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The Acquisition and Application of Absorption, Distribution, Metabolism, and Excretion (ADME) Data in Agricultural Chemical Safety Assessments

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A proposal has been developed by the Agricultural Chemical Safety Assessment (ACSA) Technical Committee of the ILSI Health and Environmental Sciences Institute (HESI) for an improved approach to assessing the safety of crop protection chemicals. The goal is to ensure that studies

are scientifically appropriate and necessary without being redundant, and that tests emphasize toxicological endpoints and exposure durations that are relevant for risk assessment. Incorporation of pharmacokinetic studies describing absorption, distribution, metabolism, and excretion is an essential tool for improving the design and interpretation of toxicity studies and their application for safety assessment. A tiered approach is described in which basic pharmacokinetic studies, similar to those for pharmaceuticals, are conducted for regulatory submission. Subsequent tiers provide additional information in an iterative manner, depending on pharmacokinetic properties, toxicity study results, and the intended uses of the compound.

INTRODUCTION

The ILSI Health and Environmental Sciences Institute (HESI) formed the Agricultural Chemical Safety Assessment (ACSA) Technical Committee in the year 2000 to design a toxicity testing scheme that would incorporate current understanding of pesticide toxicology and exposure and recognize the specificity of agricultural products. The purpose of and background for the ACSA project are described in detail in the companion paper by Carmichael et al. (2006).

As the proposed tiered testing approach for agricultural chemical safety assessment evolved, the ACSA Technical Committee and its task forces (Carmichael et al., 2006; Cooper et al., 2006; Doe et al., 2006) worked toward the following objectives:

- Provide information that can be applied to a range of relevant human exposure situations.
- Characterize effects that have the potential to damage human health at exposure levels approximating those that might be encountered in the use of these compounds.
- Avoid high doses that cause unnecessary public concern (e.g., safety assessments should focus on doses that are relevant to realistic human exposures while maintaining adequate power for the experimental studies to detect toxicity).
- Use the minimum number of animals necessary to produce a thorough safety assessment of the chemicals of interest.
- Inflict the minimum amount of distress on animals.
- Minimize excessive and unnecessary use of resources by regulatory authorities and industry, which could be used to address other issues of concern.
- Increase both the efficiency and relevance of the current safety assessment process.

Role of ADME Data in Safety Assessments

A crucial feature in the proposed ACSA tiered testing approach is the acquisition of pharmacokinetic data that would be of maximum value in the eventual human health risk assessment process. *Pharmacokinetics* or *toxicokinetics* refers to the absorption, distribution, metabolism, and excretion (ADME) of a chemical (Renwick, 2001). Current practice does not adequately address this area, nor is there a consistent set of recommendations that would aid the registrant or registering authority to decide what ADME data are best applied in risk assessments. Depending on product usage, risk assessments may address multiple exposure situations, including exposures of the public (e.g., the historical regulatory focus on long-term dietary exposure, or home application and worker exposures that may be largely intermittent dermal exposures) (Krieger and Ross, 1993). The Food Quality Protection Act (FQPA) in the United States requires consideration of aggregate (i.e., multiple exposure pathways for one chemical) and cumulative (i.e., multiple chemicals) exposures and health risks. Finally, probabilistic approaches are increasingly being applied to describe exposure, resulting in estimates of distributions for populations. These different assessment needs often result in extrapolations from a disparate route, frequency, and duration of exposure in the toxicity study to the exposure relevant to the risk assessment. The ability to address these issues would be improved through a combination of improved toxicity study designs and appropriate pharmacokinetic studies.

The purpose of this paper is to describe the recommendations of the ADME Task Force of the HESI ACSA Technical Committee for a tiered approach to ADME data development in coordination with the tiered approach to toxicity studies. A proposed tiered approach is described here, along with alternative strategies. Whether a compound represents novel chemistry or is a member of a class for which extensive information exists can impact the choices in ordering and selection of appropriate studies. In addition, early studies might be carried out using nonradiolabeled compound, though radiolabeled material facilitates a more complete understanding of mass balance. Finally, product development processes differ among organizations; consequently, the appropriate ordering may differ, though the information needed for regulatory purposes may ultimately be similar. It should be noted that the existing U.S. Environmental Protection Agency (EPA) Health Effects Test Guidelines for Metabolism and Pharmacokinetics (OPPTS 870.7485) (U.S. EPA, 1998) describe a two-tiered approach, though the studies and their ordering differs from that presented here.

