A Tiered Approach to Pharmacokinetic Studies

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Studies of absorption, distribution, metabolism, and elimination (ADME) have long been recognized as important in the evaluation of the pharmacological efficacy of pharmaceutical agents. In recent years, the importance of ADME studies in toxicology also has become increasingly apparent. In realization of the importance of ADME studies, regulatory agencies have established guidelines governing the conduct of these studies. To be of maximum utility, it is desirable that ADME and pharmacokinetic studies be closely integrated with the toxicity testing protocol. However, in many instances this is not the case, which results in ADME and pharmacokinetic studies that are often chronologically and philosophically remote from the toxicity testing protocols. An inevitable consequence of this approach is that it frequently leads to the generation of ADME data that are of limited use in the process of toxicity evaluation and risk assessment. Recently, there has been increased focus on developing testing strategies that would result in the development of ADME data with greater application to toxicity testing and risk assessment. An example of such an approach is the concept of a tiered approach to the conduct of ADME studies. An important aspect of the tiered approach is generating ADME data at an earlier stage during the toxicity testing of a chemical. This could be effected by acceptance of the concept of a minimum experimental data set for a chemical. This minimum data set could be conducted in a timely and economic manner and would develop data addressing three fundamental questions: Is the chemical absorbed? Is the chemical metabolized? Does the chemical persist? The data generated under a minimum data set scenario would not be designed to provide sufficient information for utility in risk evaluation. However, it would provide important information at a much earlier stage of toxicity testing than currently generated under existing testing strategies. Such information would be of importance in the design of toxicity testing studies. Additional ADME and pharmacokinetic information could then be conducted when a specific concern (e.g., toxicity) becomes apparent. The advantage of this approach is that it allows the design of these additional follow-up studies to be tailored to the particular toxicity or risk-evaluation end point (e.g., target organ, species extrapolation, route evaluation, etc.). The specific of the experimental aspects of the design of ADME and pharmacokinetics studies are discussed. In this development of alternate, and more efficient procedures, for the conduct of metabolism studies, it has become apparent that the potential use of ADME data obtained under studies designated by the regulatory guidelines is often of little use in addressing the major concerns of risk assessment (i.e., species, dose, and route extrapolation). In considering alternate approaches it has become apparent that increased use of dosimetry models such as physiologically based pharmacokinetic models could have significant utility in improving the risk assessment procedure. In recent years there has been growing support for the pharmacokinetic modeling approaches and, in particular, physiologically based pharmacokinetic (PBPK) models have been increasingly used in risk assessment by providing a unified description of the dynamics of chemicals and their metabolites in the blood, specific tissues and excreta. In addition to providing a dosimetric of the relationship between the exposed concentration and tissue dose, these models can also be linked to so-called biologically based dose-response models. These latter models are being developed to incorporate information on our understanding of toxicological mechanisms. Such models, in conjunction with PBPK models provide an improved biological basis for examining the relationship between chemical exposure and effect. The advent of these models heralds the prospect of reducing the uncertainty in the risk assessment process. — Environ Health Perspect 102(Suppl 11):5–11 (1994)

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Introduction

Studies of the metabolism and pharmacokinetics of chemicals are recognized as critical elements in the safety evaluation of chemicals (1). Regulatory guidelines for the conduct of metabolism and pharmacokinetic studies exist under the U.S. Environmental Protection Agency (U.S. EPA [FIFRA]), Food and Drug Administration (FDA), the Organization for Economic Cooperation and Development (OECD), the European Economic Community (EEC) and in Japan. Metabolic and pharmacokinetic studies conducted on specific chemicals which fall under the jurisdiction of the EPA are typically addressed in EPA/TSCA (Section 4) Test Rules for the respective chemical and EPA/FIFRA testing for pesticide registration. Recently, the Office of Toxic Substances of the EPA issued a proposed generic draft guideline for the conduct of metabolism and pharmacokinetic studies. Prior to the issuance of this guideline, a number of chemical companies had formed an association (The Pharmacokinetics Group) under the auspices of the Synthetic Organic Chemical Manufacturers Association (SOCMA). The purpose of this SOCMA Pharmacokinetics (PK) Group was to provide a discussion forum for approaches to the study of the pharmacokinetics and metabolism of chemicals and the application of this data in safety evaluation. The SOCMA PK Group strongly supported the incorporation of PK and metabolic considerations into the risk assessment process and the need for a pharmacokinetic guideline under TSCA. The SOCMA PK Group, however, was concerned that flexibility be incorporated into any proposed guideline since there is an implicit need for PK studies to be tailored to the specific chemical, its proposed use, and route of potential human exposure. In addition, the SOCMA PK Group hoped that harmonization of the proposed TSCA guideline with other metabolism and PK guidelines would be possible. Recent TSCA section 4 test rules had raised concern that much of the PK data requested by the EPA was not tailored to the proposed use of the chemical and, therefore, was not of maximal utility in risk.
assessment. Following discussion with the EPA about these concerns, it was decided to convene a workshop that would provide a dialogue on the design and conduct of pharmacokinetic studies and to reach clarification and agreement on technical issues regarding the design of pharmacokinetic studies.

