

US EPA ARCHIVE DOCUMENT



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

7275 PATHOLOGY (1-1-91)
#4
5329-3
1991

CONFIDENTIAL

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: SACB review of pathology report on tissues from rats dosed via intravenous injection with MVP Bioinsecticide (MYX 7275) [Submission: S388013; ID No. 053219-G; MRID No. 417039-01, -02; HED Project No. 1-0604; Caswell No. 714G].

TO: Willie Nelson/Phil Hutton (PM-17)
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FROM: Roy D. Sjoblad, Ph.D., Microbiologist
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R. D. Sjoblad

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

Reto Engler

Background: On 10/16/90, Mycogen Corporation met with representatives of OPP to discuss explanation of the mortality in rats after intravenous injection of MYX 7275. On 11/07/90, the Agency received the written report of the Mycogen Corporation pathologist. SACB received the report for review on 2/4/91. SACB will not rereview any of the intravenous studies, since they have been reviewed in other Memorandums.

SACB Conclusions: SACB agrees with the finding of the pathologists that the test material when injected i.v. at levels as high as 5×10^{10} killed cells/animal caused shock, not due to the levels of fixative materials injected, and probably due to reaction to antigenic components of the bacterial cell wall. There is little concern for risk since deliberate injections of massive amounts of MVP would be needed to initiate pathology.

Data/information submitted:

1. "Supplemental data: pathogenesis of toxicity caused by the intravenous injection of a killed MPCA in rats"; by A.C. Singer and W.C. Tacon, of Battelle, Columbus Operations, Ohio; MRID No. 417039-02; study completed on November 2, 1990.

The information in this portion of the report was submitted to address issues raised by SACB on the cause of lethality/toxicity of MYX 7275 after 5×10^{10} killed cells were injected into the tail veins of rats. The study previously has been reviewed by SACB (see 4/25/89 Memorandum from R. Sjoblad and W. Hazel to P. Hutton and W. Nelson). The relevant information in the pathologist's report is summarized as follows:

3/9 males died and 0/9 females died during the study. Clinical signs of toxicity included decreased activity, lacrimation, nasal and ocular discharge, diarrhea, and epistaxis. Necropsy findings showed

A subsequent intravenous study was done with injection of 1×10^7 killed cells per rat. No deaths or significant toxicity were observed. The study demonstrated that toxicity was dose-related, but did not resolve the cause of the lethality at the higher dose level. [Note: SACB did not request this study]

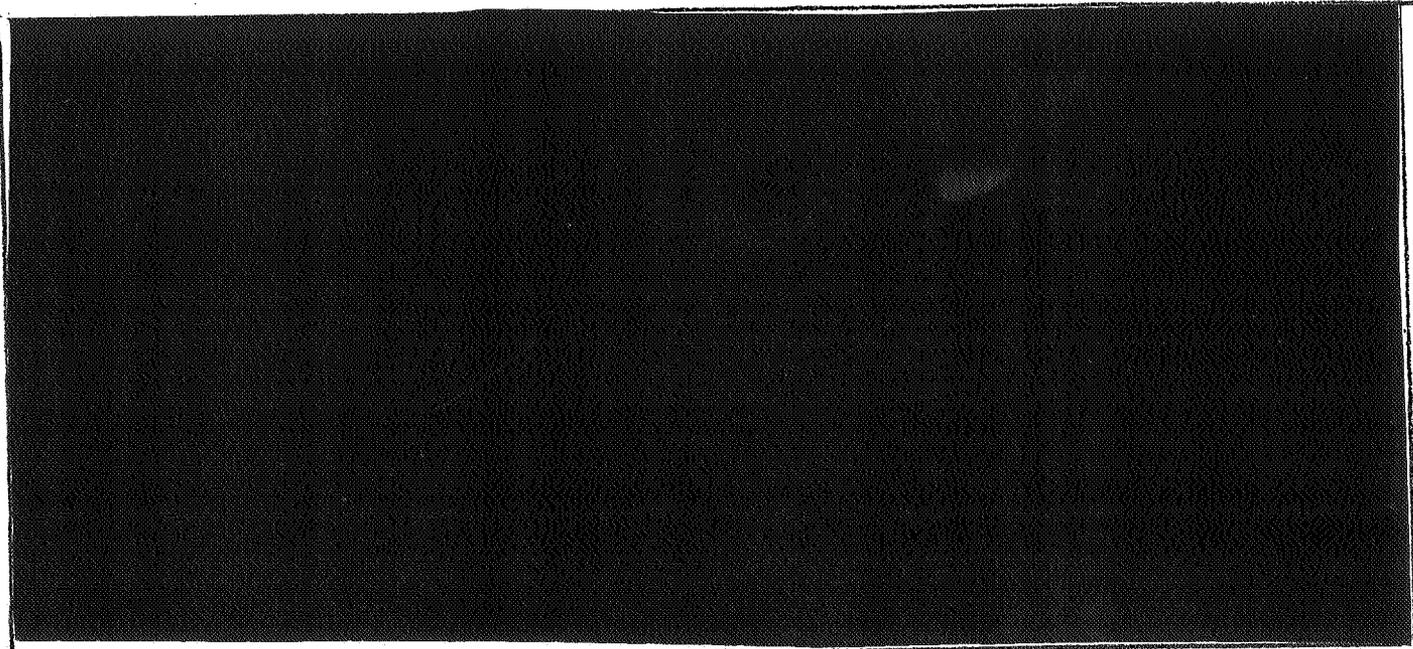
A third study was initiated where [redacted] fixed killed cells were injected into the tail veins of rats, at a dose level of 1.4×10^7 killed cells/animal. No deaths or significant toxicity were observed [Note: SACB did not request this study].

