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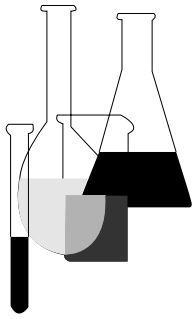
Health Effects Test Guidelines

OPPTS 870.7600
Dermal Penetration



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 870.7600 Dermal penetration.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, et seq.) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** This guideline was developed in the Office of Pesticide Programs of and published as OPP 85–3 Dermal Absorption Studies of Pesticides (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982. Dermal absorption studies are not routinely required.

(b) **Discussion.** (1) Dermal absorption studies are complex kinetic studies which, in and of themselves, provide no information on the biological activity (toxicity) of a compound. 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 percent dermal absorption does not produce an adequate margin of exposure (MOE). That is, a risk assessment must be performed to determine the need for a dermal absorption study. Dermal absorption studies cannot substitute for general dermal toxicity studies of up to 90 days of dosing (OPPTS 870.3200 and OPPTS 870.3250), which must be performed by the dermal route to assess direct toxicity to the skin (and systemic toxicity as well).

(2) It is recommended that studies showing significant toxicological effects, first identified by the oral or inhalation route, be repeated by the dermal route where practical, rather than performing a dermal absorption study. Examples are studies of organ, system, or physiological process specific short term toxicity such as developmental toxicity, immunotoxicity, or neurotoxicity studies. Low-effect and no-effect doses from such studies can be used directly in the calculation of and MOE without the necessity of kinetic evaluations. This approach is preferred since the dermal route may produce differences in distribution, metabolism, storage, and excretion from the oral or inhalation routes which can produce qualitative as well as quantitative differences in the toxicity of a compound. Such differences are not easily identified by kinetic or metabolism studies and can have significant effects on the systemic toxic response.

(3) Information on dermal exposure is necessary in order to determine doses and durations of exposure to be evaluated in the dermal absorption study. It is expected that this information will have been gathered in order to perform the risk assessment and to make the basic decision as to whether the study is required.

(4) An oral kinetic study in the rat is strongly recommended in order to allow full utilization of the dermal kinetic study in risk assessment.

It is recommended that the oral kinetic study be performed before the dermal absorption study is undertaken. The study must determine the portion (percent) of the oral dose that is absorbed and the rate of absorption, the rate of tissue distribution, the routes and rate of excretion and be sufficient to allow half-life determinations for each of the rates.

(c) **Purpose.** Data from dermal absorption studies allow the Agency to make risk determinations in cases where the toxic effect has been determined by the oral or inhalation route in the experimental animal and the exposure to humans is by the dermal route. Such determinations may vary in degree of complexity ranging from the 100 percent assumption used to justify performing the dermal absorption study to a complete kinetic analysis. A complete kinetic analysis essentially enables an investigator to convert oral or inhalation low-effect and no-effect doses into dermal low-effect and no-effect doses, thus allowing the calculation of an MOE or risk for systemic toxic effects which have not been or cannot be tested practically by the dermal route. The analysis can be performed in several ways. The first involves calculating the maximum systemic doses produced by the oral (or inhalation) no-effect and low-effect doses and calculating dermal doses that will produce the same maximum systemic doses. The calculated dermal no-effect and low-effect values are then compared with the dermal exposures from the risk assessment. In a second method the dermal exposure is converted to a maximum systemic dose which is compared with the maximum systemic doses equivalent to the oral no-effect and low-effect doses.

(d) **Material to be tested**—(1) **Compound.** The compound should be of known chemical purity and radiolabeled, usually with ^{14}C, in a position which is part of the ‘‘core’’ of the compound. The label should follow the compound and its major metabolites until excreted. To the extent practical, the label should not be exchangable nor should it be metabolically removed to $C0_2$ or become part of the one-carbon pool of the organism. Other radioactive isotopes such as ^{35}S, ^{36}Cl, and ^{113}Sn or stable isotopes such as ^{15}N and ^{18}O may be used, particularly if the element is responsible for, or is a part of, the toxic portion of the compound. Labeled compound may not be required if a sufficiently selective and sensitive physical/chemical test for identifying the compound is used. The specificity and sensitivity of the test must be demonstrated in the biological systems (organs, tissues, and body fluids) being analyzed and a report of this validation must be included in the report of the dermal absorption study. Use of a physical/chemical test may require the use of control (untreated) animals if background interference with the procedure is observed.

