

compatibility, and for aflatoxin production using thin layer chromatography and appropriate standards. Quality control standards are zero tolerance in the starter cultures and in the formulated product for aflatoxin production, and for *Aspergillus flavus* not identified as *Aspergillus flavus* isolate AF36. *Aspergillus flavus* AF36 has never been found to produce aflatoxin.

#### C. Mammalian Toxicological Profile

An acute oral toxicity test was performed whereby a single oral dose of 5,000 milligrams/kilogram body weight (mg/kg/bwt) per animal of *Aspergillus flavus* AF36 colonized wheat seed was administered by gavage to five male and five female Sprague Dawley rats. The oral LD<sub>50</sub> of *Aspergillus flavus* AF36 was determined to be greater than 5,000 mg/kg rat body weight. No clinical signs were observed during the 14-day study and no abnormalities or adverse effects were observed in any of the rats upon necropsy.

An initial pulmonary rat study resulted in lethality in a significant number of animals treated with either the live *Aspergillus flavus* AF36 in Tween 80 or heat killed *Aspergillus flavus* AF36 in Tween 80. Onset of symptoms was rapid after dosing with all deaths occurring by day 4 of the study. All rats surviving to day 4 of the study recovered and all rats sacrificed (as scheduled) on day 8 or day 15 of the study had totally eliminated viable *Aspergillus flavus* AF36 from the lungs, caecal contents, and feces. There was no evidence of infectivity. The aetiology of deaths was unclear. It appeared that *Aspergillus flavus* AF36 prepared as a test substance with Tween 80 caused a severe acute inflammatory response. Retrospective literature review and consultation with a toxicologist supported the theory that the responses were a result of a synergism with Tween 80 and/or of Tween 80 breakdown products formed during preparation of the spore suspension test substance.

A second rat pulmonary study was therefore undertaken. In the second study the conidia were both washed from the wheat and suspended in sterile physiological saline instead of Tween 80. Animals (2 male and 2 female for each treatment level) were dosed at 0, 10<sup>5</sup>, 10<sup>6</sup>, 10<sup>7</sup>, and 10<sup>8</sup> colony forming units per rat. There were no clinical signs in any of the treatment groups considered to be associated with the test substance. Rats were sacrificed at day 8 without treatment associated mortality. No abnormalities were observed in any of the animals at the macroscopic examination at termination.

Based on these two mammalian studies, the petitioner concludes that *Aspergillus flavus* AF36 does not present either a toxicological or an infectious risk to mammals. Data waivers were requested for the following toxicology studies: Acute dermal toxicology/pathology, primary dermal irritation, primary eye irritation, and acute intraperitoneal toxicology/pathology effects of the microbial pesticide. The following rationales were used as a basis for the data waiver requests:

- Researchers and other workers have worked with *Aspergillus flavus* AF36 at the Southern Regional Research Center for over 10 years and in commercial fields (1996 to 1998) and in hand-picked field plots (1989 to 1994) without report of any adverse health effects.

- *Aspergillus flavus* AF36 is widely distributed in the environment and its occurrence is natural.

- The label will require applicators and other handlers to wear Personal Protective Equipment (PPE) such as waterproof gloves, a dust/mist filtering respirator with the appropriate NIOSH approval prefix N-95, P-95, or R-95, coveralls, long sleeved shirt and long pants, and shoes plus socks, and goggles, to mitigate against dermal and primary eye irritation exposure.

The pesticide is to be applied aerially by mixers/handlers and applicators who are licensed and trained to handle restricted materials. At the 10 lb/acre application rate of the formulated material, the total amount of active ingredient is less than 0.01 lb/acre. Applications of AF36 do not significantly impact the total amount of *Aspergillus flavus* in the soil or crop, but only change the proportion of the AF36 strain in relation to the overall soil population. Since the product is applied to cotton fields as a granular formulation on colonized wheat seeds, exposure from drift is minimal.

In addition, the following rationales were advanced in support of the data waiver requests for acute dermal toxicity and primary dermal irritation. These studies were waived during the experimental use program, based upon the lack of toxicity in animals dosed orally. While other *Aspergillus flavus* strains have been reported to be dermal sensitizers, this testing is not warranted, since the aerial method of application and the PPE required on the label will mitigate dermal exposure to workers and pesticide handlers. The acute intraperitoneal study was waived based upon the lack of toxicity in animals dosed orally and by pulmonary/intratracheal instillation.

Genotoxicity, reproductive and developmental toxicity, subchronic toxicity and chronic toxicity testing were not performed, since no adverse effects were observed in the acute toxicology study Tier 1 studies. Tier II (885.3550), subchronic toxicology study (EPA OPPTS 885.3600) and chronic feeding studies (guideline 152-50) are only required if triggered by adverse effects observed in Tier I studies.

#### D. Aggregate Exposure

##### 1. Dietary exposure—i. Food.

*Aspergillus flavus* AF36 is a naturally occurring organism, which does not produce aflatoxin and is thus safer than the aflatoxin-producing *Aspergillus flavus* isolates. Proposed uses and application rates will not result in increases in the total population of *Aspergillus flavus* on the mature crop beyond naturally occurring background levels. FDA does not allow cottonseed products containing aflatoxin at 20 ppb or higher to be used in dairy rations. FDA regulations also do not allow cottonseed products containing aflatoxin levels above 300 ppb, to be used for feeding beef cattle.

*Aspergillus flavus* AF36, when applied to the soil just prior to bloom, has been shown to significantly reduce the levels of aflatoxin in cottonseed at harvest. Furthermore, the proposed use and application rate will not increase exposure of humans to *Aspergillus flavus* by dietary means, since cotton itself is not a food product for human consumption. There is minimal dietary exposure to *Aspergillus flavus* from cottonseed. There is no mechanism for *Aspergillus flavus* to be transferred from the seed to animal products and there is no evidence that the fungus readily contaminates meat or milk. Seed is typically extracted for oil with hexane and that process kills the fungus. Furthermore, applications of *Aspergillus flavus* AF36 do not increase the indigenous populations of *Aspergillus flavus* associated with the harvested crop. The applications merely alter the composition of the fungal community associated with the mature crop so that aflatoxin producing strains are far less frequent. The result is a much lower incidence of aflatoxins in the crop and in the environment associated with the developing and mature crop.

ii. *Drinking water.* *Aspergillus flavus* AF36 is a naturally occurring organism that is already widespread in the environment and is not considered to be a risk to drinking water. Both percolation through soil and municipal treatment of drinking water would reduce the possibility of exposure of *Aspergillus flavus* through the drinking