DATA EVALUATION RECORD

Reviewed by: **Susan Chang, M.S., Toxicologist, Oak Ridge National Labs**

Secondary Reviewer: Carl Etsitty, M.S., Microbiologist

STUDY TYPE:	Acute Pulmonary Toxicity/Pathogenicity - Rats (OPPTS 885.3150)
MRID NO:	457982-01
DP BARCODE NO:	D286705
CASE NO:	062458
SUBMISSION NO:	S624885
TEST MATERIAL:	*Aspergillus flavus* AF36
PROJECT NO:	UAR/006
SPONSOR:	USDA, ARS, Southern Regional Research Center, New Orleans, LA
TESTING FACILITY:	Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England
TITLE OF REPORT:	*Aspergillus flavus* AF36, Acute Pulmonary Toxicity and Pathogenicity to the Rat
AUTHOR:	Emma L. Blanchard
STUDY COMPLETED:	August 6, 2002
GOOD LABORATORY PRACTICE:	UK and OECD GLP Compliant (except for test substance characterization, stability and homogeneity)
CONCLUSION:	*Aspergillus flavus* AF36 appears to have initial dosing effect resulting 1 female and 3 male rat deaths on day 2. The rest of test animals showed an initial response but then rapid recovery indicating no toxicity, and that AF36 was non-infective, and/or pathogenic to rats when dosed at 1.93-2.90 x 10^8 cfu/rat, with observable clearance patterns.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN:

1. **Test Material:** Biological pest control agent *Aspergillus flavus* AF36 on wheat seed (harvested spore suspension containing 6.90 x 10^8 cfu/mL, assessed by Dept of Microbiology). No batch/lot number was not given.

2. **Test Animals:** Twenty-five male and 25 female Sprague-Dawley rats [Hsd:Sprague-Dawley(CD)] were received from Harlan U.K. Ltd., Bicester, Oxon, England. The rats

(approximately 8-10 weeks old) were assigned and weighed 230.0-271.9 g (males) and 200.8-226.4 g (females) on the day of dosing. The test animals were housed in groups of up to five of the same sex in metal cages with wire mesh floors. The rats had free access to drinking water (*ad libitum*) and a standard laboratory rodent diet (Special Diet Services RM1(E) SQC expanded pellet. The environmental conditions of the animal room were as follows: temperature, 22±3°C; relative humidity, 40-70%; and photoperiod, 12 hour light/dark cycle. The number of air changes per hour was not reported.

3. **Methods**: Rats were identified by tail-tattoo and assigned to treatment groups:

Sex	Sacrifice Day	Group 6A	Group 6B	Group 6C	Group 6D	Group 6E	Group 7	Group 8	Total
M	1	21-23							3
	4		24-26						3
	8			27-29					3
	15				30-32				3
	22					33-37	38-40	41-43	11
	Total	**3**	**3**	**3**	**3**	**5**	**3**	**3**	**23**
F	1	44-46							3
	4		47-49						3
	8			50-52					3
	15				53-55				3
	22					56-60	61-63	64-66	11
	Total	**3**	**3**	**3**	**3**	**5**	**3**	**3**	**23**

M = Male; F = Female;
Groups 6A-6E = *Aspergillus flavus* AF36 treated groups.
Group 7 = Inactivated *Aspergillus flavus* AF36 (active *Aspergillus flavus* AF36 was autoclaved) treated group.
Group 8 = Shelf control group refers to untreated rats housed in the same room as the treated rats.

The rats were quarantined 8 days prior to dosing. Wheat seeds colonized by the test organism *Aspergillus flavus* AF36 were stored at 2-8°C. The culture was held at 37°C for 5 days to initiate growth from colonized seed and the spores were harvested by addition of sterile physiological saline (0.85% saline). The suspension was filtered, sonicated, and standardized by viable plate count using Potato Dextrose Agar (PDA) and stored at 2-8°C for 2 days prior to being prepared for use as the dose suspension. On the day of dosing, the suspension was allowed to warm to room temperature and provided for dosing at a concentration sufficient to enable a dose of 10^8 cfu/animal. The harvested *Aspergillus flavus* AF36 spore suspension was analyzed and the number of viable spores was determined to be 6.90×10^8 cfu/mL. The viable counts of the pre-dose and post-dose suspensions were 9.67×10^8 cfu/mL and 1.03×10^9 cfu/mL, respectively. The dose suspension was administered by a single intratracheal dosage. The rats received a 1.2 mL/kg dose of *Aspergillus flavus* AF36. Body weights for the surviving rats were recorded on days 1 (prior to dosing), 4, 8, 15, and 22. The test animals were observed for mortality and clinical signs of toxicity at frequent intervals post dosing and at least daily thereafter for the duration of the study. Samples of feces from the survivors in Group 6E were collected on days 4, 8, 15, and 22. All surviving rats due for sacrifice were first rendered unconscious (via isofluorane anaesthetic) to collect blood samples (≈ 1 mL) in heparinized containers followed by carbon dioxide asphyxiation for sacrifice and necropsy, except group 6A (sacrificed approximately 1h after dosing). The