(2) **Vehicle/solvent**. The vehicle system used should duplicate that under which field exposure occurs. The basic vehicle is usually the material used in the commercial formulation (formulation blank). Dilutions are made with the field vehicle, usually water, to produce a solution or suspension. In cases where exposure is to the chemical and not a formulation

or dilution thereof, a neutral suspending agent which does not interact with the test chemical or affect the permeability of the skin, such as carboxymethylcellulose, may be used. However, organic solvents or special solubilizing/suspending agents must not be used. Formulations applied dry (dusts, granulars, etc.) should be moistened with water to assist in the application a quantitative dose. This moisture mimics to some measure the presence of perspiration in field use.

(3) **Dose preparation.** Dose solutions/suspensions should be prepared so that sufficient quantities of radiolabel are present in each dose to allow sufficient sensitivity for that dose. The sensitivity required for a dose depends upon how small a quantity of the test compound is considered necessary to produce a toxic effect. If it is not possible to detect a significantly small fraction of that quantity, it will not be possible to determine if the quantity absorbed allows an acceptable MOE or produces a toxicologically acceptable risk. The dosing solutions/suspensions may be obtained by any practical and reliable method that the laboratory can devise. Predictability of dose preparation is most important—it is critical to know the actual concentrations obtained and that the dosing material is homogeneous so that the actual dose administered is known. It is not critical that the nominal dose be precisely administered.

(e) **Test procedure**—(1) **Variation of procedure.** The basic study described is designed to cover the entire range of doses and durations of exposure expected for a pesticide designed for a wide variety of uses. It is frequently possible to cover the use pattern at risk for a particular pesticide with a lesser number of doses and durations of exposure. In the case of pesticides having a limited pattern of use, Registrants may, after consultation with the Agency, perform only those doses and durations of exposure that are applicable to the use pattern which is being considered for risk assessment.

(2) **Test animal**—(i) **Species and strain.** The laboratory rat is the required species because this guideline has been designed and validated for the rat. Other animal species have been considered and rejected. It is recommended that the rats be of the same strain as those used for the metabolism and toxicology studies on the test compound. The rat is not intended as a model of absorption through the human skin but rather as a test system for dermal absorption. It is possible to use the absorption rates determined as a modest overestimate of human dermal absorption or to perform a kinetic evaluation as described in paragraph (c) of this guideline for risk assessment purposes.

(ii) **Age.** Young adult animals should be used.

(iii) **Sex.** The male rat should be used. The choice of a single sex allows consistency and comparison from study to study.

(iv) **Numbers.** A total of 24 animals is used at each dose level. This is based on four animals per dose per exposure duration, the minimal experimental unit. Studies using fewer exposure durations will use fewer animals per dose but the same number (four) per duration of exposure.

(3) **Dose levels and dose selection.** (i) Four doses are recommended; at least three doses should be used. Experience has shown that absorption is not dose-linear—as the dose per unit area increases the flux increases to a lesser degree. Some compounds have shown saturation of the absorption process (no increase of flux with dose). Thus, the quantity and rate of absorption determined at one dose is generally not valid at a dose which is greater or less by more than approximately one-half of a log unit. Compounds that are caustic or are dermal irritants should be tested only at doses which do not show such effects. Direct dermal toxicity is considered an unacceptable risk and compounds which show such effects are regulated using the low-effect and no-effect doses for direct dermal toxicity.

(ii) Doses should be at log intervals (i.e. 1.0, 0.1, 0.01 and 0.001 mg/cm^2) and span the range of doses expected in field exposure. The numbers are examples—the interval is the important factor. The maximum practical dose is on the order of 1 mg/cm^2—larger doses tend to fall off the skin or exceed saturation of the absorption process. When only three doses are qiven the highest dose should be on the order of 0.1 mg/cm^2. When different forms of the compound are tested (salts, esters, etc.), comparative doses should be equimolar to the doses of the parent compound.

(iii) Doses must be determined on the basis of quantity per unit area of exposed skin (expressed as milligrams per square centimeter, not on the basis of quantity per unit of body weight (milligrams per kilogram). In this study the skin is being dosed to determine its permeability to the test compound which is dependent on the dose to the skin. The test animal per se is not being dosed.

(iv) The maximum dose volume should not exceed 10 $\mu L/cm^2$. Larger volumes of liquid have been found to flow on the skin and produce uneven dose distribution on the dosed area.