The Pharmacokinetics Workshop was organized by the Office of Toxic Substances (OTS) and the SOCMA Pharmacokinetics Group. The workshop was held at the Key Bridge Marriott, Washington, DC, on March 7 and 8, 1990. Over 50 experts in various aspects of metabolism and pharmacokinetic studies participated.

Following introductory remarks from L. Tahan (U.S. EPA, OTS), R. Zendzian (U.S. EPA, OPP), and A. Wilson (Monsanto Company) and SOCMA Pharmacokinetics Group attendees participated in focused breakout sessions that covered the following topics: minimum data set requirements, conduct of dermal pharmacokinetic studies, conduct of inhalation route pharmacokinetic studies, conduct of pharmacokinetic studies by oral and other routes, consideration of dose and animal selection, metabolite identification and characterization, and integration and sequencing of pharmacokinetic studies.

Pharmacokinetic Decision Tree—Tier System

Metabolism and PK studies are often chronologically and philosophically remote from the toxicity testing protocols. The lack of correlation between the two types of tests significantly affects the utility of metabolism and PK information in safety assessment. To be of maximal utility, metabolism and PK studies must be integrated with the toxicity studies for the particular chemical and must be targeted to a toxicologic end point. Under the present TSCA section 4 test rules, this is rarely possible because metabolism and PK studies are typically conducted consecutively with toxicity studies. Therefore, potential target sites for toxicity are generally not known at the time the metabolism and PK studies are conducted.

The drawback to this approach is that in the event of toxicity the metabolism and PK studies would frequently be of little use in addressing the toxicity concern. In the event of a toxic response, additional metabolic studies typically would be needed to develop data that would have a greater probability of addressing any safety concerns of the chemical. To address this question of more closely integrating metabolism and PK studies with the toxicity testing and the risk assessment of a chemical, the concept of a decision tree approach for the conduct of metabolism and PK studies was developed. The basic outline of this tiered approach is presented. Initial studies (tier 1 or minimum data set) could provide the basis for decisions concerning the need for additional pharmacokinetic studies. If required (pharmacokinetic or toxicologic considerations), then additional studies (tier 2 and 3) would provide data useful in risk assessment.

Minimum Data Set (Tier 1)

An essential component of this tiered approach is the concept of a minimum data set. The specific components of the minimum data set are outlined in the following section. It was recognized by the group that this minimum data set would not, in many cases, be sufficient to assist in the design of toxicity studies or interpretation of toxicity data. Rather the concept for the minimum data set (tier 1) was conceived as being the minimum that would be acceptable for assessing critical elementary characteristics of the chemical, and would provide the basic information for decisions concerning the need for additional pharmacokinetic studies. The minimum data set constitutes tier 1 of a three-tier system (Table 1, Figure 1). The specific study requirements for the minimum data set are outlined below.

The Work Group agreed that the minimum data set requirement (tier 1) would ask three fundamental questions: Is the chemical absorbed? Is the chemical metabolized? Does the chemical persist? These questions are outlined as follows and discussed in detail below.

- Is the chemical absorbed? Suggested: iv/additional route excreta and/or blood
- Is the chemical metabolized? Suggested: separation of parent from metabolites (e.g., excreta, blood)
- Does it persist? Suggested: whole-body elimination and/or blood kinetics

Is the Chemical Absorbed?
The study should be conducted using the iv and an additional route. The additional route can be the oral or the most likely route of human exposure. This study would in most cases be conducted using radiolabeled material, although a nonradiolabeled material would be acceptable providing a sufficiently sensitive analytic procedure exists. The extent of absorption could be determined by any acceptable and validated procedure, for example:

Measurement of the Levels of Radioactivity in Urine or Expired Air. If feces are to be used to measure absorption, then verification that the material present in the feces is either a metabolite or that the parent chemical can be absorbed and excreted unchanged must be demonstrated.