Tiered Approach to Pharmacokinetic Studies
Tier 1: Basic Tier

Tier 1 studies would always be conducted, unless technically infeasible for a particular compound, for submission along with

the first tier toxicity studies (e.g., 28-day rat dietary study—see Doe et al., 2006). These studies may be useful for toxicity study design, but they also provide the basis for virtually all applications to risk assessment. The most basic data set would consist of blood time-course measurements in adult rats of both sexes following a single intravenous and oral dosing to establish oral bioavailability. In such studies, parent compound (and total radioactivity, assuming radiolabeled compound is used) would be quantified. The intravenous studies determine distribution and clearance in the absence of impacts of oral absorption and first-pass clearance. Studies of the dose-dependence of pharmacokinetics, the impact of repeated dosing, and the extent and identity of metabolites and routes of elimination are well-established follow-on studies. In a significant change from current practice, it is also proposed that blood concentrations be determined in the toxicity study animals or satellite groups (e.g., in the 28-day rat feeding study) to provide data directly under the conditions of the toxicity study.

While studies of lactational or placental transfer could prove valuable for designing and interpreting toxicity study results, such study designs are less well established. One can find examples, however, particularly for pharmaceuticals, in the published literature. Therefore, although these studies would ideally be conducted in Tier 1, from a practical perspective, they may be done later until more experience is gained on how best to carry out these studies.

Tier 2: Intermediate Tier

Tier 2 involves studies that would be completed as appropriate to build the necessary database for study interpretation and risk assessment purposes. Studies of tissue distribution, biliary elimination, enterohepatic recirculation, serum protein binding, and in vitro metabolism in rodents and other species would be conducted as required based on the characteristics of the compound. Not all chemicals undergo metabolism, biliary elimination, or enterohepatic recirculation; consequently, such studies would be triggered by findings in the first tier. Compounds that are metabolized would be subject to in vitro metabolism studies, and such studies would be considered essential prior to controlled human exposure studies. Studies with species other than rats, for example, dogs and rabbits as recommended for specific toxicity studies, are included in this tier because they are anticipated to be done after rat pharmacokinetic studies and relevant toxicity studies have been completed. If the toxicity studies in these other species will potentially drive the risk assessment, it becomes more important to collect additional pharmacokinetic data than if the risk assessment is based upon the rat studies, though the comparative data could still be useful.

Tier 3: Advanced Tier

Studies in this tier would include those that assist in understanding the mode of action for a compound and develop in-formation for improved risk assessment, particularly in terms of route and interspecies extrapolations. In this respect, PBPK modeling can be particularly valuable. Dermal studies are an important exposure route for many pesticides, although, for a select group of compounds, inhalation pharmacokinetics might also be needed. In addition, various kinds of studies in humans are described. To provide an adequate scientific and ethical rationale to conduct controlled human exposures, the studies in the previous tiers in animals and in vitro studies, such as metabolism in human tissues, would have been completed. In addition, a stronger case for controlled human exposures can be established through the development of predictive pharmacokinetic models, often PBPK models. These would be used for improved study design, and the resulting data would be used to determine if the model based on animal and in vitro data provided an adequate representation of pharmacokinetics in humans for use in risk assessment.

Alternative Tier Strategies

Several options for alternative strategies for each tier may be useful. The logic of data collection is dependent on whether the compound under consideration is a novel chemical class or a member of a well-studied existing class. The logic also depends on whether the compound is undergoing initial development and toxicity testing, which would impact a company's development strategy, or whether it is an existing compound for which additional information is being obtained for re-registration purposes and to improve the estimation of human risks.

A similar tiered approach has been described arising from efforts to better link pharmacokinetic and toxicity studies for synthetic organic chemicals (Wilson et al., 1994). The authors proposed an initial minimal tier to address three questions: Is the chemical absorbed? Is the chemical metabolized? Does the chemical persist? This minimal tier is a limited set of studies that are included in Tier 1 presented in this paper; it represents the basic starting point, particularly for a new chemical. Much of the remainder of the basic tier described in this paper appears in the second tier proposed by Wilson et al. (1994). Useful perspectives are presented on both the ordering of information collection and experimental design issues. One difference is the assumption by Wilson et al. that the male rat is the most appropriate sex and species with which to work (largely reflecting needs related to subchronic and chronic bioassays), while the ACSA tiered testing proposal has somewhat greater focus on the significance of female rats and early life stages (reflecting concerns about children and needs related to reproductive and developmental toxicity testing) (Cooper et al., 2006). Overall, many of the same issues are identified for better linking pharmacokinetics to toxicity study design, interpretation, and risk assessment applications.