(4) **Duration of exposure.** In the full study four animals per dose are exposed for durations of 0.5, 1, 2, 4, 10, and 24 h. For an abbreviated study, designed for a single exposure scenario, the recommended minimal durations of exposure are 1, 10, and 24 h. The evaluation with time is recommended since experience has shown that skin deposition (wash-resistant) and penetration are rarely linear with time, the greatest variation occurring in the first 1 or 2 h of exposure.

(5) **Administration of the test substance**—(i) **Animal preparation.** The back and shoulders of the rats are clipped free of hair and the area wiped with acetone 24 h prior to dosing. A soap and water wash may

be used but care must be taken to rinse the area throughly to remove residual soap. A soap and water wash is generally harder on the skin than an acetone wipe. Clipping and wipe produce a standardized skin condition at the time of dosing. Use of a shorter interval between skin wipe and dosing has been shown to increase dermal penetration. Animals showing damage to the skin at the time of dosing should not be used.

(ii) **Dose application.** The measured dose of the compound is applied to a measured area of the rat's skin of no less than 10 cm^2. Because most dose forms are suspensions, this minimal area is necessary for even spreading. The material is spread evenly until a film is formed over the application site. The spreader should be checked for retention of material and the actual dose applied should be determined by subtraction of retained material from the total dose. Particular attention must be paid to determining the actual dose applied.

(iii) **Site protection.** The application area must be covered to prevent loss of compound through falling off, being rubbed off, or being licked. The cover must allow air circulation over the application site to allow normal evaporation of surface water from the skin. A combination cover (protective device) consisting of a spacer (a rubber, plastic or glass rectangle, square, or ring glued to the skin) to outline the application site and a filter paper or gauze cover glued to it is recommended. The spacer should be impervious to the test solution. The paper or gauze should not be in direct contact with the test material or the skin. See paragraph (g)(5) of this guideline for information on volatile chemicals.

(6) **Animal processing.** The treated animals are placed individually in metabolism cages. Expired air should be collected if a metabolism study shows that label is expired. Total urine and feces are collected separately (a single collection for the entire duration of exposure). At the exposure intervals (0.5, 1, 2, 4, 10 and 24 h for the standard study) four animals per dose are anesthetized, exposed skin is washed with a mild soap/detergent solution followed by several water rinses, to mimic human washing, and the protective device is removed. The skin at the exposure site must be washed before it is removed from the animal; contact of the wash solution with the cut edge or undersurface of the skin has been shown to produce artifactual binding of test compound. Liquid detergent designed for dishwashing is suggested for the wash solution. The animals are killed and a blood sample collected from the heart or post cava. Residual urine is collected from the bladder and added to the collected urine. The exposed skin, selected organs (if part of the experimental design), and the remainder of the animal (carcass) are collected and prepared for determination of the quantity of compound therein. See paragraph (g)(4) of this guideline for recommendations on organ/tissue collection.

(7) **Sample analysis.** A total material balance must be obtained for each animal. Total compound must be determined in each of the following

samples: Urine, feces, blood (for consistency, it is recommended that blood mass be assumed equal to 7 percent of body weight (see paragraphs (i)(1) and (i)(2) of this guideline), wash from the skin, material in or on the protective device, material remaining in or on the washed skin, material in selected organs (if collected), and residue in the carcass. Concentration of the test compound should be determined in the blood and any organ samples collected.

(f) **Data and reporting**—(1) **Data to be reported.** The study report should include the following information/data derived from the experimental procedure on all animals in all groups:

(i) The method of determination of the limit of sensitivity and the limit of sensitivity for each sample type in each dose group.

(ii) The actual quantity of test compound administered to each animal and the mean for each experimental group of four animals.

(iii) Count (disintegrations per minute per gram), quantity of isotope, quantity of compound and percent of actual dose administered in each sample for each experimental animal in each experimental group and the mean of those values for each experimental group of four animals.

(iv) The concentration, quantity, and percent of dose of test compound in the blood and all tissues analyzed for each animal and the mean of that value for each experimental group of four animals.

(v) Mass balance totals for each animal in each experimental group and for the means for each experimental group.

(vi) Determination of the quantity and the percent absorbed for each animal and for the group (mean). The quantity absorbed is that portion of the dose which enters the systemic compartment of the organism. The quantity in/on the skin is localized in the epidermis (mainly the stratum corneum) and is not available for systemic distribution and toxicity until it enters the vascular dermis. This determination is based on the following distribution of the administered dose:

(A) Not absorbed—quantity in skin wash, and on the protective cover.