Determination of Radioactivity in Blood Samples. Blood samples should be collected at sufficient time intervals to permit estimates of extent of absorption and time course of elimination from the blood area under the blood concentration and time curve.

Is the Chemical Metabolized?
To determine whether a chemical is metabolized, it would be required to analytically demonstrate whether the material analyzed was parent or metabolite(s). It is not, however, required at the tier 1 stage to characterize or identify metabolites. It is forseen that a characterized separation system, capable of distinguishing parent material from metabolites, would be sufficient to answer the question. The matrix for analysis would be flexible but would in most cases include excreta or blood.

Does the Chemical Persist?
An assessment of the potential for the chemical or its metabolites to persist in the body is highly recommended. Determination of whole body elimination would be sufficient and could be determined using excreta data. Direct tissue or blood analysis could also be used to address the persistence of a chemi-
cal. Examples of kinetic parameters that would relate to persistence would be half-life (t1/2) of elimination or percent of dose remaining in the body at a given time point after dosing. The 90% eliminated or 7-day postdosing time point was generally considered appropriate.

**Minimum Experimental Design for Tier 1 Studies**

A minimally experimental design was developed for tier 1 studies.

- 3 animals per dose level
- 3 dose level
- 3 sex, male preferred
- Healthy young adults, matched for age and body weight
- Rat preferred, unless other species used in toxicity study
- 7 days or until 90% eliminated
- Relevant route, plus iv (absorption determination)

It was recognized that in most cases the same experiment or experimental design would suffice to answer all three questions posed in tier 1. The minimally acceptable design for a tier 1 study was a) three animals per dose level; b) one dose level (When possible, this dose level would be equal to that used for iv study); c) one sex with preference given to the male; and d) animals should be healthy young adults matched for age and body weight. (In general, it is recommended that animals should not be less than 8 weeks of age and would preferably be 10 to 12 weeks of age).

The preferred species is the rat unless different from the species to be used in toxicity studies, then the latter would be the species of choice. No a priori restrictions were placed on the dose level to be used in the iv study; however, it was recognized that solubility and toxicity considerations might be limiting. Time for collection of excreta and blood samples would depend on the individual chemical, but should span a sufficient time period to permit accurate determination of extent of absorption. The criteria of 90% eliminated or 7 days were viewed as appropriate to terminate collection.

**Factors Influencing Need to Do More than Minimum Data Set**

Pharmacokinetic evaluations, in addition to those obtained under the minimum data set (tier 1), will be required in many cases. The factors that should be considered to trigger metabolism and PK studies in addition to that obtained by the minimum data set studies are outlined below:

- Pharmacokinetic data needed for toxicity study design or interpretation
- Exposure consideration
- Significant findings in tier 1 (i.e., absorption, metabolism, persistence)

No consensus was reached in this workshop for a quantitative evaluation of what constitutes a significant finding. However, for the minimum data set, the level would typically be assessed to be significant absorption, metabolism, or persistence. It is recognized that minimal levels of absorption could be of toxicological concern. In this case, the need for additional pharmacokinetic studies would be triggered by the toxicity data.

**Tier 2 Studies**

The following were some general views of the Work Group on the aspects to be considered under tier 2 studies. The studies delineated by the other Work Groups (Appendices I–V) describe the specific components in greater detail.

**Kinetics and Dose Response**

This study would monitor parent material or dose surrogate. The prime objective of this study would be to determine possible nonlinearities in kinetics of absorption, distribution, metabolism, and elimination. The proposed number of dose levels would be three with the dose range spanning a minimum of two orders of magnitude where possible. The low dose would be equivalent to the human exposure level or the dose level used in the intravenous study, while the high dose should be related to the LD50 (e.g., 1/10 LD50). The medium dose should be appropriately placed between the high- and low-dose groups.

**Repeated (Multiple) Dosing**

The question addressed here is: Does the pharmacokinetics of the chemical change upon repeated (multiple) dosing? The recommended procedure is a 7- to 14-day repeat dosing with unlabeled material followed by a single dose of radioactive substance. The Work Group considered a 7-day predose period sufficient and in many cases 5 days would suffice. The Work Group also supported the view that these repeat dosing studies could be coupled with ongoing toxicity studies, in which case animals would be administered trace levels of radioactive material at specified intervals after study initiation. Typically, the animals would be a satellite group in addition to those used in the standard toxicity studies.