sacrificed rats were examined *post mortem* by opening the cranial, abdominal, and thoracic cavities in the order of shelf controls (days 22 only) followed by treated groups. Brain, kidney, spleen, liver, lymph nodes, heart, lungs, and cecum were removed, macerated and placed in petri dishes. The number of viable colony forming units (cfu) of the test organism/g of tissue, blood, cecal contents, and feces was determined by serial dilution and plating on PDA containing 60 µg/mL nalidixic acid. The plates (triplicates) were examined for growth of the test organism following incubation (32°C for 2-5 days).

II. RESULTS:

1. **Mortality**: Mortality is given in the follow table. One female rat (No. 48) and three male rats (Nos. 24, 32, and 33) were found dead on day 2.

Groups	6A	6B	6C	6D	6E	7	8
Males	0/3	1/3	0/3	1/3	1/5	0/3	0/3
Females	0/3	1/3	0/3	0/3	0/5	0/3	0/3

2. **Body Weights:** Although some surviving rats lost weight intermittently, all surviving rats gained weight prior to scheduled sacrifice.

3. **Clinical Observations:** Irregular respiration, rales, hunched posture, and/or piloerection were noted from the decedents prior to death. Rales were noted from 11/34 rats treated with the test organism (7 males and 4 females) and two males treated with the heat-killed test organism on the day of dosing. The survivors recovered by day 2. One male treated with the heat-killed test organism had rales on days 5 to 8. Hunched posture was noted from 4/34 rats treated with the test organism, 5/6 rats treated with the heat-killed test organism, and 1/6 rats in the shelf control group on days 2 with recovery by day 9 or later. These clinical signs were not considered due to the test organism.

4. **Gross Necropsy:** The decedents had pallor and enlarged, swollen or thickened tissues of the lungs. No observable abnormalities were noted from any surviving rat.

5. **Infectivity Results:** No test organisms were detected in any samples from the shelf control (Group 8) and inactivated test organism treated rats (Group 7). The test organism was detected in the lung of the treated rats. Clearance from the lungs was established by day 4 in males and day 8 in females after dosing. Results are shown below. The test organism was not detected (< 10 cfu/g, the detection limit) in the cecal contents of the rats from Groups 6B, 6C, 6D, 7, and 8. In the feces from group 6E, AF36 was recovered from feces on day 4 (749 CFU/g for males and 2,328 CFU/g for females), but none at any later collection.

Recovery of *Aspergillus flavus* AF36 in tissues from male rats[a]					
Tissues	**Sacrifice Day and Mean Viable (CFU/g) Recovery**				
	1	4	8	15	22
Lungs	1.19×10^6	< 10	< 10	< 10	< 10

Data taken from Appendix 4, p. 53, MRID 45798201.
[a]Animals deceased prior to scheduled sacrifice not included.

Recovery of *Aspergillus flavus* AF36 in tissues from female rats [a]					
Tissues	Sacrifice Day and Mean Viable (CFU/g) Recovery				
	1	4	8	15	22
Lungs	1.23×10^{6}	1.64×10^{5}[b]	< 10	< 10	< 10

Data taken from Appendix 4, p. 53, MRID 45798201.
[a]Animals deceased prior to scheduled sacrifice not included.
[b]One animal had 1.13×10^{2} cfu/g and another animal had 3.27×10^{2} cfu/g.

III. DISCUSSION: The presented data show no clinical signs in rats that were considered to be due to the test organism. *Aspergillus flavus* AF36 was detected in the lungs with clearance by day 8 after dosing. No test organisms were detected in any samples from the shelf control or inactivated test organism treated rats. Therefore, based on the presented/submitted data, the test organisms were not toxic, infective, or pathogenic to rats. The packet classification is **ACCEPTABLE.**

A preliminary study (Groups 1, 2, 3, 4, and 5) was included in this study report and also in MRID 45739101. An evaluation report is presented in 45739101.der.wpd and is not repeated in this DER.

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R141815

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HED File Code:	41500 BPPD Tox/Chem
Memo Date:	8/6/2002
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Accession #:	000-00-9002

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