(B) Absorbable—quantity in/on the washed skin.

(C) Absorbed—quantity in the urine, cage wash, feces, expired air (if present), blood, organs (if collected), and the remaining carcass.

(2) **Final report format and content.** The final report should contain the items that are listed.

(i) **Cover page and regulatory documentation.** A cover/title page and additional documentation (i.e., requirements for data submission, good laboratory practices (GLP) statement and statement of data confidentiality

claims), if relevant to the study report, must precede the content of the study format. These requirements are described in 40 CFR parts 158 and 160.

(ii) **Table of contents.** The table of contents should include a listing of the e1ements of the final report such as the summary, an introduction, the materials and methods, results, discussion, bibliography, tables, figures, appendices and key subsections as appropriate.

(iii) **Body of the report.** This item should include such detail that the reviewer can assess the quality of the study and conformity to the dermal absorption guidelines or an approved variation of the guidelines. It should contain the following sections:

(A) *Summary.* The test report should contain a summary including a brief description of the study protocol, chemical used, the animals tested, and the highlights of the results of the study. Any deviations from the intended protocols should be noted.

(B) *Introduction.* Include the objectives of the study and the Guideline reference. The overall experimental design should be explained.

(C) *Materials and methods.* (*1*) Test substance. An identification of the material tested and vehicles used to include the following:

(*i*) Test material used in test (chemical name, CAS No.)

(*ii*) Properties of the test substance: Chemical structure, form, radiolabeled, technical label-position, source, radiopurity, lot number, source, purity, lot number purity, state (liquid or solid), ionization constant (if applicable), pH (if applicable), solubility in various solvents (if known), octanol/water partition coefficient (if known)

(*iii*) Vehicles used (if a formulation vehicle is used, it must be identified but its confidential composition need not be included in the report), source, lot number.

(*2*) Test animals. An identification of the experimental animals used to include the following: Species and strain, sex, source, body weight range, pretest condition, housing conditions.

(*3*) Experimental design. The experimental design, as performed in the study, must be described in complete detail. A step-by-step description of the entire study in sufficient detail to allow precise understanding of how the study was performed is required. The description should include, but not necessarily be limited to, the following: Doses used, number of animals per dose group, duration of exposure, preparation of the application site, area of the application site, dose preparation, dose application, dose quantitation, method of protecting the application site, urine, feces and expired air collection, termination method, sample collection methods,

skin wash, protective device, skin at application site, blood, individual organs (if collected), residual carcass, urine, feces.

(*4*) Evaluation procedures. A detailed description of the methodology of the study to include, but not necessarily be limited to, the following: Method of assignment of animals to test groups, method of verification of radiopurity, method of determination of actual dose method of sample collection, storage and analysis, detailed secondary procedures, such as sample analysis, should be included in the appendices.

(*5*) Deviation from protocol. Deviations from the protocol or from an approved variation thereof must be described along with the rationale for the changes.

(D) *Results*. This section should provide a narrative summary of the results of the study. Data generated are best presented in summary tables and figures included in paragraph (h) of this guideline.

(E) *Discussion.* (Optional) This section should provide an assessment of the results of the study, an interpretation of the observations and should try to explain unexpected findings. The impact of any deviation from the guideline protocol should be discussed.

(F) *Bibliography.* (Optional) Complete citations of any documents referred to in the report. Referenced documents may include previous reports, correspondence to and from the Agency, publications in the technical literature, and Agency guidelines. Each citation must be sufficiently complete so as to allow identification and retrieval of the document.

(G) *Tables/Figures.* These tables and figures should summarize and illustrate the results of the study by presenting mean values for each experimental group of four animals. Examples are given at the end of this guideline.

(H) *Appendixes*. These should include individual animal data, analytical methods, results of analysis of the test substance and the dose formulation, protocol, sample calculations and other information as appropriate.

(g) **Additional dermal absorption studies.** Additional, more specific studies may be necessary to clarify important points raised in toxicity, metabolism or kinetic studies of a compound. The studies presented below provide a beginning or outline for designing compound-specific studies but the individual studies must be designed specifically for the test compound and the toxicology or kinetic issues of concern. Consultation with the Agency before performing such a study is strongly recommended. The individual sample collections/special treatments may, where practical, be combined with or added to the basic study. Additional studies are as follows:

(1) **Significant quantity of residue remaining on the washed skin.** (i) For some compounds the washed skin has been found to retain significantly more material than was absorbed during the exposure period. In some cases the difference between absorbed and retained material is sufficient to convert an acceptable risk into an unacceptable risk if all the retained material is considered as absorbed. This study is designed to determine the fate of that residual material, in particular the portion absorbed and the rate at which it is absorbed.