**Routes of Exposure**

The effect of route of exposure can be delineated at any time and would be conducted in a manner consistent with that outlined by the consensus reached by the individual Work Groups addressing the relevant route of exposure.

**Tier 3 Studies**

The studies outlined in this tier would generally be initiated as the result of toxicity considerations or specific exposure questions. Some of the areas that would be considered in this tier are:

- **Metabolite Characterization.** One of the prime objectives of metabolism studies is to relate information on metabolic biotransformation to toxicity. To fulfill this end point, it was not considered necessary to provide detailed characterization or identification of metabolites or metabolic pathways unless this is deemed necessary to address the specific toxicologic concerns; considerable flexibility was stressed in ensuring that the metabolite analysis be tailored to the issue of concern.

- **Other Approaches.** The Work Group listed several other factors and end points that may be applied to a specific toxicologic concern: a) DNA and protein binding; b) species, sex, strain, and age considerations; c) human data; and d) effect of formulations and vehicles. The Work Group did not spend any further time outlining these since they would depend on the specific toxicologic problem.

**Pharmacokinetic Modeling.** The Work Group strongly endorsed the development and application of physiologically based pharmacokinetic (PBPK) modeling. They felt that this technology was advancing at a rate where it will in the foreseeable future play a significant role in both the prospective design of toxicity studies and retrospective analysis of toxicity data. The Work Group encourages the U.S. EPA to use wording in the Pharmacokinetics Guidelines that would endorse the development and utilization of this technology by industry to support regulatory compliance. Furthermore, the group endorsed the view that scientifically validated PBPK modeling could be used to augment the more standard pharmacokinetic approaches.

**Appendices**

Presented in the following Appendices are the specific experimental designs to cover studies in tier 2 and 3 of the decision tree. These experimental designs stand alone and are not part of the minimum data set experiment protocol.
Appendix I: Dose and Animal Selection

Dose Selection. The participants addressed the following issues with regard to dose and animal selection in PK studies:

a) What factors influence the selection of dosage routes?

Primary consideration should be given to the route of human exposure to the chemical, and that this exposure route should be reproduced in the PK investigation. When PK studies are designed to aid in the interpretation of an existing or anticipated toxicity study, then the exposure route used in that study should also be used for the PK study. While both toxicity and PK studies are conducted ideally by the environmentally relevant route, this is not always done in chronic and subchronic studies because of practical considerations. To enable route-to-route comparisons, the PK study should ideally be performed by both the relevant route and by the route used in the toxicity study. When such comparisons are to be made, however, an appropriate dose surrogate must be identified for use in making comparisons, such as the area under the curve (AUC) of the parent compound in blood. It was recognized that this approach goes beyond the minimum approach. A probe study conducted by the oral gavage route, for example, may be useful for obtaining large quantities of metabolites to aid in metabolite identification. The iv route of administration is generally useful as a comparison route for generating PK parameter estimates in the absence of absorption considerations, but it is recognized that solubility of the test chemical in aqueous media and local injection effects (e.g., sclerosis) may preclude the iv route for some chemicals.

b) How many dose levels are necessary for PK studies by the iv, oral, dermal, and inhalation routes?

One dose level is generally sufficient for i.v. studies. For studies where the question of nonlinear dose effects are to be addressed, two or three dose levels should be used. If the PK study is designed to help set dose levels for a toxicity study, then a minimum of three dose levels should be used. If, on the other hand, the PK study is conducted to help interpret data obtained from a toxicity study, then two doses set at the low- and high-dose levels used for the toxicity study may be sufficient. Because the dermal route of exposure often results in relatively low blood levels of test compound, nonlinearity may be better determined using the oral or inhalation routes. Therefore, one dose level may be sufficient for dermal studies depending upon the aim of the investigation. Additional dose levels in dermal studies may yield useful information if the chemical is rapidly absorbed or metabolized by the skin.

c) What criteria should be used in setting dose levels for iv, oral, inhalation, and dermal studies?

Doses selected should not induce toxicity upon a single administration. The highest dose may be selected to produce minimal toxic effects upon repeated administration, and may, therefore, be equivalent to the mean therapeutic dose of a chronic or subchronic study. The lower dose(s) should typically be 10- to 100-fold lower than the high dose to discriminate first-order (linear) pharmacokinetic patterns. Doses (i.e., concentrations) for vapor inhalation studies should not exceed 50% of the lower explosive limit. Inhalation studies, doses that significantly overload pulmonary clearance should be avoided. In dermal studies, doses selected will depend on the protocol followed (i.e., finite versus infinite dosing) and the specific aims of the investigation.

