CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Dermal Absorption Study Protocols Proposed for Paclobutrazol
Tox. Chem. No. 628C; Tox. Proj. No. 7-0600.

TO: Robert J. Taylor, Product Manager 25
Registration Division (TS-767C)

THRU: Judith Hauswirth, Ph. D., Section Head Judith W. Hauswirth 6/29/87
Review Section 6
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: Roger Gardner, Toxicologist
Review Section 6 Roger Gardner 6-29-87
Toxicology Branch
Hazard Evaluation Division (TS-769) 6/30/87

Action Requested

In a letter dated April 9, 1987, the Registrant (ICI Americas Inc.) submitted two protocols for dermal absorption studies of Paclobutrazol in rats. The Registrant requested comments on the protocols from the Toxicology Branch.

Comments

Protocols A and B are generally consistent with that recommended by the Toxicology Branch (see Attachment below). However, the following points should be considered:

1. In addition to the procedures described in Protocols A and B, the shaved skin of test animals should also be washed with acetone (see Attachment, Page 2, "Methods", first paragraph).

2. The vehicle used in either protocol should be the same as the one encountered under field conditions for Paclobutrazol (see Attachment, Page 2, "Methods", third paragraph).

3. The dose range in Protocol B should be lower; 10, 1, and 0.1 mg/rat is recommended since it is a range that is more appropriate to field conditions of exposure.

4. The site of application should be protected in a nonoclusive manner that minimizes or prevents the test substance from falling off or being rubbed off the skin (see Attachment, Page 2, "Methods", fourth paragraph).

5. The application site should be washed with a mild soap and water followed by water rinses rather than distilled water as stated in the submitted protocols.

6. Radioactivity found in the skin of the application site is considered to be potentially absorbable rather than unabsorbed.

ATTACHMENT

"Procedure for Studying Dermal Absorption"
by Robert P. Zendzian, Ph. D., Pharmacologist
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Procedure for Studying Dermal Absorption

Robert P. Zendzian PhD
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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.

Materials

Twenty-four young adult male rats, 225-250 grams in weight, are used at each dose point. It is preferred that the rats be of the same strain used for metabolism studies on the test compound.

The compound should be chemically pure and radiolabeled, usually with carbon-14, in a position which is part of the "core" of the compound. The label should follow the compound and its major metabolites until excreted. The label should not be exchangeable nor should it be metabolically removed to CO_2 or become part of the one-carbon pool of the organism.

Methods

Twenty-four hours prior to dosing the back and shoulders of the rats are clipped free of hair and the area washed with acetone. Do not damage the skin.

Twenty-four animals are used per dose. A minimum of three but preferably four doses, at log intervals should be used. The doses should span the range of dose per unit area of skin which can be expected to occur in human exposure. Experience has shown that the highest useful dose is in the order of 10mg/rat with descending doses of 1, 0.1, and 0.01mg/rat. If less then four doses are used it is preferred that the lower dose range be used.

The compound is applied to a measured area of the rat's skin, at least 10 cm^2, in the form applied in the field utilizing the field solvent. When no solvent is specified, as for the technical material or a dust, the compound is dissolved or suspended in water. Organic solvents should not be used. The material is spread evenly until dry. The spreader should be checked for loss of material. The treated area is covered with a nonocclusive cover to prevent loss by falling or being rubbed off.

Experience has shown that the application area must be covered. A combination cover consisting of a rubber ring glued to the skin and a filter paper or gauze glued to the ring appears to be most effective.

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 and a blood sample taken. The animals are killed and residual urine collected from the bladder and added to the collected urine. 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. 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. 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 or 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 blood concentration of the compound can be used for a direct comparison with other studies on the compound.

Graphs relating dose, time and amount absorbed may be constructed and used to calculate absorption for doses which are not directly studied. Using proper assumptions one may extrapolate to estimate human absorption under conditions of normal exposure.

Additional procedures

1) Procedure to define compounds which are essentially not absorbed.

Results from a study of a compound expected to have little or no dermal absorption have suggested the necessity of treating an additional group of rats. In the study, analysis of the dermal residue indicated no absorption to a limit of 0.1 percent of the dose. This limit was defined by the variability of recovery of compound from the skin. The blood showed no radioactivity at any dose and duration of exposure. The urine showed radioactivity which did not appear to follow the dose and duration of exposure relationship expected. In only one of nine treatment groups were the results internally consistent with all four animals showing similar positive results. In the other eight groups the number of animals having radioactivity in the urine ranged from zero to three with a mean of 1.5. These results appear indicative of contamination of the urine rather than dermal absorption.

Under such circumstances an additional group of four rats should be treated with the high dose at the 10 and 24 hour durations of exposure. These animals should have their urinary bladders cannulated to avoid contamination of the urine collected during the exposure period. Samples of blood, urine and carcass should be counted for the longest practical time in order to produce the lowest possible limit of dermal absorption. In the case where no absorption occurs under the experimental conditions the limit of dermal absorption will be defined solely by the sensitivity of the method for detecting the radio tracer.

2) Procedure for examining compounds which show a major residue on/in the washed skin.

Several compounds have been tested which show a significant residue on/in the skin despite vigorous washing. The concentration has appeared in short exposures and shows little or no increase with time and often does not appear to increase to any large extent with increase of dose. This suggests a binding process.

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.

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 sacrificed and 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 original study and the animals followed for at least an additional 24 hours.

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

A balance comparison of the various residues should give some indication as to whether or not the quantity in the washed skin can be absorbed and some 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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