(ii) Selected dose levels, as determined from the risk assessment, are administered to groups of four animals. At 10 h one group from each dose is terminated as in paragraphs (e)(6) and (e)(7) of this guideline. In the remaining groups the skin is washed at 10 h, the wash sample collected for analysis and the groups carried for one or more additional days, up to 14 days, in metabolism cages. A minimum of 14 days has been suggested by absorption data and a maximum of 21 days has been suggested by cornified epidermal turnover time in the rat. Suggested exposure durations, per group of four rats, are 10 h, and 1, 2, 7, 14, and 21 days. Urine and fecal samples are collected for 24 h intervals. Expired air is collected if labeled material is expired. At termination samples are collected from each animal as in paragraphs (e)(6) and (e)(7) of this guideline. Samples should include any organs/tissues collected in the original study.

(2) **Determination of metabolites.** Experience has shown that both qualitative and quantitative production of metabolites of foreign compounds can vary significantly with route of administration. When testing compounds for which it has been determined that specific metabolites are of toxicological concern, urine and fecal samples collected in the basic dermal absorption study may be analyzed for the specific metabolites and their proportionate production. It may be necessary to dose additional animals to provide sufficient excreta samples. Blood concentration of label in dermal absorption studies has shown that compound concentration is usually too small to allow metabolite identification.

(3) **Blood/plasma kinetics study**. (i) This study is designed to provide data to be compared with blood/plasma concentrations at effect and no-effect doses by the route by which the toxic effect was originally identified (usually oral). The same species must be used for oral and dermal determinations. When using these data it is not necessary to obtain and utilize absorption and excretion parameters in determining MOEs by different routes of administration. Kinetic comparisons of the effect of route on biological activity are performed by comparing concentrations of active chemical at the active site following administration by different routes. This information is extremely hard to obtain, so that obtaining it for any chemical other than the most important of human drugs is usually impractical. The most practical surrogate data are blood/plasma concentrations following different dosing routes. An oral blood/plasma kinetics study must be performed, using at least the effect and no-effect doses from the

critical toxicology study and data on blood/plasma concentration with time as well as volume of distribution and disappearance parameters (half-lives) obtained before the dermal kinetic study is performed.

(ii) The test compound must be radiolabeled and the radioactivity in each dose must be sufficient to detect the same minimal concentration in the blood/plasma. That is, the limit of detection for each dose must be the same in mass of test material per unit mass of blood/plasma. The limit of detection must be at least one-hundreth of the maximum blood/plasma concentration observed following the no-effect oral dose to allow detection of a hundredfold MOE. A proportionately smaller limit of detection is necessary for a proportionately larger MOE. If the appropriate limit of detection cannot be practically obtained the study should not be performed.

(iii) Dermal doses should be selected to bracket the doses (expressed as milligrams per square centimer) reported for the exposures at risk and should be at log intervals. Dose preparation, application and protection of the active site should follow the procedures in the basic dermal absorption study. The only samples collected for analysis are dosing material (to determine concentration, homogeneity, and dose applied) and blood (to determine blood/plasma concentration with dose and time). Analysis of both whole blood and plasma concentrations is recommended. The rat has been found to bind many chemicals to the erythrocyte and these data will allow detection of this process which can have significant effects on the apparent whole blood kinetics of the bound chemical.

(iv) A minimum of four animals should be used for each dose-duration-of-exposure data point. Depending upon the size of the blood sample collected and the time between collections, it may be possible to collect more than one sample from each animal. However, because of the limited blood volume in the rat, it is recommended that individual groups of four rats be used for each exposure (blood sample collection) period.

(v) It is impossible to predict the timing of blood sample collection following dermal dosing that will be necessary to define the blood/plasma concentration curve for a particular chemical. Therefore, a preliminary study is recommended. Use the highest dose proposed for the study, one or two animals per dose, and collection intervals of 1, 2, 4, 10 and 24 h. Sample collection time can be adjusted from these data to better fit the expected blood/plasma concentration curve. It must be possible to identify the peak blood/plasma concentration and characterize the curve leading to and following it. Note: If blood/plasma concentration is below the limit of detection for this high dose, it will not be necessary to run lower doses. No detectable radioactivity can be expected at the lower doses and the MOE will be derived from the limit of detection following the high dermal dose.

