Definitive Protocol

Study Title	(^{14}C)- Desmedipham : Absorption, distribution, metabolism and excretion following repeated oral administration to the laying hen
Sponsors' Monitor	Philip Fisher
Sponsor	Aventis CropScience Ltd 355 rue Dostoïevski BP 153 F-06903 Sophia Antipolis cedex France
Regulatory Guidelines	US EPA Sub-division O, Series 171-4b OPPTS 860.1300 (August 1996) EU 96/68/EC Annex I, Section 6.2 (October 1996)
Study Director	Trevor Hardwick
Test Facility	Covance Otley Road, Harrogate North Yorkshire HG3 1PY ENGLAND
Sponsors's Reference Number	27788
Covance Study Number	1849/016
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The radioactive dose to be administered to the hens was omitted from the study protocol in error. The radioactive dose will be 1 MBq/day.

It has been discovered that the test substance is not stable in acetonitrile. Acetonitrile will not be avoided, if possible, in any HPLC method or extraction procedure developed.

The stability of the test substance in the capsules will be measured at 0, 4, 7 and 14 days after preparation and not 0, 3, 7 and 14 days as stated in the study protocol.

The responsible person for quality assurance is D Kirkland, Vice President of Consultancy and Regulatory Services and not K de-Salis, Head of Quality Assurance, as stated in the study protocol.

Environment

Animals will be housed in the livestock unit which is designed to provide a temperature of between 10 and 25°C and to maintain humidity between 40 and 80% (temperature and humidity are recorded daily). Fluorescent lighting will be cycled 16 h light (07:00 - 23:00) and 8 h dark. The unit is designed so that there are 10 air changes per h.

Diet and drinking water

Hens will be offered a total of 150 g of a mixture of a commercially available ground concentrate and grit per day. The ratio of concentrate to grit will be determined during acclimatisation and maintained throughout the study. Actual food consumption will be recorded daily. The diet suppliers will be recorded in the raw data. Periodic analysis of water is undertaken for bacteria, heavy metals and chlorinated hydrocarbons. It is the responsibility of the sponsor to notify Covance Laboratories Ltd if any contaminants expected to be present in food or water will affect the integrity of the study.

Body weight

Body weights will be determined during the initial acclimatisation period, on the day of the first dose occasion and at necropsy.

Pre-experimental procedures and acclimatisation

The hens will be acclimatised for a minimum of 10 days and de-wormed if required. During this period, the hens will be placed into individual metabolism cages suitable for the separate collection of excreta and eggs and acclimatised for at least 7 days prior to the first dosing occasion. Whilst being acclimatised food consumption will be measured in order to determine the daily feed ration for the study. The appearance and behaviour of the animals will be observed daily (*am* and *pm*).

Identification of the test system

On arrival, the hens will be arbitrarily allocated a stock number, or suppliers number. During the initial acclimatisation period, individual subject code will be allocated. Hens will be uniquely identified by numbered leg tags.

In addition to the above, metabolism cages will be coded by coloured cards giving information including study number, subject code and animal number. The study room or pen will be identified by a card on the outside door giving information including room number and study number.

EXPERIMENTAL PROCEDURES

Dose level and frequency of administration

Capsules will be orally-administered following the morning egg collection for 14 consecutive days. For a dose level equivalent to 10 ppm diet each hen will receive 1500 µg of desmedipham per day. This is based on an assumed consumption of 150 g of ground concentrate and grit/day.

Sample collection, storage and processing

The order of sampling and dosing is shown in Appendix 2. Excreta will be collected at 24 h intervals and up to necropsy. Eggs will be collected immediately before dosing and in the afternoon, between 5 and 8 h after dosing. Egg yolk and white will be separated and pooled by animal for each 24 h collection period. Cage debris will be removed at each excreta collection and pooled over the duration of the study. At approximately 23 h, but less than 24 h, after the last dose occasion, the animals will be removed from the metabolism cages and killed by cervical dislocation. A terminal blood sample will be taken and collected into lithium heparin tubes. A portion will be centrifuged to produce plasma. Metabolism cages will be rinsed with methanol and then water to provide a final cage wash. The following tissues will be taken or sampled:

- Muscle (maximum amounts of breast and thigh)
- Fat (maximum amounts of peritoneal and perirenal)
- Liver (whole organ)
- Skin (to include subcutaneous fat)