In the finite dose protocol, a limited amount of chemical is applied to the skin and the percent absorbed is determined. In the infinite dose protocol, a large excess of chemical is applied to the skin so that the concentration of chemical at the skin surface is very high (i.e., essentially infinite) during the course of the experiment. The maximum feasible dermal dose is a useful approach for obtaining absorption rate information for many chemicals. However, if the chemical produces excessive irritation or ulceration upon dermal exposure to high concentrations, then more dilute solutions should be used. The surface area of exposure used in dermal studies should always be specified.

d) How is the effect of repeated (multiple) administration on PK and metabolism best studied?

Repeated administration (at least 7 consecutive days) of unlabeled test chemical followed by administration of radiolabeled test chemical is a common approach for examining the effect of enzyme induction or inhibition on the metabolic pattern and kinetics. Bioaccumulation, on the other hand, is not addressed by this approach but may be predicted by modeling the data from a single administration. Repeated dermal PK studies are generally not recommended because there are no widely accepted procedures for repetitive application, containment, and recovery of chemicals by the dermal route. The question of enzyme induction by dermally applied chemicals may be addressed by other approaches, such as the direct determination of enzyme levels after repeated application. The development of methods for repetitive dermal PK studies was recommended as an area needing further research.

e) What radiochemical purity is feasible and sufficient for a meaningful study?

The difficulties encountered in attaining highly pure radiochemicals were discussed. The recommendation was made that compounds should be 95 to 99% radiochemically pure and that any 2% and greater impurity should be chemically identified (e.g., by comparison of retention time with known standards).

Animal Selection. The following issues are pertinent questions regarding the appropriate selection of the test species to be used in PK studies:

a) What factors influence the selection of test species and strain?

The rat is the species of choice for PK studies. Additional or alternate species may be selected if there is a specific need. The selection of rat strain should be guided by the strain used in chronic or subchronic toxicity investigations.

b) What factors influence the age selection of animals?

The age of the experimental animals must be consistent (e.g., + 1 week) within each PK experiment. The sexually mature adult is generally the animal of choice; however, depending on the study objectives, older animals may be preferable. If it is necessary to obtain serial blood samples, consideration should be given to the size of the animals so that the overall volume of blood withdrawn does not adversely affect the health of the animals or the pharmacokinetic parameters (e.g., < 10% of the blood volume).

c) When should PK studies be conducted on both sexes?

It was recommended that one sex generally be used, males preferred. Females should be used when indicated by use or exposure data. Both sexes should be used where toxicity data suggest that sex related differences exist.

Appendix II: Metabolite Characterization and Identification

Definitions. To establish consistent referencing for commonly used terminology in the analysis for test material and metabolites, the following definitions were agreed upon: Metabolite identification is defined as the elucidation of chemical structure. Metabolite characterization is defined as
determination of chemical or physical properties or both of a metabolite and is distinct from structure elucidation. Metabolite profiling is defined as chromatographic separation that is of sufficient resolution to determine chromatographically separable components arising from the test substance, including unchanged test material.

**Biologic Media for Metabolite Characterization.** Data from the intravenous and additional routes should guide selection of biologic media for use in metabolic characterization studies (i.e., minimum data set). The following general guidelines should be employed: Urinary metabolites should be profiled when urinary elimination is significant. Test material, or metabolites, in expired air should be characterized whenever present in significant amounts. Fecal metabolites should be characterized when this is a major route of elimination (≥10% of absorbed dose). Bile metabolites serve equally well for characterization and may have identification advantages over fecal samples. Determination that feces is a major route of excretion following absorption would come from the iv study or from bile cannulation studies. Blood is not generally recommended as a media for routine metabolite characterization unless knowledge of blood metabolites would answer specific questions.

**Sample Selection for Analysis.** Specific recommendations concerning frequency and timing of metabolite characterization were: As a general rule, chromatographic profiling should be conducted on a daily basis from pooled (by dose level, route of administration and sex) excreta samples until the daily output (per pooled sample) is less than about 10% of the administered dose. Total radioactivity in each individual sample also should be determined.

