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

SEP 24 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: SACB Review of Request from Novo-Nordisk A/S for Waiver of Certain Product Identity and End-use Product Toxicology Data for B. thuringiensis var. tenebrionis. (ID #s 058998-EUP-R, and 058998-RA; MRID Nos. 418075-01, -02, -03; HED Project No. 1-1072; Caswell No. 066).

TO: Mike Mendelsohn/Phil Hutton (PM-18)
Insecticide-Rodenticide Branch
Registration Division (H7505C)

FROM: Roy D. Sjoblad, Ph.D., Microbiologist
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

THROUGH: Reto Engler, Ph.D., Chief
SACB, HED (H7509C)

Background: SACB has reviewed Product Characterization and Mammalian toxicology data on the TGAI of Novodor™ (see 8/20/90 Memorandum from F. Chow to P. Hutton/M. Mendelsohn). SACB concluded that there were no concerns for the toxicity or pathogenicity of the TGAI. An inhalation study with the TGAI allowed for placement of the material in TOX Category III. SACB also recommended that evidence should be submitted that demonstrates the organism does not produce B-exotoxin under fermentation conditions, or that the fermentation product does not contain B-exotoxin (as per CFR 180.1011).

Novo-Nordisk A/S has now submitted additional information to support a request for waiver of certain data/information for the characterization of the product, and for toxicology studies on the end-use formulation (Novodor ™ Flowable Concentrate). Consideration of the rationales presented for waiver of toxicology data for the end-use formulation is appropriate for determination in the Precautionary Review Section, RD. M. Mendelsohn also requested that SACB make a determination on whether the acute pathogenicity/toxicology studies submitted to support the Registration of the technical grade of strain NB125 also would suffice to support the registration of the TGAI of NB176.

Conclusion: Strain NB176 was sufficiently well characterized and except for a larger delta-endotoxin crystal, and plasmid size differences, is identical to NB125 for all other parameters tested. Therefore the mammalian pathogenicity/toxicity data generated for NB125 can be used to support NB176. The beta-exotoxin assay still needs to be run after a typical fermentation run, and if negative then dose not need to be done after 6 months of storage. [redacted]

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

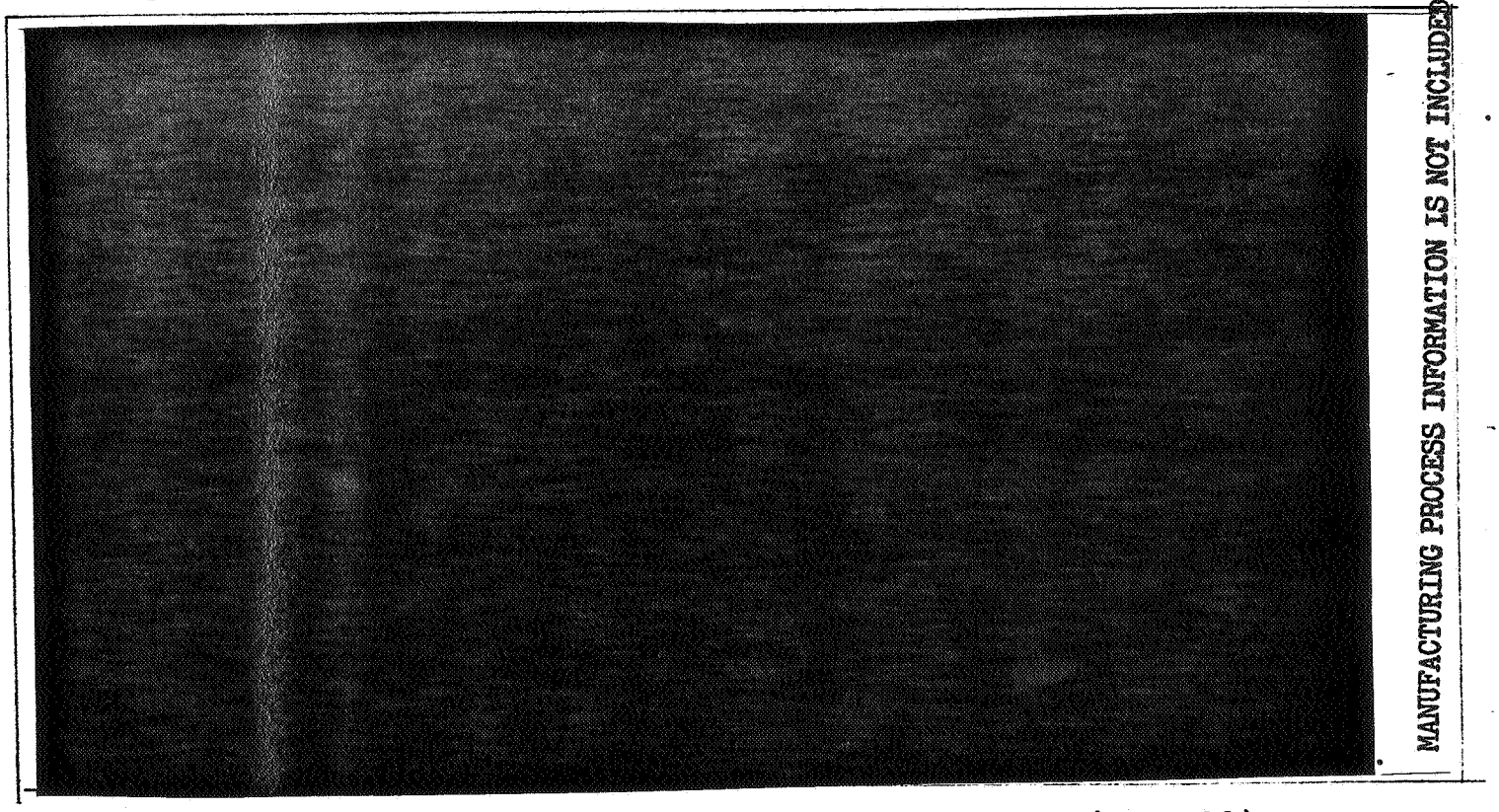
Data/information presented:

