case was B. thuringiensis seen in gram stains of peripheral blood nor in kidney cultures, which is strong evidence that a bacterial septicemia due to B.t. did not occur. In order for B.t. to be responsible for the spontaneous rat deaths, organism replication accompanied by infectious disease symptoms must occur, when in fact no disease symptoms were noted. The fact that spontaneous deaths occurred in approximately equal numbers in in control and treated animals suggests that the cause of these deaths was not treatment related. The exact cause of all the spontaneous rat deaths was not evident, however, two group 3 female animals, and 1 group 1 female probably died due to possible intubation error. No B. thuringiensis related effects were observed in food consumption, body weight and clinical pathology data. An increase in male (1-3 x 10^9 spores) leukocyte counts was seen at test weeks 9 and 13, and a very slight increase over time in serum glucose for the (1-3 x 10^9 spores/kg) females was observed.

Massive tissue infectivity with Bacillus thuringiensis did not occur. No massive invasion by spores or vegetative cells from the gastrointestinal tract to other tissues was observed throughout the study. Low recoveries of B. thuringiensis from the lungs of most animals and occasionally in the liver and spleen were found. No organisms were routinely observed in the kidney or blood smear.

Conclusions:

No systemic toxicity or any evidence of infectivity occurred from administration of Thuricide daily for 90 days to rats at dose levels up to 1-3 x 10^9 spores/kg.

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NOEL for B. thuringiensis administered by the oral gavage route to rats for 90 days > 1-3 x 10^9 spores/kg (HDT).

Classification: Core-Minimum Data

5) a. Maximal challenge infectivity, and eye irritation study by Dr. J. Shadduck at Southwestern Medical School, University of Texas, Dallas (Summary report only).

Conclusions:

1) Injection of Bacillus thuringiensis var. israelensis H-14 (B.t.i.) is not harmful unless more than 10^6 viable organisms are introduced directly into the brain.

2) Multiplication of B.t. H-14 does not occur in mammalian tissues in vivo. The bacteria persist for three to four weeks at sites of injection and collect in the spleen where they can be destroyed.

3) Dry B.t.i. H-14 powder dusted into the eyes of test animals is only slightly irritating but the bacteria can persist for at least one month after the end of the exposure period. Finite clumps of B.t.i. H-14 placed directly into the conjunctival sac are much more irritating; adverse effects quickly resolve when the material is removed.

Classification: Supplementary Data (Report presented as a summary without procedure details).

b. Dr. H. de Barjac reported to the World Health Organization that B. thuringiensis israelensis H-14 has all the biological and morphological characteristics common to all strains of B.t.i.; however, it does not produce the B exotoxin, and does produce a endotoxin, to which blackfly and mosquito larvae are susceptible.

Dr. de Barjac states that preliminary data from studies in progress in mice, rats and rabbits indicates that in no test was B.t.i. H-14 able to replicate in mammals (replication is prerequisite to expression of virulence and microbial pathogenicity).

Classification: Supplementary Data (Summary only, procedure details not presented).

William S. Woodrow 7/9/82

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