Various criteria for pooling of samples from animals were recognized as having value. Clear preference for any one procedure was not found. As guidance, one should use equal percentages of excretory output from each animal by either a mass, volume, or radioactivity basis. Random pooling is to be avoided. Further characterization should be considered for any chromatographically separable metabolite that exceeds, in toto, about 5% of the administered dose, regardless of route of excretion biologic media, sex, or dose level. An important exception to this 5% rule is where it is suspected that a metabolite at a lower level is responsible for the toxic effects of a chemical. In such cases attempts should be made to characterize this metabolite and should be limited only by the sensitivity of the analytic methodology. It is recommended that this further characterization should at least include an indirect characterization method such as enzymatic or acid hydrolysis applied to a pooled sample. It is further suggested that positive controls be used for enzymatic procedures such as β-glucuronidase/sulfatase.

**Test Material.** It is strongly recommended that radiolabeled test material be used for the majority of these studies. Unlabeled material can be used provided it is documented that its use is consistent with fulfilling the objectives of the studies. This documentation should typically include validation of extraction and analytic methods for tissues and excreta. The position of the radiolabel in the test molecule should be selected based on the least vulnerable moiety for metabolic change (e.g., on the core of the molecule) and should be consistent with achieving the objectives of the studies. In some circumstances, it will be necessary to use a test material with the label in a second position or one that includes a second type of label. General labeling with tritium is not recommended because of the possibility of label exchange. The label position in the test substance should be documented in the study records.

**Appendix III: Oral and Other Routes**

**General Discussion.** The exposure routes considered included intravenous (bolus and infusion), oral (gavage, feeding, and drinking water), intradermal/subcutaneous, intraperitoneal, ocular, and intratracheal. In addition to dosing routes, there was also discussion on the purity of test material, which gender to use, number of animals, fasted versus nonfasted animals, animal species, dose volumes, and treatment of data. The group concluded that only oral gavage (po) and bolus intravenous (iv) dosing needed discussion. The other routes were considered applicable only under special circumstances.

The group agreed that the rat should be the primary species, with alternative species being considered based upon available data. Rats should be fasted before dosing, in order to have the amount of food in the gastrointestinal (GI) tract at the same level in all animals. In general, the test material was considered to contain a radiochemical label. The position of the label must be in a location to accomplish the overall goals of the study; labels in more than one position may be required. Intravenous dosing experiments were considered critical and were called “guide-post” studies. Lack of stability in saline or water does not exclude iv dosing a priori very small amounts of undiluted, high-specific activity test material dosed intravenously can provide very important information on overall rates and routes of elimination. A minimum of one dose level is required. The dose volume should be kept under 1 ml/kg, must be compatible with the biologic system, and should not produce any overt signs of toxicity. It is recognized that the nature of the test material may exclude the iv dosing route.

**Study Design Considerations.** Samples collected include blood (plasma, serum), urine, feces, and expired gases. Blood sampling should continue through 72 hr post-dose, and excreta should be collected through 7 days; the study may be stopped earlier if 90% or more of the dose can be accounted for. Blood sampling volumes and techniques should not compromise the health of the animals. It was recognized that different animals might be used for bleeding and excreta collection. The carcasses of animals used for excreta collections should be used for material balance calculations. No evaluation of individual organs is recommended. The group felt that data from three animals per sex should be presented. In the general session, several other groups had decided that only data from males were needed unless there were toxicity data that indicated a possible sex difference.

Data based on total radioactivity in the urine, feces, and expired gases are indicators of the important routes of elimination of systemic test material, and these should be used to determine the general rates of elimination. Total radioactive measurements in the blood (plasma) or excreta can be used to estimate elimination rates. All blood (plasma) pharmacokinetic calculations based on total radioactivity are limited and must not be overinterpreted. Investigators are encouraged to assay the blood (plasma) samples for parent test material.

With the basic data from the guide-post study available, an appropriately designed oral study can be conducted. Pilot studies can be useful for further refining the design of definitive gavage studies. Gavage was recommended as the preferred route of oral exposure. Because of the complications involved, administration through food or drinking water should only be used to answer specific questions, such as the effect of food interactions on bioavailability. Encapsulation of the test material may be
acceptable if the encapsulation does not prevent absorption.

The gavage study should include single-dose studies (a low- and a high-dose) and a multiple dose study. The low single dose would mimic a dose that a human might consume in the water or diet; it should not produce signs of toxicity. The highest single dose should be determined according to three criteria: a) does not exceed 1000 mg/kg, b) can be administered in a volume of 5 ml/kg or less, and c) produces minimal or no toxicity. The multiple-dose study should be conducted using the dose level used for the single low-dose study.