1. Physical and Chemical Properties (151A-16).

The Flowable Concentrate formulation of Novodor™ is a light brown to brown aqueous suspension, which has a characteristic odor of bacterial fermentation products. The specific gravity is 1.05-1.15 at 25C, and the pH is 5.0-6.5. It retains its original potency after 6 months storage at either 5C or 25C. It has an upper viscosity limit of 4500 cps, and is not corrosive to polyethylene plastic storage containers.

2. Sample Analysis (151A-13, and 151A-15).

Active crystal protein was determined using a photometric immunoassay; and the activity is expressed as KBTTU (equivalent to 1000 Btt units relative to a Btt crystal protein standard). The relative potency of Novodor™ FC batches is determined by bioassay on Leptinotarsa decemlineata second instar larvae. Potency is expressed as Novo Biokontrol Units/gram (NBU/g).



3. Product Identity and Disclosure of Ingredients (151A-10).

The strain of Bacillus thuringiensis used is designated subspecies tenebrionis strain NB 176, which is "...a high yielding variant of...strain NB 125 which is the strain identified in the registration package for the Novodor Technical manufacturing use product...". The Registrant states that the newly submitted taxonomic data show that the only detectable difference between

strain NB176 and NB125 is that NB 176 produces more insecticidal toxin protein, and this is evidenced by the production of larger crystals in strain NB176. The identification information on strain NB176 is summarized as follows.

The bacterium is a Gram positive spore forming rod containing parasporal crystals. The oval spore is centrally located, and the crystals are flat and plate like and are quadrangular to rhomboidal in outline. NB176 is positive for the following characteristics: catalase, anaerobic growth, acid produced from glucose, nitrate reduction and hydrolysis of casein and starch. It was weakly positive in the Voges Proskauer test. Lecithinase was not assayed for.

Strain NB 176 is negative for: acid from arabinose and from mannitol, and from xylose; and, gas from glucose.

A numerical system (F. G. Priest and B. Alexander, 1988, J. Gen. Microbiol. 134:3011-3018) also was used and allowed for placement of the bacterium as a B. thuringiensis/B. cereus with a Willcox probability of 0.999879. Production of parasporal bodies allows for the distinction of the strain as B. thuringiensis. Characteristics in the numerical analysis for which NB176 scored as positive were: elastin, starch, salicin, anaerobic, nitrate, and Voges Proskauer. The strain was negative for: cellobiose, fructose, galactose, lactose, mannose, raffinose, and xylose; and growth at 50°C and growth in 10% NaCl.

