

DBT Study Title: Acute Oral Safety Limit Study In Rats and Mice

Dow AgroSciences LLC Study Title: Cry1F-(Synpro) Microbial Protein + Cry1Ac-(Synpro) Microbial Protein: Acute Oral Toxicity Study in CD-1 Mice, Unpublished Report of Dow AgroSciences LLC (Study ID 011127).

Introduction

The purpose of this study was to determine the median oral lethal dose (LD₅₀) of a Cry1F microbial protein + Cry1Ac microbial protein.

Test Guidelines. OECD Guideline No. 401 Acute Oral Toxicity, 1987. EPA Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity, 1998. JMAFF Acute Oral Toxicity Study, 2000. EEC Methods Number B.1 Acute Toxicity (Oral), 1992.

GLP Standards. This study was conducted in accordance with the US Environmental Protection Agency-FIFRA GLPs Title 40 CFR, Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Good Laboratory Practice Standards (Final Rule); the Japanese Ministry of Agricultural, Forestry and Fisheries (JMAFF) 11 NohSan, Notification No. 6283 – 1 October 1999 revised by 12 NohSan, Notification No. 8628 – 6 December, 2000; the Organization for Economic Co-Operation and Development (OECD) OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1, OECD Principles on Good Laboratory Practice (as revised in 1977) ENV/MC/CHEM(98)17; the European Community (EC) EC Directive 99/11/EEC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999); and the Standard Operating Procedures of Toxicology & Environmental Research and Consulting, The Dow Chemical Company.

Quality Assurance. The study conduct, data, protocol, protocol changes/revisions, and final report were inspected by the Quality Assurance Unit, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.

Archiving. The data, protocol changes/revisions, and final report are archived at Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.

Materials and Methods

Selection of Animal Species. Male and female CD-1 mice obtained from Charles River Laboratories Inc. (Raleigh, North Carolina) were used in the study. Females were nulliparous and non-pregnant. Mice were born on May 13, 2001, and dosing began on July 24, 2001. At the commencement of the study, the weight variation of animals used was minimal (within 20% of the mean weight for each sex).

Accommodation and Husbandry. Upon arrival at the laboratory, the mice were examined by the laboratory veterinarian for their health status and acceptability for study purposes. Animals were housed two per cage in stainless steel cages in rooms that were designed to maintain adequate environmental conditions concerning temperature, humidity, photocycle, and air exchanges. The

relative humidity and room temperature were maintained within a range of 40-70% and $22 \pm 3^\circ \text{C}$, respectively. A 12-hour light/dark photocycle was maintained for all animal rooms. Room air was exchanged approximately 12-15 times/hour, and the water lines automatically bled every six hours.

Animals were provided LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in pelleted form. Feed and municipal water were provided *ad libitum*. They were acclimated to the laboratory environment for at least one week prior to the start of the study.

Preparation of Animals. Five mice per sex were randomly assigned by body weight to dose groups using a computer program. Mice were identified by a code number transmitted by a subcutaneously implanted transponder (BioMedic Data Systems, Inc., Maywood, New Jersey).

Test Protein Dose Preparation. An ~50:50 mixture of Cry1F microbial protein + Cry1Ac microbial protein as a 10% mixture in 0.5% aqueous methylcellulose was prepared.

Test Protein Dose Administration. Animals were fasted 1 hour prior to dosing. Animals were weighed and a detailed clinical observation (DCO) was conducted for all mice prior to test substance administration. Animals were administered 5000 mg/kg of a 1:1 mixture of the Cry1F microbial protein preparation (15% AI w/w) + Cry1Ac microbial protein preparation (14% AI w/w) resulting in a dose of 375 and 350 mg AI per kg, respectively. The volume of the test material in methylcellulose exceeded 20 ml/kg body weight; therefore, the test material mixture was administered as three fractional gavage doses given approximately one hour apart. After the third administration of the substance, feed was provided to all mice.

Observations. Animals were observed a minimum of two times on the day of treatment. A DCO was done each day (including weekends and holidays) during the study. Hand-held and open-field observations included a careful physical examination. For scored DCOs only observations other than those typical expected were recorded. Observations were dictionary based, and the dictionary contained the most of the common physical and neurological abnormalities seen in toxicity studies. Since not all potential observations were contained in the dictionary, free-field descriptions also were allowed. Each animal was weighed pre-study, the day of treatment, and on test days 2, 8, and 15.

A necropsy was performed on all animals. Animals submitted alive for necropsy were anesthetized by inhalation of carbon dioxide and were euthanized by decapitation after clamping the trachea. A complete necropsy was conducted on all animals by a veterinary pathologist assisted by a team of trained individuals. The eyes were examined *in situ* using a moistened glass microscope slide applied to the corneal surface. Following inspection of the externum and body orifices, the nasal, cranial, oral, thoracic, and abdominal cavities were opened and the visceral organs were examined both *in situ* and following dissection, and tissues were not saved.

Results

Mortality results are shown in Table 1. All mice survived the two-week observation period with no mortality noted among treated animals.

Table 1. Mortality Results for Male and Female Mice

Dose (mg/kg)	#/Sex/Dose	#Dead		Approximate Observed Time of Death (Day)	
		Males	Females	Males	Females
5000	5	0	0	----	----

---- No deaths noted.

There were also no remarkable clinical observations or detailed clinical observations noted for any animal throughout the study. All mice gained weight over the duration of the study and there were no gross pathologic or visible lesions for any animal in the study.

Conclusion

The acute oral LD₅₀ of the mixture of Cry1F and Cry1Ac microbial proteins in male and female CD-1 mice in this study was greater than 5000 mg/kg (containing 375 mg Cry1F protein/kg and 350 mg Cry1Ac protein/kg).

Key References

EEC (1992). Official Journal of the European Economic Community: Methods for the Determination of Toxicity, Acute Toxicity (oral), Directive 92/69/EED. Part B.1.

U.S. Environmental Protection Agency. Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity, August 1998.

JMAFF (2000). Ministry of Agriculture, Forestry and Fisheries, Requirements for Safety Evaluation of Agricultural Chemicals, 59 NohSan, Acute Oral Toxicity Study.

OECD (1987). Organization for Economic Cooperation and Development – Guideline for Testing of Chemicals, Acute Oral Toxicity 401, 24 February, 1987.