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:	457981-01
DP BARCODE NO:	D286705
CASE NO:	062458
SUBMISSION NO:	S624885
TEST MATERIAL:	*Aspergillus flavus* AF36
PROJECT NO:	UAR/004
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:	April 18, 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 in 14 deaths by day 4, but was not infective or pathogenic with clearance by day 8, when dosed with 1.2 mL/kg at 5.30 x 10^8 cfu/mL (or 1.28-1.63 x 10^7 cfu/animal) to the test animals.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN:

1. **Test Material:** Biological pest control agent *Aspergillus flavus* AF36 on wheat seed (containing 1.83 x 10^9 cfu/mL – assessed by Dept of Microbiology). The test material did not contain lot/batch number.

2. **Test Animals:** Twenty-six male and 26 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 218.2-256.5 g (males) and 201.8-232.7 g (females) on the day of dosing. The test animals were housed in groups of

five of the same sex in metal cages with wire mesh floors. The rats had free access to drinking water (*ad libitum*) and 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 A	Group B	Group C	Group D	Group E	Group F	Group G	Total
M	1	1-3							3
	4		4-6						3
	8			7-9					3
	15				10-12				3
	22					13-17	18-20	21-23	11
	Total	**3**	**3**	**3**	**3**	**5**	**3**	**3**	**23**
F	1	24-26							3
	4		27-29						3
	8			30-32					3
	15				33-35				3
	22					36-40	41-43	44-46	11
	Total	**3**	**3**	**3**	**3**	**5**	**3**	**3**	**23**

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

The rats were quarantined 5 days prior to dosing. Wheat seeds colonized by the test organism *Aspergillus flavus* AF36 were stored at 2-8°C. The culture growth was at 37°C for 6-8 days to initiate sporulation and the spores were harvested by addition of sterile distilled water containing 0.5% Tween 80. 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 centrifuged and the pellet was resuspended in 0.1% sterile physiological saline containing 0.1% Tween 80 (SPST) at a concentration sufficient to enable a dose of 10^8 cfu/animal. The harvested *Aspergillus flavus* AF36 suspension was analyzed and the number of viable spores was determined to be 1.83 x 10^7 cfu/mL. The dose suspension (5.30 x 10^8 cfu/mL) 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 E were collected on days 4, 8, 15, and 22. All surviving rats due for sacrifice first rendered unconscious (via isofluorane anaesthetic) to collect blood samples (≈ 1 mL) in heparinized containers followed carbon dioxide asphyxiation for sacrifice and necropsy, except group A (were 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 (day 22 only) followed by treated groups. Brain, kidney, spleen, liver, lymph nodes, heart, lungs, and

cecum were removed 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 were 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). Limit of detection for this procedure is 10 viable test organism/mL or gm.

II. RESULTS:

1. **Mortality:** Mortality is given in the follow table. Eleven rats died, were found dead, or were sacrificed due to severity of clinical signs on day 2; three additional rats died, were found dead, or were sacrificed due to severity of clinical signs on day 4.

Groups	A	B	C	D	E	F	G
Males	0/3	2/3	1/3	2/3	1/5	0/3	0/3
Females	0/3	2/3	2/3	0/3	3/5	1/3	0/3

2. **Body Weights:** One surviving male (No. 5) treated with the test organism lost weight prior to sacrifice on day 4. Two (No. 10 and 28 test animals' body weights were not recorded at death. All other surviving rats gained weight prior to scheduled sacrifice.

3. **Clinical Observations:** Groups A-E: Rales, underactive behavior, piloerection, and fast respirations were noted from the viable test organism treated rats immediately after dosing. With the exception of rales in a male rat (No. 16), all surviving viable test organism treated rats recovered from rales and fast respirations by day 2. Underactive behavior and/piloerection continued up to the day of sacrifice or until days 8 or 9. After day 1, hunched posture was also noted, but was not persistent. Group F: One male (No. 20) and all females treated with the heat-treated test organism showed rales after dosing with recovery within one hour. Underactive behavior, piloerection, fast respiration, and hunched posture were noted from the decedent female (No. 43) that was treated with the heat-treated test organism. Group G: No significant findings were noted.

4. **Gross Necropsy:** Thirteen decedents had enlarged, swollen or thickened tissues and dark mottled patches on the lungs and the other decedent showed gaseous distension of the stomach and small intestines and fluid in the abdominal cavity. Enlarged, swollen or thickened tissues with dark mottled patches or nodular white lesions and mottled appearance in the lungs were noted from three surviving rats (Nos. 5, 9, and 16). No observable abnormalities were noted from any other surviving rat. A deviation to the protocol: On day 4, the caecum was removed after the heart and lungs during the removal of the organs for microbiological analysis.

5. **Infectivity Results:** No test organisms were detected in any samples from the shelf control (Group G) and inactivated test organism treated rats (Group F). The test organism was detected in the lungs of the *Aspergillus flavus* AF36 treated rats. Clearance from the lungs was established by day 8 after dosing. Results are shown below. The test organism (10^2-10^6 cfu/g) was detected in the cecal contents of 3/3 animals on day 4 with clearance by day 8. The test organism (25-10^4 cfu/g) was detected in the feces 6/6 animals on day 4 with clearance by day 8.

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	2.06×10^6	5.70×10^5	< 10	< 10	< 10[b]

Data taken from Appendix 5, p. 45, MRID 45798101.
[a] Animals deceased prior to scheduled sacrifice not included.
[b] One animal had 5.46×10^2 cfu/g.

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	3.82×10^6	35	< 10	< 10	< 10

Data taken from Appendix 5, p. 45, MRID 45798101.
[a]Animals deceased prior to scheduled sacrifice not included.

III. DISCUSSION: The presented data show transient clinical signs in rats. *Aspergillus flavus* AF36 was detected in lungs, cecal contents, and feces on day 4 with clearance by day 8 after dosing. No test organisms were detected in any samples from the shelf control and inactivated test organism treated rats. The study author indicated that the etiology of the deaths on the study is not clear and maybe due to dosing the test organism in Tween 80 causing "a severe acute inflammatory response leading to death." Based on the presented/submitted data, the test organisms were not toxic, infective, or pathogenic to rats. The packet classification is ACCEPTABLE.

MRID 45739101 in the same submission includes "Comments on Use of Tween 80 in Protocol UAR/004" and gives the explanation that toxicity observed is attributable to using Tween 80 as the vehicle.

MRID 45798201 in the same submission includes a similar study using the same test organism without using Tween 80 as the vehicle.

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R141863

Chemical: Aspergillus flavus 36 colonized wheat seed

PC Code:
006456
HED File Code: 41500 BPPD Tox/Chem
Memo Date: 4/18/2002
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