Flagellar antigen serotype analysis showed that the strain possesses the 8a8b H-antigen, and therefore is B. thuringiensis var. morrisoni. Activity against coleopteran insects further differentiates the strain as B. thuringiensis var. tenebrionis (i.e., pathovar C within the serovar morrisoni).

Strain NB176 was susceptible to neomycin, tetracycline, doxycycline, erythromycin, clindamycin, fucidin, streptomycins, gentamicin, chloramphenicol, and nitrofurantoin. It is resistant to penicillin, ampicillin, carbenicillin, methicillin, cephalosporins, polymixins, and trimetoprim. It shows intermediate patterns of susceptibility to sulfonamides, trimetoprim+sulfa, and rifamycins.

Nine plasmid bands were detected ranging from 105 to 10 Mda. An 88 Mda plasmid was believed to encode for the coleopteran toxin. Major bands were found at 35, 36, 88, and 105 Mda, and additional bands were estimated at 26, 23, 20, 17, and 10 Mda.

Strain NB125 had bands at 35, 72, 88, and 105 Mda. Smaller size plasmids may have been present but these were not reported. It was stated the the loss of the 76 Mda band from NB 125 and the appearance of the 36 Mda band in NB176 may have been due to a deletion mutation.

The toxicity to non-target insects was reported as follows:

Test material	Test larva	Highest Concentration	No. dead/No. live larvae
BMN 9004	T. ni	3.9% (equiv. to 585 BTTU/g)	0/49
NB176	A. aegypti	1:100 dilution	0/40

The LC_{50} of BMN9004 to the target insect, Leptinotarsa decemlineata, was about 50 ug/ml (range of 25 to 70 ug/ml in 4 tests) for 1st instar larvae, and about 100ug/ml for 2nd instar larvae.

Results on mouse (NMRI mouse) mortality after intraperitoneal injection of NB176 were reported as follows. A broth culture of the bacterium (at $3x10^9$CFU/ml) was suspended in sterile 0.9% saline and 0.2 ml were administered to animals at 10^6, 10^7, and 10^8 CFU/animal. Control groups were dosed with 0.2 ml saline or with autoclaved NB176 at 10^8 CFU/animal. There were 5 mice/sex/dose group. Body weights were determined at dosing and at 7 days after dosing, and gross necropsy was performed on each animal at 7 days, or if found in extremis or dead during the study. Cageside clinical observations were made daily.

There were no deaths in mice dosed with 10^6 or 10^7 viable CFU, or with mice dosed with 10^8 units of autoclaved bacteria. At 10^8 CFU viable cells/animal 3/5 males and 4/5 females died within 4 hours after dosing. By day 2, 5/5 males in this dose group were found dead. The remaining female remained alive until study termination.

Mean body weight gains of mice in all dose groups where there were no deaths were similar, excepting a slight decrease in the weight of the autoclaved material dose group.

Clinical signs of toxicity observed in the animals that subsequently died included inactivity and abdominal pain. Animals in the 10^8 autoclaved CFU group showed similar, but less pronounced, signs. Activity decrease was seen in the 10^7 CFU dose group. The surviving female in the 10^8 CFU dose group showed slightly distended abdomen. Lesions observed in the animals that died included peritonitis, dilation of the small intestine, and hyperemia and edema in the cranial part of the jejunum. One animal showed prolapse of the rectum. Small yellow foci on the liver, spleen and diaphragm were noted in animals dosed with inactive material, and an enlarged inguinal lymph node.

