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:	457391-01
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 (Interim Report)
AUTHOR:	Emma L. Blanchard
STUDY COMPLETED:	March 27, 2002
GOOD LABORATORY PRACTICE:	Not stated
CONCLUSION:	This study was a preliminary study to investigate a suitable concentration of *Aspergillus flavus* AF36 to be used in a main study for an acute pulmonary toxicity and pathogenicity to the rat. This study concluded that 10^8 cfu/rat would be a suitable dose level.
CLASSIFICATION:	SUPPLEMENTAL. No upgrade necessary since this is an interim report of a range finding study.

I. STUDY DESIGN:

1. **Test Material:** Biological pest control agent *Aspergillus flavus* AF36 (containing 6.23×10^8 cfu/mL)

2. **Test Animals:** Twelve male and 12 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 234.9-283.4 g and 209.0-232.7 g, respectively, on the day of dosing. The test animals were housed one or two rats of the

same sex per metal cage with wire mesh floor. The rats had free access to drinking water 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 1	Group 2	Group 3	Group 4	Group 5	Total
M	8	1, 2	3, 4	5, 6	7, 8	9, 10	10
	Total	**2**	**2**	**2**	**2**	**2**	**10**
F	8	11, 12	13, 14	15, 16	17, 18	19, 20	10
	Total	**2**	**2**	**2**	**2**	**2**	**10**

M = Male; F = Female;
Group 1 = Vehicle (0.85% saline) control
Groups 2-5 = Treated with *Aspergillus flavus* AF36 (2.18-2.52 x 10^5, 2.18 - 2.45 x 10^6, 2.16-2.81 x 10^7, and 2.07-2.65 x 10^8 cfu/rat in 0.85% saline, respectively, for groups 2, 3, 4, and 5)

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 growth was at 37°C for 5 days to initiate sporulation 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). Due to page 12 of the study (MRID 45739101) being missing, the test substance dose and quantification are unknown. The test material (0, 10^5, 10^6, 10^7, and 10^8 cfu/rat in 0.85% saline, respectively, for groups 1, 2, 3, 4, and 5) 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) and 8. 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. All rats were necropsied.

II. RESULTS:

1. **Mortality**: One female (No. 16) dosed with 10^6 cfu/rat died approximately two hours after dosing. All other rats survived the study.

2. **Body Weights:** All females dosed with 10^8 cfu/rat lost weight, but the other surviving rats gained weight by the end of the study.

3. **Clinical Observations:** The decedent had rales, lethargy, and pale skin prior to death. Piloerection was noted on most of the rats including the controls within 30 minutes after dosing. Fast respirations was noted on all rats dosed with 10^7 and 10^8 cfu/rat immediately after dosing and one male and two females in the 10^5 cfu/rat within 30 minutes after dosing. One 10^5 female had an abnormal gait approximately two hours after dosing. Rats recovered from all clinical signs by day 2.

4. **Gross Necropsy:** The decedent had enlarged, swollen or thickened tissues and a dark red appearance of the lungs. No observable abnormalities were noted from any surviving rat.

III. DISCUSSION: This study was a preliminary study to investigate a suitable concentration of *Aspergillus flavus* AF36 to be used in a main study for an acute pulmonary toxicity and pathogenicity study using the rat. This study concluded that 10^8 cfu/rat would be a suitable dose level. Since this is a dosing finding study, no classification is assigned.

MRID 45739101 includes "Comments on Use of Tween 80 in Protocol UAR/004" and gives the explanation of toxicity observed in UAR/004 (MRID 45798101) attributed to Tween 80 as the vehicle. It also includes a number of Tween 80 literature confirming the attribute toxicity and potential vehicle of use for membrane absorption.

13544

R141886

Chemical:	Aspergillus flavus 36 colonized wheat seed
	PC Code: 000456
HED File Code:	41500 BPPD Tox/Chem
Memo Date:	3/27/2002
File ID:	DPD286705
Accession #:	000-00-9002

HED Records Reference Center
4/13/2007