Additional Considerations. Depending on the study objectives fasted or nonfasted animals may be used. The study should include measurements of test material and metabolites (when appropriate) in blood and excreta. If excretion represents greater than 95% of the administered dose, there is no reason to suspect that the substance is accumulating in a particular tissue or organ. Under such situations, determination of tissue distribution may not be necessary. When tissue distribution is determined, it should include the major metabolic organs: liver, kidney, brain, fat, bone, thyroid, muscle, heart, GI tract, gonads, known targets organs, and residual carcass.

The multiple dosing study should be done over a period of at least 7 days. The animals should be given nonradiolabeled doses until they receive the final dose, which should be radiolabeled. These animals may be fasted before receiving this final dose, if preferred. Measurements should include blood and excreta concentrations of test material and material balance. The observation period should be determined according to the single-dose group data.

The vehicle used for delivery of the radiochemical should not interfere with normal metabolism. The preferred vehicle is saline; the second choice is an aqueous suspension. The third choice, which should only be used when other vehicles are not practical, is a nonpolar solvent, such as corn oil. The group recognized that corn oil causes undesirable metabolic effects, but could not identify an acceptable alternative in all cases.

Appendix IV: Dermal Route Pharmacokinetics Studies

Study Design Considerations. The following questions regarding the general design considerations for a dermal pharmacokinetics study were discussed:

a) Should vehicles be used versus neat applications?

Whenever possible, compounds should be applied in vehicles that represent actual human exposure conditions, (i.e., field experience). Ideally, a vehicle should neither impair nor enhance absorption of a compound, should not change concentration (e.g., evaporate) appreciably during the application, and should not alter the permeability properties of the skin. If solid material applications are required, the test material should be applied in a manner consistent with the anticipated human exposure. If necessary, pilot or in vitro skin penetration studies should be conducted to determine if solid material will absorb into the skin. In the application of cutaneous doses, dose per unit area should be the major consideration. The criteria for selecting surface area also should consider the analytic sensitivity of available methods of detection. The surface area for application should be appropriately determined and reported; it is typically in the range of 5 to 10 cm² for the rat. For irritating chemicals, a vehicle may be introduced to dilute the dose applied to reduce irritation.

b) Determination of the extent of absorption following cutaneous application.

For purposes of determining the extent of absorption of test chemical, mass balance studies are sufficient. When more detailed pharmacokinetic data are required, it is recommended that the systemic dose over time be determined by measuring the concentration of unmetabolized chemical in the plasma. The fraction of dose absorbed can be estimated from AUC measurements derived from administration of the same dose by both the iv and dermal routes and can be compared with the absorbed dose from the mass balance studies.

c) What is the most effective way to handle removal (wash-off) of material from the application site?

The discusants recognized that a purpose of the dermal washing efficiency study is to determine the efficiency of washing by soap and water at the end of a work day. The discussion group recommended that washing not be done using a solvent. The use of anesthesia for the washoff procedure in animals, while not completely relevant to either toxicity study exposure regimens or to estimations of human exposure, may be warranted for the purpose of determining quantitative recovery and to minimize stress to the test animal. The use of anesthesia should generally be left to the discretion of the investigator and to ensure that humane procedures are employed.

d) At study termination, what is the most effective procedure for recovery of test material from the application site?

The discussants agreed that removal of the treated skin area from the test animal at the time of sacrifice, followed by analysis of a piece of this skin for residual radioactivity, was an appropriate method to determine the amount of chemical remaining at the site of application.

e) What are the best means of preparing the surface area and applying the dose, and what types of coverings or coverings should be used to protect the dose site?

The decision to occlude the exposure site should be based on the exposure scenario being modeled. It is recognized that occlusion will generally increase and enhance dermal absorption. Pilot studies should be conducted to determine the interaction of occlusive and containment materials with the test substance. The discussants further recommended that the exposure site be washed no sooner than 6 hr after application of the test substance. It was agreed that it may not be practical for volatile substances to keep the test substance on the skin for a minimum of 6 hr. The discussants could not reach a consensus regarding the occlusion of volatile materials and further identified that there is currently no generally recognized procedure for studying skin absorption for volatile chemicals. In such cases, in vitro methods may be useful in estimating skin penetration versus evaporation.

f) Problems encountered and effective resolutions of low radioactivity recoveries from dermal study segments.