4. Additional information on Novodor™ Flowable Concentrate.

[redacted] The activity is declared at 15 KBTTU/g, equivalent to 16,400 KBTTU/quart.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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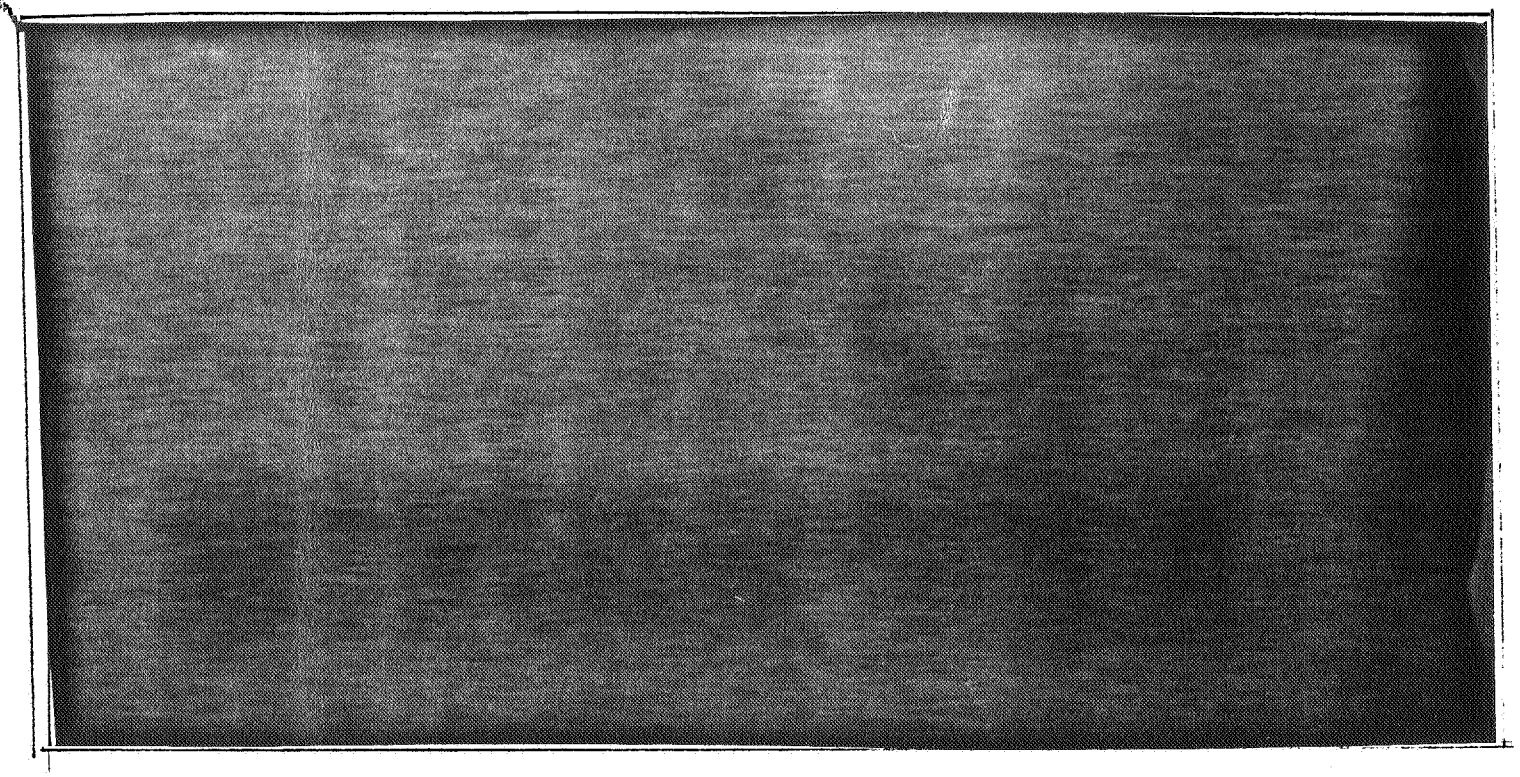

5. Beta-exotoxin determination.

A housefly larvae assay was used to evaluate the ability of a culture of NB176 to produce beta-exotoxin. The inoculum source for the assay was a freeze-dried ampoule of the bacterium. [Note: SACB is assuming that the bacteria from the ampoule are grown in the culture broth although this was not specified]. In the assay system used, NB176 did not produce beta-exotoxin. However, the request by SACB that the assay be done after a typical fermentation has not been addressed. This assay should be done. A waiver has been requested for the 6-month storage stability determination for B-exotoxin. This was based on the lack of B-exotoxin in the freeze-dried preparations as described above;

SACB Discussion:

The strain NB176 was sufficiently well-characterized to show that except for a larger delta-endotoxin crystal, and size differences of at least one plasmid, it was identical to NB125. SACB believes that the acute toxicology data submitted to support an EUP or registration of the TGAI of NB125 could be used also to support an EUP/registration of NB176. A beta-exotoxin assay should be done after a typical fermentation run, and if these results are negative then the Registrant need not perform a 6-month stability study for the beta-exotoxin.