Many of the studies in the first two tiers of the ACSA proposal would be advantageously conducted using radiolabeled compound. This would permit quantification of parent compound

and total radioactivity; the difference provides some perspective on the pharmacokinetics of the metabolites. Mass balance can be determined in excretion route and tissue distribution studies when radioactivity is used. However, because this can be a nontrivial synthetic task, it may be useful to collect either pharmacokinetic or in vitro data at a phase of product development prior to preparation of radiolabeled compound.

Because dermal exposure is a major concern for risk assessment, early evaluation of dermal absorption in vitro could be included in the first tier of studies during product development. This might be a critical factor for evaluating which of several compounds to take forward for further development. Similarly, in vitro metabolism studies are more readily conducted than many of the in vivo studies. Consequently, in vitro studies might be included in the first tier, particularly because they may play a role in confirming the appropriate toxicological test species.

These recommendations provide a clear role for ADME in study design, interpretation, and risk assessment. To highlight the uses for the data, the studies and illustrative examples are described next with a focus on their applications, rather than the precise ordering described here for the tiers.

PHARMACOKINETIC STUDIES IN LABORATORY ANIMALS FOR IMPROVED TOXICITY STUDY DESIGN

Paradigm Shift

Animals are useful predictors of chemical risks to humans. One of the continuing dilemmas faced by the scientific and regulatory communities when conducting human health risk assessments for pesticides is the need to extrapolate virtually all relevant safety data across species because of the lack of an adequate human database. Extrapolations are used for most endpoints tested under the umbrella of toxicokinetics and toxicodynamics (e.g., data on animal metabolism, oral and dermal bioavailability, toxicity data, etc.). Each extrapolation reflects limited confidence in the available data used to predict the situation in humans. This limited confidence results in the application of several uncertainty factors for risk assessment.

For other biologically active chemicals, such as pharmaceuticals, significantly lower safety factors are applied with greater confidence because much of the data are directly generated in the target species. Exceptions to this rule are data on genotoxicity, carcinogenicity, and reproductive toxicity, which even for pharmaceuticals largely depend on animal data. Nevertheless, for many of these endpoints, the dose selection and the selection of the "most human-like test species" in the bioassay are based on considerations of pharmacokinetics and metabolism. Therefore, in contrast to pesticides, toxicokinetic data are used on a standard basis in drug development to assist in candidate selection, appropriate species selection for toxicity testing, and dose selection for toxicity studies, as well as in safety assessment, by comparing experimental animal versus human systemic exposure (Clark and Smith, 1984). International harmonization of guidelines on toxicokinetics and dose selection in toxicity studies for pharmaceuticals has been achieved, and those guidelines can be reviewed on the U.S. Food and Drug Administration (FDA) homepage (http://www.fda.gov/cder/guidance/index.htm). Efforts to achieve this paradigm shift for industrial and agricultural chemicals have been ongoing as the field of pharmacokinetics has matured (Wilson et al., 1994).

Because the selection of appropriate dose levels in toxicity studies is crucial to the interpretation and assessment of test results, it is worthwhile to use a range of information, not just toxicity, in dose selection. While the driver for testing at the maximum tolerated dose (MTD) is to obtain greater power with a limited number of animals, the primary concern is that the MTD can saturate or overload pharmacokinetic processes, resulting in a systemic exposure fundamentally different than would be observed with real-world human exposure scenarios for which risk assessments need to be derived (Counts and Goodman, 1995; Slikker et al., 2004a, 2004b). This artificial exposure scenario may result in toxicities that would not arise at relevant dose levels. Another issue with the current approach is that, based on the generation of a carcinogenicity database obtained with MTD-based studies, a significant amount of additional mechanistic animal experimentation may be triggered, resulting in unnecessary use of animals. Therefore, availability of data on pharmacokinetics and metabolism of the test compound at different dose levels could fundamentally change and inform the current approach to dose level selection. Consideration of all relevant data is paramount in determining the most appropriate bases for selecting the doses for toxicity studies for pesticides.

Objectives

Setting Dose Levels—Pharmacokinetic Endpoints in Dose Selection

Pharmacokinetic studies describing the behavior of a compound over a range of doses can assist in the selection of doses for toxicity studies. Selecting doses that produce a range of blood levels rather than very similar blood levels (Example A) is advantageous to obtain more informative dose-response data from the toxicity study. Decisions about dose selection in light of available saturable pharmacokinetics and toxicity data require expert scientific judgment rather than fixed rules (Foran, 1997). Valuable information also may be available for the class of compounds under study, e.g., whether the parent or metabolite is the likely active form for inducing toxicity. Because choices of doses involve judgment, dose selection should be a point of discussion between study sponsors and the regulatory agencies that would use the data. The goal is to obtain a reasonable balance among a variety of factors, including the power of the study within reasonable practical and cost constraints, the potential for dose-dependent changes in mode of action, concerns for palatability of compound in food or drinking water that may adversely effect animal nutrition, and other factors described in some detail in a number of review articles (Foran, 1997; Slikker et al., 2004a, 2004b; Bus and Reitz, 1992).

