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CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

FEB 28 1990

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: CYPROCONAZOLE - DRAFT PROTOCOL FOR A DERMAL PENETRATION STUDY

TO: SUSAN LEWIS/CARL GRABLE
PRODUCT MANAGER (21)
REGISTRATION DIVISION (H7505C)

FROM: LINDA L. TAYLOR, PH.D. Linda Lee Taylor 2/28/90
TOXICOLOGY BRANCH II, SECTION II
HEALTH EFFECTS DIVISION (H7509C)

THRU: K. CLARK SWENTZEL K. Clark Swentzel 2/28/90
SECTION II HEAD, TOXICOLOGY BRANCH II
HEALTH EFFECTS DIVISION (H7509C)

AND

MARCIA VAN GEMERT, PH.D. Marcia Gemert 2/28/90
CHIEF, TOXICOLOGY BRANCH/HFAS/HED (H7509C)

REGISTRANT:	SANDOZ CROP PROTECTION CORPORATION
CHEMICAL:	CYPROCONAZOLE
SYNONYM:	SAN 619 F
PROJECT NO.:	0-0757
CASWELL NO.:	272E
RECORD NO.:	260053
IDENTIFYING NO.:	55947-RGG
MRID NO.:	NOT APPLICABLE
ACTION REQUESTED:	PLEASE REVIEW AND COMMENT ON PROPOSED PROTOCOL.

COMMENT: THE REGISTRANT IS SEEKING AN EXPEDITED REVIEW OF THIS PROTOCOL IN ORDER TO INSURE THAT THE STUDY IS CONDUCTED ACCORDING TO EPA REQUIREMENTS, SINCE THE EUROPEAN GROUP CONDUCTING THE STUDY IS HOPING TO INITIATE THE STUDY IN MARCH, 1990.

THIS DRAFT PROTOCOL IS NOT ACCEPTABLE. AS DISCUSSED IN THE ATTACHED PAPER BY ZENDZIAN, THE MINIPIG IS NOT AN ACCEPTABLE ANIMAL TEST SYSTEM FOR THIS TYPE OF STUDY. ADDITIONALLY, ONLY ONE ANIMAL IS PROPOSED PER DATA POINT AND, GIVEN THE VARIABILITY OF RESULTS AMONG ANIMALS, THIS TOO IS NOT ACCEPTABLE. I DISCUSSED THIS PROTOCOL WITH DR. ZENDIAN, WHO INDICATED THAT OTHER PROTOCOLS FOR THIS TYPE OF STUDY HAVE BEEN SUBMITTED TO EPA FROM THIS TESTING FACILITY (INVERESK RESEARCH INTERNATIONAL LIMITED) AND HAVE BEEN EVALUATED AND FOUND ACCEPTABLE. THUS THE TESTING FACILITY IS AWARE OF EPA'S DATA REQUIREMENTS FOR THIS TYPE OF STUDY.

I HAVE ATTACHED A COPY OF THE PROCEDURE FOR STUDYING DERMAL ABSORPTION, WHICH SHOULD BE SENT, ALONG WITH THE PAPER, TO THE REGISTRANT.

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Skin Penetration Method Suggested for Environmental Protection Agency Requirements

ROBERT P. ZENDZIAN

ABSTRACT

The Environmental Protection Agency has circulated a protocol for examining dermal absorption of pesticides in rats. This protocol will be considered as a guideline for determining the dermal absorption of pesticides. Approximately 40 pesticides have been evaluated with this protocol. Male rats are dosed dermally with labeled pesticide. Doses, in mg/cm², are applied to the shaven skin of the back as the use product, diluted with water if necessary. The application site is protected with a nonocclusive device. Four rats per dose are exposed for 0.5, 1, 2, 4, 10, or 24 hrs. Samples collected are soap and water wash, skin at the application site, blood, total urine and feces, carcass, and selected tissues. Mass balance calculations include determination of pesticide that can be removed with soap and water, pesticide bound on or in the skin, total pesticide absorbed with time, blood concentrations with time, pesticide accumulation in target tissues, and pesticide excreted.

INTRODUCTION

EXPOSURE TO PESTICIDES CAN OCCUR during application, while working with pesticide-treated materials, or in treated areas, and when eating pesticide-treated food. Exposure during application and from treated materials or areas is mainly via the skin, although some inhalation exposure does occur. In contrast, the majority of animal toxicology studies use the oral route, either by direct dosing or in the feed. This route is the easiest to use and provides data for assessing the safety of oral exposure but makes it necessary to provide a connection to human dermal exposure. Providing this connection is the purpose of the dermal absorption study.

Dermal absorption studies are not routinely required. The 1982 Environmental Protection Agency (EPA) Pesticide Guidelines for Toxicology Studies state:

> Dermal absorption studies may be required on an individual basis for compounds having a serious toxic effect, identified by oral or inhalation studies, for which a significant route of human exposure is dermal and for which the assumption of 100% absorption does not produce an adequate margin of safety.[1]

Health Effects Division, Office of Pesticide Programs, United States Environmental Protection Agency, Washington, D.C.

size and genetic variability. It also has a highly permeable skin compared to that of humans and other species.