(4) **Organ/tissue collection.** (i) If a target organ for the toxic effect of the test compound has been identified, it may be useful to determine test compound concentration, with time and dose, in that tissue following dermal absorption. Such information can used directly in risk assessment by comparing the maximum concentration in the target organ following dermal administration with the maximum concentration following the effect and no-effect doses by the route used in the critical toxicology tests. Because of the possibility of saturation of absorption by the dermal route, it may be impossible to reach a toxic concentration in the target organ by dermal dosing indicating that there is no risk of concern associated with dermal exposure.

(ii) Organs should be collected during, as part of, the basic dermal absorption study. Both concentration and total amount of test material in the organ (and/or tissue sample) must be obtained; concentration for comparison between organs and blood in order to detect evidence of bioaccumulation in a particular organ and total amount as part of the material balance for the dermal absorption study. Organs/tissues suggested for collection are: The target organs, liver and kidney as metabolic/excretory organs; heart, lungs, spleen, gonads, adrenals, pancreas, as discrete critical organs; brain as indicative of the effect of the blood brain barrier; muscle as a fast storage tissue; and fat as a slow storage tissue.

(5) **Volatile compounds.** (i) Dermal absorption studies of volatile compounds can be compromised by inhalation of the vapor, condensation of the vapor in the metabolism cage with ingestion and condensation of the vapor on additional areas of the skin. Use of an impermeable cover on the protective device to counter these effects will produce unrealistic absorption data. A cover on the protective device consisting of filter paper impregnated with activated charcoal (or a material such as XAD4 amberlite resin) has been shown to be effective in trapping vaporized organic test material. A preliminary in vitro test is recommended to determine the effectiveness of activated charcoal or resin for a particular compound. For such a test the spacer is glued to a glass plate, a small measured dose of test material placed within and covered with the charcoal or resin cover. This model is placed in a metabolism cage maintained at rat body temperature with a trap on the air outflow to determine whether any test material escapes the charcoal. After 1 to 2 h the distribution of the dose is determined from glass plate, spacer, charcoal or resin cover, metabolism cage wash, and trap. If the dose is confined to the glass plate, spacer and cover, the study can proceed. If material is found in the cage wash and/or the trap it may not be possible to perform the study and advice should be sought from the Agency.

(ii) The experimental design of the dermal absorption study should follow that of the basic dermal absorption study or an appropriate variation thereof. A trap may be necessary on the air outflow of the metabolism

cage if the test compound is excreted by respiration. It should also be determined if the test material evaporates from the excreta.

(6) **Infinite dose studies.** (i) This study is designed to address dermal absorption of the test compound while swimming or bathing, during which the individual is exposed dermally to a constant concentration of pesticide from an essentially unlimited source. This is a very tricky study and should be designed in close consultation with the Agency.

(ii) In this study a reservoir is glued to the skin of the rat, filled with a water solution of the test compound and covered with an impervious cover. The exposure area is defined while the dose is varied by varying the concentration of test compound. Concentrations tested should be at log intervals and selected to bracket the expected field exposure. Exposure durations should be chosen to match time in the water for the swimmer or bather.

(iii) Test compound, test animal, animal preparation, animal processing, and sample analysis should be essentially the same as in the basic dermal absorption study or an appropriate variation thereof. Results of this type of study are expressed as flux (mass per unit area per unit time).

(h) **Explanatory Documentation.** For explanatory documentation of the Guideline write, Public Docket and Freedom of Information Section, Field Operations Division, Office of Pesticide Programs, Environmental Protection Agency, Washington, DC 20460, or call (703) 305–5805. Ask for OPP–00369 (no charge).

(i) The following references should be consulted for additional background material on this test guideline.

(1) *Laboratory Animal Medicine.* Fox, J.G., Cohen, B.J., and Loew, F.M., eds. Academic (1984).

(2) *The Laboratory Rat.* Baker, H.J., Lindsey, J.R. and Weisbroth, S.H., eds. Volume I, Biology and Diseases. Academic, p. 108 (1979).

(3) Zendzian, R.P. Skin Penetration Method Suggested for Environmental Protection Agency Requirements. *Journal of the American College of Toxicology* 8:829–835 (1989).