Another approach is to develop a comparison with expected human internal exposure. A systemic exposure representing a large multiple of the expected human exposure, perhaps characterized using the area under the curve (AUC) in blood, may also be an appropriate basis for dose selection for chronic studies as is currently done for pharmaceuticals. Based on the expected use scenarios and residue levels, it should be possible to establish reasonable estimates of human exposure, even at the stage of development when the decision for long-term study dose level selections are made. However, as such estimates are inherently uncertain, it is important that the level of animal systemic exposure be sufficiently great to provide reassurance of an adequate test for chronic toxicity, including carcinogenicity.

Example A. Dose selection based on saturation of absorption One example of using this approach is saturation of absorption as demonstrated by achieving a plasma level plateau despite increasing dose levels. High-dose selection based on saturation of absorption can be used as a tool for decision making in study design.

Plasma concentration data on the parent compound (experimental herbicide) generated in rats and dogs showed that systemic exposure was limited by saturation of absorption kinetics at high dose levels (Figure 1). In the 13-week dog study, maximum systemic exposure was already achieved at 7000 ppm compared to the limit dose of 50,000 ppm in dogs. A similar situation was seen in rats where a dietary concentration of 10,000 ppm could be considered a dose for which systemic exposure cannot be increased significantly, even when doubling this dose level (Bayer, unpublished data).
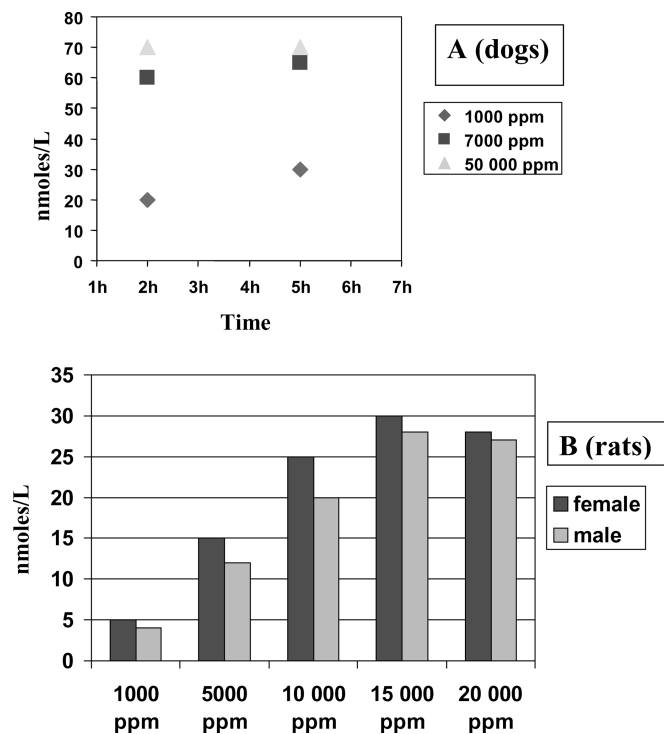

FIG. 1. Systemic exposure (i.e., plasma concentrations) of an experimental herbicide as a function of dietary levels in dogs.

Data such as these should be used to limit the high dose in long-term studies to the dose level generating the maximum achievable systemic exposure levels. Those data can usually be generated in the course of regular toxicity studies without the need for additional use of animals. The data may have to be generated in each individual species and sex for which long-term studies are to be conducted.

Routes and Rates of Elimination and Metabolism Over Dose Range of Interest
Understanding the clearance of compounds, such as by urinary elimination or metabolism, provides insights into potential persistence and the comparability of the animal models to humans at different dose ranges. Persistence and bioaccumulation arise from slow clearance, and are often but not always combined with lipophilicity.

Saturation of metabolic and transporter-dependent elimination pathways is another confounding factor potentially resulting in the generation of data for toxicity endpoints, including carcinogenicity, which may not be relevant for actual human exposure conditions. The qualitative systemic exposure (for example, ratio between parent compound and metabolites) achieved under such exposure conditions in animals may be significantly different compared to the human situation at, or even severalfold above, real-world human exposures, such as an acceptable daily intake (ADI) or acceptable occupational exposure level (AOEL). In vivo and in vitro studies on metabolism should therefore be conducted at different dose levels to identify saturable pharmacokinetics. These would range from exposure levels expected to occur in occupational and dietary situations up to the range of dose levels at which toxicity studies are conducted (or an appropriate limit dose). It should also be recognized that differences in dose rate, as often occur with oral gavage versus dietary or drinking water exposure, typically need to be accounted for in describing saturable phenomenon. Dose levels producing a substantially different metabolic profile due to saturation of metabolic pathways would be considered inappropriate for testing if they would be substantially different that expected in humans. In vitro studies could also help to determine the most "human-like" species in terms of metabolites generated or transporter activities, and provide more confidence in the animal data generated. Animal species with a different metabolic profile compared to humans could be considered inappropriate for toxicity studies and excluded from long-term testing. These dose or species-dependent differences are often quantitative (i.e., varying extents of production of metabolites) rather than qualitative; consequently, selection of conditions analogous to the human situation requires judgments to be made.

