CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: DEET: Review of the Proposed Protocols for Pharmacokinetics and Comparative Dermal Absorption Studies

Caswell No. 346 HED Project No. 9-1355
EPA ID No. CSMA-DEET-20 Record No. 244321

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INTRODUCTION

In response to the Data Call-In Notice for DEET for a metabolism study and a age-related dermal absorption study, the registrant, the DEET Steering Committee of Chemical Specialties Manufacturers Association, submitted the protocol for these two studies with rats. The metabolism study with oral administration proposes to examine the absorption, distribution, metabolism, and excretion of DEET. The dermal absorption study also evaluates the absorption, distribution, metabolism, and excretion patterns of the test article as a function of age.

DISCUSSION

The summary of the study design is excerpted from the report and presented in Table 1. The proposed protocol for oral administration follows closely the recommendations in Subdivision F Guidelines for metabolism study with the exception of the procedure for repeated oral administration. The protocol proposed to administer the unlabelled DEET in feed for 13 days whereas the Guidelines recommend that the test agent be introduced by gavage. This reviewer has discussed this matter with Gerald Schoenig,

Ph.D., the toxicology consultant for the DEET Steering Committee. It would be more precise and simpler to administer the unlabelled DEET by gavage than by feed. In addition, with daily administration by gavage, an experimenter can adjust the dose daily according to the changing body weight of the rats. Therefore, this reviewer recommends that the repeated dose of the unlabelled DEET be administered by gavage.

For the comparative dermal absoption study, the protocol would be acceptable if more dose levels were to be tested. A set of produres prepared by a senior pharmacologist, Robert Zendzian, for dermal absorption study is attached. The registrant is encouraged to follow the experimental procedures presented in the Attachment, Procedure for Studying Dermal Absorption.

The registrant should be commended for the proposed evaluations of the tissue concentrations of the radioactivity at peak blood levels in both oral administration and dermal absorption studies.

Table 1*

Study Design for Definitive Portion of DEET

Pharmacokinetic and Comparative Dermal Absorption Study

Group No.	Route of ^{14}C-DEET Administration	^{14}C-DEET Dosage Level (mg/kg b.w.)	Number of Rats: Male	Female	Age of Rats	Time of Sacrifice After ^{14}C-DEET Administration
1	Oral	100	5	5	Adult	Seven days
2	Oral	500	5	5	Adult	Seven days
3	Oral[1]	100	5	5	Adult	Seven days
4	Oral	100	-	5	Adult	Peak ^{14}C blood levels
5	Dermal	100	5	5	Adult	Seven days
6	Dermal	100	-	5	Adult	Peak ^{14}C blood levels
7	Dermal	100	5	5	Weanling	Seven days
8	Dermal	100	-	5	Weanling	Peak ^{14}C blood levels

[1]Animals in this group will be fed diets containing technical grade DEET at a concentration of 1000 ppm (equivalent to 100 mg/kg b.w.) for 13 days prior to administration of a single oral dose of ^{14}C-DEET.

* Summary excerpted from the report (EPA ID No. CSMA-DEET-20)

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ATTACHMENT

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.

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. Double labeling may sometimes be necessary. Unlabeled compound may be used if a sufficiently specific and sensitive test is available.

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 10 mg/rat with descending doses of 1, 0.1, and 0.01 mg/rat. If less then four doses are used it is preferred that the lower dose range be used. Doses must be mass/unit area of skin (mg/cm^2) and not mass/body weight (mg/kg) since the rate of absorption is directly related to mass/unit area.

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. Usually the use product (emulsifiable concentrate, flowable powder etc.) is used for the highest dose and is diulted with water for the lower doses. 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 and to prevent the animal eating the test material.

Experience has shown that the application area must be covered. A combination cover consisting of a 'spacer' glued to the skin and a filter paper or gauze glued to the ring appears to be most effective. The 'spacer' will outline the application site and be sufficiently thick to hold the cover from contact with the site.

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.

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 appeared 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. 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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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.