While recoveries of >90% are desirable for material balance studies, in dermal studies, lower recoveries are often obtained and the causes for the lower recovery are not always understood or apparent. The acceptability of lower dermal recoveries should be considered on a case-by-case basis. These problems are generally related to difficulties in recovering radioactivity from containment devices and materials or omission of volatile organic chemical traps or both. Also, it was recognized that this latter question may be resolved by conducting a probe study prior to the definitive study.

Additional Discussion—Use of Probe Studies. These additional recommendations were made to be consistent with the concept of a minimum data set (tier 1). It was agreed that probe studies can be used for a) the establishment of an appropriate experimental design (e.g., intervals after dose application for blood and excreta sam-
Appendix V: Inhalation Route Pharmacokinetics Studies

Dosimetry and Sampling Considerations.

For several reasons, most notable of which is the feasibility of monitoring, dose and exposure should be specified in terms of chamber concentration rather than deposited or delivered dose. Chamber concentration of the test agent should always be measured and in the case of aerosols, particle size distribution of the test substance should be characterized. The plasma AUC for the parent compound should be used as a surrogate for the delivered dose. An iv dose study (if feasible) needs to be conducted to provide a reference AUC value for the parent compound. The discussion group agreed that plethysmography should not be routinely required. The work group suggested that highly irritating doses of test substances that might alter the breathing patterns are properly considered to produce overt toxicity and are thus doses that are more than minimally toxic. With regard to explosive organic vapors, for safety reasons the test doses should not exceed 50% of the lower explosive limit (LEL). Pulmonary clearance of deposited particulates is not an issue for minimal PK testing; however, it should be considered for longer term (14- and 90-day) tests.

The group strongly endorsed pilot studies to aid in the design of blood sampling schedules. They agreed that blood sampling is important to carry out during exposure. After discussing the difficulties of multiple sampling during any test (because of reduced blood volume considerations), the discussion group recommended that PK studies should be conducted on adult rats up to 16 weeks old. Because of the difficulty of simultaneously sampling breath, urine, feces, and blood, the group agreed that two test groups are needed—one to study blood concentrations, the other to study excreted material (in breath, urine, feces). Two test groups may not be needed if pilot studies indicate minimal parent compound and metabolite exhalation.

Methods to maintain volatile compounds in urine and feces were discussed, and it was concluded that cryogenic trapping techniques are available and should be used. Measuring volatile compounds in blood during exposure and in blood, urine, feces and expired air collected postexposure would provide sufficient data unless special circumstances dictate otherwise. Collection of urine and feces during exposure was not considered necessary.

Study Design Considerations. With regard to whole-body versus head-only systems, dermal absorption and preening complicate the pharmacokinetics profile from animals exposed in whole-body chambers. The group, therefore, recommended using head-only exposures. However, it was recognized that closed-loop recirculating systems can provide useful information under certain circumstances.

The group agreed that 6-hr exposures are a good standard for PK evaluation. A minimum of two inhalation exposure concentrations are necessary to detect disproportionate changes in the pharmacokinetic profile. The group suggested that the multiple exposure studies could be of the seven consecutive daily exposure format or alternative schedules of repeat exposure if adequately justified. The group concluded that some flexibility in criteria for cessation of a test should be available. The criterion of 90% elimination of the administered dose is difficult to ascertain in inhalation studies. The group discussed the need for determining the absorbed dose immediately following the exposure. They concluded that while this was advisable, its applicability should be determined on a case-by-case basis.

Concerning aerosols, radiolabels, and insoluble particles, the group concluded that using an iv dose should not be a routine part of an inhalation PK study for insoluble particles. However, an iv dose should be used routinely for soluble aerosols. Aerosols, especially insoluble and aerosol–vapor mixtures, will often require compound– or mixture–specific consideration.

Additional Considerations. The specific times for collecting blood and excreta should be determined by pilot studies. Sampling of different levels of the respiratory tract was discussed at length but was not recommended for typical PK studies. Mixtures raise extremely complex questions, and they should be considered on a case-by-case basis.

REFERENCE

1. Frantz SW, Beatty PW, English JC, Hundley SG, Wilson AGE. The use of pharmacokinetics as an interpretive and predictive tool in chemical toxicology testing and risk assessment: position paper on the appropriate use of pharmacokinetics in chemical toxicology. Regul Toxicol Pharmacol 19:317–337 (1994).