Example B. Selection of appropriate species for toxicity testing based upon metabolism Only a few standard animal species are available for toxicity testing. In many cases, significant quantitative

TABLE 1
Comparisons of blood levels of parent compound and metabolites in monkeys and mice (Bayer, unpublished data): AUC values
in rhesus monkeys and mice for AI, M03, and M11 (μg · h/L)

	Rhesus monkey				Mouse			
	1.6 mg/kg		16 mg/kg		2.4 mg/kg		30 mg/kg	
Substance	Day 1	Day 10	Day 1	Day 10	Day 1	Day 14	Day 1	Day 14
AI	21.5	29.2	311	226	91	102	816	614
M03	1030	1330	13,200	9330	2386	2424	15,096	14,472
M11	NQ	NQ	63	46	7152	24,000	85,440	288,000

Note. NQ, not quantifiable.

and qualitative differences in toxicity between individual test species are observed. It is standard practice to use the most sensitive species for risk assessment and apply an additional 10-fold interspecies safety factor to account for potentially even greater sensitivity of humans. As illustrated in Table 1, however, it may be possible to identify the basis for the species differences when they arise from differences in absorption and/or metabolism of the test compound. It is therefore recommended that in vitro screening on compound metabolism be conducted to compare different experimental animals and humans. For long-term testing, it seems appropriate to exclude animal species that largely deviate from the human situation, as the results will not allow a good prediction for human risk assessment. This approach may avoid animal testing in species not relevant to human risk assessment.

After short- and long-term exposures to Compound A, the mouse was identified as the most sensitive species, with a no-observed-effect level (NOEL) about 10-fold below the rat. A specific metabolite with significantly higher toxicological potency when compared to the parent compound and other metabolites was identified in mice. Comparative toxicokinetic studies showed that mice had much higher systemic exposure to this metabolite when compared to the rat, dog, and rhesus monkey. Comparative in vitro metabolism work on mouse, rat, and human primary hepatocytes showed that human cells form only minimal amounts of this metabolite. Consequently, mouse toxicological data are nonrelevant for human risk assessment (Bayer, unpublished data).

Example C. Selection of appropriate species for toxicity testing (2,4-D) Comparative differences in the capacity for urinary elimination of the phenoxyacetic acid herbicide 2,4-D have been used in the United States and Europe to base risk assessments on toxicity data from rats rather than dogs (Timchalk, 2004). Dogs demonstrated toxicological effects at lower exposures than rats, but measurements of plasma levels found dog results to have a significantly higher area under the curve (AUC) than for rats due to slow urinary elimination in dogs (Van Ravenzwaay et al., 2003). Following dosing with 5 and 50 mg 2,4-D/kg body weight, the plasma AUCs for dogs were 125- and 15-fold higher than for rats. Regulatory actions were based upon the results of the rat toxicity studies because humans clear this compound more similarly to rats than dogs.

Limiting the Number of Routes Used for Toxicity Studies

Toxicity potentially needs to be evaluated following exposure by the oral, dermal, and inhalation routes, depending on the different exposure routes for humans. The dermal route is the primary route of exposure for operators (mixing and loading, application) and reentry workers. Worker or consumer exposures to aerosols or volatiles may be by inhalation, while exposure to residues in food or water would be oral. Use of route-to-route extrapolation based on pharmacokinetics can provide a sufficiently comprehensive database for systemic toxicity without conducting each toxicity study by multiple routes of exposure. Some studies by the appropriate route may be required for assessing contact site toxicity. In general, most toxicology studies are conducted via the oral route. Approaches for dermal extrapolation are discussed later (see Improving Human Health Risk Analysis Through Applied Pharmacokinetics).