The young adult rat is of convenient size: the back provides sufficient area for dosing and the body mass does not require excessive amount of radiolabeled compound. Since it is used for the majority of toxicity studies, there is usually a good data base on the pesticide's toxicity and metabolism in the species. It is the first species of choice for metabolism studies. Rat skin is generally considered more permeable than that of humans.

The genetically hairless mouse has a skin that is histologically very similar to human skin and the young adult has been used for prototype dermal absorption studies. The area of skin available for dosing is small, which can give rise to problems in consistency of dosing, per unit of area, particularly when suspensions are used. Such a small animal can be hard to control for the duration of exposure while the application site is protected.

The sum of these considerations has led to a confirmation of Du Pont's choice of the laboratory rat as the experimental animal for in vivo studies of dermal absorption of pesticides.

EXPERIMENTAL DESIGN

Young adult male rats weighing 225–250 g are used. Four animals are used for each dose and exposure duration.

Twenty-four hours prior to dosing, the back and shoulders of the rats are clipped free of hair and the area washed with acetone. The acetone wash removes skin debris, waxes, and oils and the 24 h interval before dosing allows secretion of a relatively consistent layer of natural "skin oils." A measured area, of at least 10 cm^2, is marked on the skin to delineate the application area.

The test material is of known chemical purity and usually radiolabeled with carbon 14. The position is selected so that the label will follow the compound and/or its major metabolites until excreted. The vehicle for the high dose should be the vehicle of the use product, whether an emulsifiable concentrate (EC), a dust, or some other form. A liquid formulation can be applied neat: a dry form should be suspended in water. Lower doses are produced by diluting this material with the field solvent, usually water. A dose selected to determine absorption from crop residue should be prepared in water.

The dose must be applied quantitatively and as evenly as possible to the entire application site. The application site must be covered to prevent the animal licking the site and to prevent material from falling into the urine/feces collection. The cover must be nonocclusive to avoid concentration of moisture from the skin, resulting in a change in skin permeability. A combination cover consisting of a "spacer" glued to the skin and a filter paper or gauze glued to the "spacer" has been shown to be most effective. The "spacer" outlines the application site and is sufficiently thick to keep the cover from contact with the site.

The basic exposure periods are 0.5, 1, 2, 4, 10, and 24 h. The exposure periods cover a range of exposure to pesticides from activities such as short-duration homeowner use, mixer/loader exposure, half- or whole day exposure, and exposure of persons who wash infrequently. Each dosed animal is individually caged in a metabolism cage and the total urine and feces collected for the entire exposure period.

At the end of the exposure period, the rat is anesthetized for ease of handling, the protective cover removed, and the application site washed with soap and water. The rat is sacrificed, the application site skin collected, a blood sample collected, residual urine from the bladder collected and added to the urine collection, and the remaining carcass preserved. In some cases individual organs, which have been identified as targets of the test compound, are collected.

The samples analyzed for test material (radiolabel) are

1. Materials used to apply the dose

2. The protective cover
3. The skin wash
4. The skin of the application site
5. Total urine
6. Total feces
7. Blood
8. Total carcass
9. Individual organs, if collected

DOSE SELECTION

Doses are selected to span the range of doses per unit area of skin that can be expected to occur in human exposure. Whole log intervals between doses produce the most usable data. The highest useful dose is on the order of 1 mg/cm^2 with descending doses of 0.1, 0.01, and 0.001 mg/cm^2, on a minimum area of 10 cm^2. The lower dose range is generally the most use[illegible] Doses must be given as mass/unit area of skin (mg/cm^2), since the rate of absorption is dir related to mass/unit area and the selection of the proper dermal absorption rate to be used in the transfer of rat dermal absorption data to human exposure data will be based on comparable mass/unit area.

The highest concentration of active ingredient to which the applicator is exposed is the concentrated form sold by the manufacturer. This material, with the pesticide suitably labeled, should be used for the high dose. The lowest concentration of active ingredient to which one can usually expect the applicator to be exposed is the field mix. The field worker may be exposed to a still lower dose from plant residue. The experimental doses should span the exposure range as determined by field exposure studies. All concentrations less than the concentrated form should be made by diluting the high-dose material with the field solvent, usually water. Most pesticide is applied in water as a suspension and its absorption should be tested in a suspension.

DATA ANALYSIS

Actual dose applied

The actual dose applied for each animal is calculated by subtracting the quantity of compound remaining on the materials used to apply the dose from the measured quantity in the syringe. Because of the problems frequently seen in applying suspensions, this determination is critical. It is more important to determine the actual dose applied than to obtain the nominal dose. This dose is used as 100% in calculating the percentage absorption.

Material not absorbed

The total amount of test compound that was not absorbed by each animal is the sum of the quantity remaining on the protective cover and the quantity that can be washed off the application site with soap and water.