SACB noted that the above studies did not resolve the toxicity/ lethality issue. Also, SACB did not believe that the explanation of pathogenesis in the original study - physical blockage of capillaries - was shown from the data provided.

A fourth intravenous study was done, in which [redacted] fixed or [redacted]-fixed killed cells - at 5×10^{10} killed cells/animal - were administered intravenously to rats. In addition to the observations made in the first study, histopathology was done on kidney, liver, spleen, and lung. Mortality, signs of toxicity, and necropsy results from animals dosed with [redacted] fixed cells were similar to those in animals from the the [redacted] fixed cells, and were similar to the toxicity signs observed in the original study. An additional sign of toxicity in the fourth study was observation, at necropsy, of congested mesenteric vasculature.

Histologic slides from the study were reread, and the pathologists found general agreement with the original pathology report. To summarize early death animals showed peracute inflammatory infiltrates and hepatic necrosis. Chronic hepatic inflammatory changes were noted in rats scheduled for interim and terminal sacrifice. Splenic necrosis/congestion and intravascular bodies (probably test material) were noted in early death animals only. Pulmonary vessels of the spontaneous deaths were acutely congested.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED



is far less than that expected to cause mortality. The actual amount of [REDACTED] in the test material would be lower due to [REDACTED]

The pathologists suggest that antigenic components of the cell wall of the Gram-negative bacterium, Ps. fluorescens are causing the signs of toxicity and mortality, specifically those components commonly referred to as Gram-negative cell wall endotoxins. Endotoxic shock reactions (and also other shock-initiating insults) result in hypoxic cell injury and hemodynamic and metabolic disturbances. The vascular endothelium damage that follows coagulopathic over-reaction of blood to endotoxin is theorized to lead to hypoxic organ failure. Many organs are involved, but typically hemorrhage and/or necrosis are noticed in the heart, lungs, kidneys, gastrointestinal tract, and liver. Also seen may be acute tubular necrosis in the kidneys, fibrin thrombi in organs, and lipid-depleted adrenal glands.

All lesions seen in the intravenous studies are consistent with toxicity and early death being attributed to a shock syndrome, where death is attributed to failure of at least the liver and kidneys. Also, the clinical signs of toxicity (decreased activity, lacrimation, nasal/ocular discharge, epistaxis, and diarrhea) are compatible with a shock syndrome. The "...Agency was correct in requiring histopathology to confirm or dispute [the mechanical plugging of capillaries] hypothesis."

Literature studies would indicate that the dose level of 5×10^{10} Gram-negative cells injected in the intravenous studies is equivalent to the classical dose used to initiate the endotoxic shock cascade.

Intravenous toxicity of the killed bacteria should not cause concern for risk, since experimental injection of massive doses would be needed to cause pathology.

A dose level of about 10^7 units of Gram-negative bacteria is appropriate for the intravenous toxicity test as specified in Subdivision M guidelines.

SACB Discussion: SACB agrees with the findings of the pathologists, that the test material initiated a shock cascade in test animals, not due to the fixative material, but most likely to certain antigenic components of the bacterial cell wall ("endotoxin"), when the animals were dosed with excessive amounts of killed Gram-negative bacteria. Such conditions are not expected to arise under the proposed uses of MVP Bioinsecticide, since deliberate injection of large amounts of the material would be required to initiate the pathology.

2. Certification of ingredient limits. A confidential statement of formula was submitted that certifies that the encapsulated delta-endotoxin of B. thuringiensis var. kurstaki will be present in an amount not to exceed [REDACTED] as the upper limit, or less than 10% as the lower limit. This and other amendments (namely in the amounts of certain intentionally added inerts) are different from the CSF submitted for the EUP Request.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

3. Label. A label was submitted for registration of MVP Bio-insecticide. SACB would recommend that appropriate respiratory tract coverings (namely a particle or dust mask) should be worn by applicators and other exposed workers during application of aerosols of MVP. SACB believes that it is prudent to minimize exposure of the respiratory tract to aerosols containing microbial cells (even killed cells) and to other materials intentionally added to the product as inert ingredients. The label should be amended to reflect the wearing of appropriate protective clothing.