Study Designs for Basic Pharmacokinetic Data in Test Species

The overall objective of basic pharmacokinetic studies is to help design and interpret ongoing or future toxicology studies. This requires establishing a baseline pharmacokinetic understanding. The objective of these baseline pharmacokinetic studies is to establish the extent and rate of absorption, bioavailability, the general distribution of the chemical, the extent of metabolism, and the routes and rates of excretion (Clark and Smith, 1984). These experiments will provide information on any nonlinear kinetics resulting from saturation of absorption, shifts in metabolic pathways, or routes of excretion. Simple compartmental and noncompartmental kinetic analysis of the data will provide information on bioavailability, half-lives for absorption and elimination, and clearance rates (Renwick, 2001). The experimental study designs begin with highly artificial exposures (i.e., intravenous single dosing) that simplify the interpretation of the data by focusing only on clearance of the compound, move on to characterize oral absorption under controlled conditions, and ultimately characterize pharmacokinetics under conditions directly relevant to the toxicity study (e.g., repeated dietary exposures). While individual pharmacokinetic studies can provide essential information, it is really the acquisition of

knowledge across this sequence of studies that ultimately is informative for risk assessment purposes.

Generally, the rat is the test species used for initial pharmacokinetic studies due to its common use in toxicological testing and its larger size, compared to the mouse, thus making the rat more experimentally accessible. Throughout this paper, the descriptions provided are generally for rats. The strain of rat should ideally be that used in the planned or completed toxicological study. Not infrequently, however, different strains of rats are used. Male rats have historically been used most commonly for pharmacokinetic studies, but the increasing frequency of risk assessments based on reproductive and developmental toxicity outcomes makes data on females of obvious value. Similarly, young adult animals are typically used for initial studies, but toxicity results in pregnant, young, or aged animals increase the value of information across the life span. Most of the pharmacokinetic studies discussed in this article could be carried out with other species or life stages as appropriate for designing or interpreting toxicity studies or improving their use in risk assessment. Study designs may have to be adjusted for other species or life stages due to the size or number of samples that can be obtained, the feasibility of dosing methods, or other issues specific to the test species or the toxicity study in which they are used.

Studies to Establish Oral Bioavailability and the Impact of Dosing Methods

Since toxicological evaluation of most agricultural chemicals will be conducted via oral administration in the diet, it is desirable to have a fundamental understanding of the extent of oral bioavailability. Bioavailability can refer to the rate (hour^{-1}) and extent (%) of chemical absorption into the systemic circulation (Gibaldi and Perrier, 1982). Usually, bioavailability of a chemical refers to the parent compound, but it could refer to its metabolite. It considers only one chemical form. Absorption refers to the parent chemical and all its metabolites. A number of factors can influence the extent of oral absorption, including pH, presence of hydrolytic enzymes, influence of gut microflora, and the impact of food (Renwick, 1994). The difference between oral absorption (i.e., presence in gut tissue and portal circulation) and systemic bioavailability (i.e., presence in systemic blood and tissues) can arise from chemical degradation due to gut wall metabolism or efflux transport back to the intestinal lumen or presystemic metabolism in the liver, among other factors.

Frequently, bioavailability is used to describe the comparison of chemical in systemic circulation following different exposure routes, such as oral, dermal, or intravenous (iv) administration. Oral bioavailability, or the fractional absorption following oral administration, can be readily determined experimentally by conducting in vivo oral and iv kinetic studies that compare blood kinetics and the routes and rates of elimination. The generalized approach is to administer the same dosage (mg/kg) both iv and orally, and then compare areas under the blood concentration curves (AUCs) or excretion (urine, or feces) over time. A very common method for establishing the extent of oral bioavailability is to compare the blood or plasma AUCs following oral and iv administration based on the following relationship:

$$F = \frac{\text{AUC}_{\text{oral}}}{\text{AUC}_{\text{iv}}}$$

where F is the fractional bioavailability (unitless fraction). If administration of the same dose by both routes is not feasible, the AUCs are corrected for dose. It is also feasible to use urinary excretion data to estimate oral bioavailability, based on the ratio of the total amount of unchanged chemical excreted in the urine after oral administration to that following iv dosing (Gibaldi and Perrier, 1982). For some compounds, iv dosing is not feasible due to issues such as solubility, though a range of vehicles are available to help overcome such problems (e.g., emulsifiers, serum of the same species). In addition, greater toxicity may be associated with iv dosing because the entire dose is rapidly systemically available; consequently, the iv dose used may be the same as a low oral dose.

These studies ideally would be conducted using radiolabeled material with quantification of parent compound and total radioactivity; the difference between the two provides initial information on the behavior of the metabolites. In general, measurement of total radioactivity alone provides data that are unsatisfactorily nonspecific without quantification of parent chemical. However, oral bioavailability can be determined using nonspecific assay systems such as ^{14}C-labeled test material, assuming the pharmacokinetics are in the linear range (Gibaldi and Perrier, 1982). This may be useful during the early phases of pharmacokinetic evaluation when the extent of metabolism is unknown (e.g., to demonstrate that different salts of an acid are equally bioavailable and toxicity studies for each form are not required).