Material remaining on/or in the application site

The quantity of test compound that remains on/or in the skin of each animal after soap and water wash is potentially available for absorption, but may also be lost by exfoliation of the

Procedure for Studying Dermal Absorption

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Introduction

This paper presents a general procedure for dermal absorption studies on pesticides which is applicable to any compound or formulation of a compound. The study requires application of various doses of radiolabeled compound to the shaven skin of male rats followed, at specific intervals after dosing, by total urine and fecal collection, determination of blood concentration, determination of the quantity in the body and determination of the quantity remaining on the skin. It is assumed that a metabolism study of the test compound has been performed in the rat before the dermal absorption study is undertaken.

The rat is used for purely practical reasons, it is not intended as a model of absorption through the human skin but rather as a test system for dermal absorption. The domestic rat is a conveniently sized animal, which is readily available and used for most of the toxicology studies on pesticides including metabolism. Because of its small size, several animals can be used per dose and several dose levels per compound within the constraints of time and resorces. Foreign compounds in general pass more rapidly through rat skin than through human skin and thus determination of dermal penetration in the rat offers a built-in safety factor for projection to human exposure.

The study described here combines two different types of dermal absorption studies in a manner which can compensate for their individual deficiencies and simultaneously cover the full range of possible dermal absorption patterns. The first type of study involves placing a measured quantity of compound on the skin for a specific period of time. The animal is then killed and the treated skin is removed. The quantity remaining on the skin is determined and the quantity of compound absorbed is calculated by subtraction. This method works very well for small quantities of a compound which does not fall or vaporize off of the skin. Large quantities, volatile compounds or strange solvents, cannot be used in this procedure.

The second type of study measures what goes into the animal. The compound is applied to the skin in a measured dose and the quantity in the body and the quantity excreted for a specific time period is measured. The procedure has greater possibilities for error in very low doses, for compounds which are not rapidly excreted and for compounds which are completely metabolized to CO_2, water and urea.

The treated animals are placed individually in metabolism cages. All urine and feces are collected, a single collection for the entire duration of exposure. At intervals of 1/2, 1, 2, 4, 10 and 24 hours, four animals per dose are anesthetized. The exposed skin and residual compound are collected separately by washing the skin with a mild soap solution followed by several water rinses. Liquid Ivory or Dove for dishwashing is suggested. The skin must be washed before killing the animals, as up to three fold differences have been observed in the ability of skin on the live animal and skin from the killed animal to bind test compounds. The animals are killed, a blood sample taken, and residual urine collected from the bladder and added to the collected urine. Any material on the protective appliance is measured. The remainder of the animal is prepared for determination of the quantity of compound in the carcass.

For each animals the following determinations are made. Results are expressed as quantity or concentration of the parent compound and as percent of applied dose. Metabolites are not separately distinguished.

1) The quantity of the compound in/on the application device and the protective appliance.

2) The quantity of compound that can be washed from the skin.

3) Quantity of compound remaining on/in the skin at the application site which cannot be removed by washing.

4) Concentration of compound in the blood and from this the quantity of compound in the blood.

5) Quantity of compound excreted in the urine and feces.

6) Quantity of material remaining in the carcass.

Results and Conclusions

From the quantity determined in parts 1 and 2 above one may calculate, by subtraction the quantity absorbed provided that other routes of loss are not significant. Excessive variation of results within groups at the same time and dose will indicate external loss of the dose.

From the quantity in the skin, the quantity excreted, the quantity in the blood and the quantity remaining in the carcass one may obtain directly the quantity absorbed. The quantity which cannot be removed from the skin by washing is considered potentially able to be absorbed and, if the amount is large, special studies may be required to quantitate its potential for absorption.

For regulatory purposes one must assume that this material is available for further absorption. However, this may not be true particularly in cases were little or no detectable compound appears in blood, excreta and/or carcass. However, studies such as the one suggested below have shown that absorption of the residue following washing can range from none detectable to essentially all, over a period of two weeks after dosing.

In such cases the following additional study is suggested.

1) Eight rats per dose are treated for the time period which shows the maximum skin concentration (or ten hours).

2) At the end of the exposure period 4 rats per dose are treated as in the basic protocol.

3) The skin of the remaining 4 rats per dose, is washed in the same fashion used in the basic study and the animals followed for at least an additional 72 hours. A study which carried the post-wash period for up to three weeks showed maximum absorption at two weeks. This appears to be a practical limit for observation.

4) The animals are then treated as in the basic protocol.

A balance comparison of the various residues will give some indication as to whether or not the quantity in the washed skin can be absorbed and quantitation of any absorption. If absorption occurs it may be necessary to repeat this process with longer post washed periods to obtain a quantitation of absorption over time.

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California Modifications
October 9, 1985

Please note. This procedure has been developed by the experimental work performed on pesticides by Registrants in their own or contract laboratories. Their continued work provides valuable and unique information on improving the experimental design and methodology. It is strongly advised that you contact the Agency before performing a dermal absorption study on a pesticide in order to take advantage of the most recent information. You may submit your protocol, through the Registration Division, for evaluation by the author of this document.