Another important aspect of oral bioavailability is the impact of the dosing media or vehicle. Oral absorption can be a complex phenomenon involving multiple passive diffusional and active transport pathways depending upon the characteristics of the chemical (Liu and Pang, 2005; Roth et al., 1993). The trend is to use drinking-water or dietary exposures in the toxicity studies due to concerns about bolus gavage dosing. However, drinking-water and dietary exposures are difficult to characterize in terms of how much chemical and when the animal is exposed because they vary with each animal's behavior. Oral absorption pharmacokinetic studies are generally done, therefore, with bolus gavage dosing so that the time and amount of chemical to which the animal is exposed are known precisely, allowing the blood time-course behavior to inform an understanding of absorption (given knowledge of the clearance from intravenous dosing). The vehicle used for gavage dosing requires careful consideration because it is well known to impact the absorption kinetics (e.g., water, oil, emulsion). Ultimately, a few studies are often

needed to evaluate absorption under reasonably well-controlled experimental conditions and the conditions of the toxicity study.

Studies to Establish the Extent of Metabolism and Elimination Routes

During the early development phase of a new pesticide, particularly for compounds based a new chemistry, the extent of mammalian metabolism will not be known. Thus, metabolite standards will most likely not be available for the compounds under study. However, the use of radiolabeled (primarily ^{14}C-labeled) test material for pharmacokinetic evaluation makes it feasible to determine the extent of overall metabolism and to semi-quantify the metabolic products. For example, it is feasible to measure the total amount of radioactivity excreted in the urine, as well as the percentage of that material that is identified as parent. Profiling of urinary ^{14}C-labeled metabolites by HPLC using radiochemical detection can also be conducted to obtain a preliminary determination of the extent of metabolism. Likewise, by collecting exhaled air ([14]CO_2 and trapped ^{14}C-organics), urine, and feces over time, the routes and rates of elimination can readily be determined through the analysis of these samples simply by scintillation counting.

Example D. The use of radiolabeled test material for pharmacokinetic evaluations (triclopyr) Data on the herbicide triclopyr illustrate the utility of this pharmacokinetics approach. (For a detailed description of the kinetic evaluation, see Timchalk et al., 1990.) Pharmacokinetic studies were conducted with triclopyr to determine the absorption, distribution, metabolism, and excretion following oral and iv administration. Triclopyr with an analytical purity of ∼99% was used and mixed with a ^{14}C-labeled triclopyr (>98% radiochemical purity) for these studies. Groups of cannulated male and female rats (3–5/group) (data not illustrated here) were given triclopyr orally by gavage or iv via the jugular cannula at a dose of 3 mg triclopyr/kg body weight. The blood ^{14}C kinetics, distribution through 72-hours postdosing, and extent of metabolism (urine) were determined.

As illustrated in Table 2, the overall ^{14}C recovery following oral and iv exposure to triclopyr in the rat indicates that the urine is the primary, and most likely the only, route of excretion of systemically available triclopyr and related metabolites. In addition, by comparing the overall recovery of radioactivity in the urine and the urinary time

TABLE 2
Distribution of radioactivity recovered 72 hours after male rats were given oral or iv doses of 3 mg ^{14}C-triclopyr/kg of body weight (adapted from Timchalk et al., 1990)

	Percentage of administered dose	
Samples	3 mg/kg single iv	3 mg/kg single oral
Urine	94.34 ± 0.98	93.05 ± 0.93
Feces	0.82 ± 0.50	1.98 ± 0.32
Cage wash	0.42 ± 0.35	0.97 ± 0.40
CO_2 trap	0.08 ± 0.01	0.07 ± 0.01
Tissue and carcass	0.03 ± 0.01	0.30 ± 0.19
Total	95.69	96.37

TABLE 3
Radioactivity excreted in the urine during the indicated intervals in male rats given oral or iv doses of 3 mg ^{14}C-triclopyr/kg of body weight (adapted from Timchalk et al., 1990)

	Percentage of administered dose	
Collection intervals	3 mg/kg single iv	3 mg/kg single oral
0–12 hours	90.21 ± 1.51	62.53 ± 5.40
12–24 hours	3.37 ± 0.80	27.01 ± 4.27
24–36 hours	0.37 ± 0.09	2.19 ± 0.76
36–48 hours	0.16 ± 0.02	0.76 ± 0.30
48–60 hours	0.14 ± 0.16	0.35 ± 0.13
60–72 hours	0.08 ± 0.02	0.21 ± 0.03
Total	94.34 ± 0.98	93.05 ± 0.93

course of elimination (Table 3), it is readily concluded that triclopyr is well absorbed orally (100%), and the greater urinary excretion through 12–24 hours postdosing is consistent with the slower oral absorption relative to the rapid infusion following the iv dose (90%; 0–12 hours).

Following oral and iv administration of triclopyr, the ^{14}C-plasma time-course terminal half-life was calculated as ∼3.6 hours and 1–2 hours, respectively. These differences suggested that the rate of oral absorption was the rate-determining step responsible for the observed elimination half-life following the oral dose.

To determine the extent of triclopyr metabolism, urine specimens were subjected to HPLC analysis. The results are presented in Figure 2 and Table 4. One major and four minor radioactive peaks were observed; >89% of the urinary radioactivity eluted as Peak E, which had a retention time similar to that of triclopyr. The remaining radioactivity was associated with four peaks, with Peak D having a similar retention time to trichloropyridinol. Acid hydrolysis did not appreciably alter the profile. Comparison of the metabolite distribution between oral and iv groups suggests that the orally dosed animals may exhibit slightly more metabolism. This could be due to a slightly longer residence time and greater involvement of the liver (site of metabolism) following oral exposure versus the iv dose that is rapidly cleared by the kidneys. Nonetheless, these results indicate

TABLE 4
Percent distribution of ^{14}C in the urine 0–12 hours postdosing in male rats given oral or iv doses of 3 mg ^{14}C-triclopyr/kg of body weight (adapted from Timchalk et al., 1990)

Urine collection	3 mg/kg single iv	3 mg/kg single oral
Peak A	2.08	2.17
Peak B	ND	2.35
Peak C	2.02	4.17
Peak D	0.93	1.55
Peak E	94.97	89.76

Note. ND, not detected. To characterize the extent of metabolism, urine samples from the 0–12 hour and 12–24 hour collection intervals were profiled by HPLC.
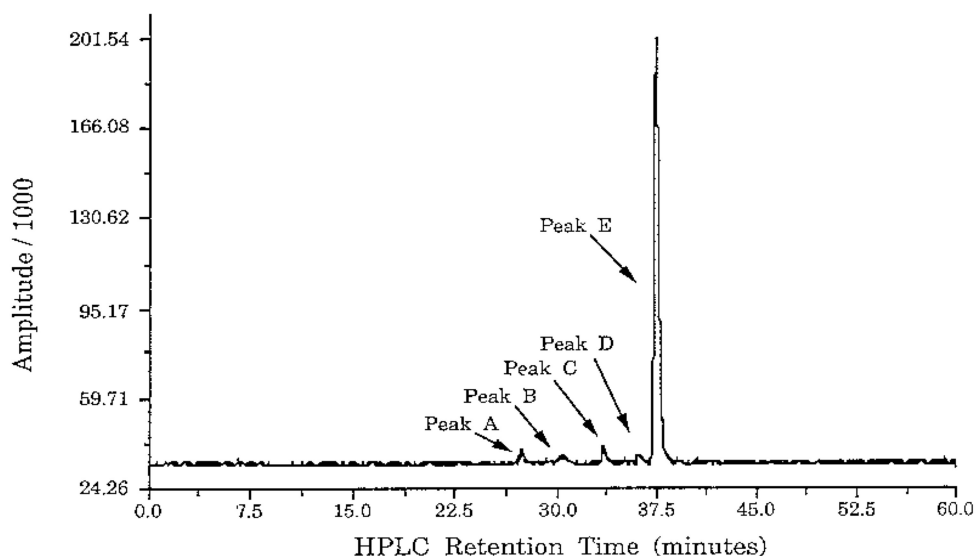
FIG. 2. HPLC radiochromatogram of urine following exposure to ^{14}C-trichlopyr. Peaks D and E are tentatively identified as trichloropyridinol and triclopyr (parent compound), respectively.

that triclopyr undergoes limited metabolism and is rapidly eliminated in the urine primarily as the parent compound. Based on chromatography, Peak D was identified as trichloropyridinol, and Peak E as the parent triclopyr.

These studies alone provide limited basic information that is critical to subsequent pharmacokinetic studies, including comparative studies. They provide the basis for the species comparison study previously described (see Example B) and the dose-dependent pharmacokinetic evaluation to assist in toxicity dose selection (see Example E later).

Studies of the Influence of Dose on Kinetics (Single Exposure)

The fundamental mechanisms underlying absorption, distribution, metabolism, and elimination of chemicals in vivo involve processes that often have a finite capacity for interactions, such that at high chemical concentrations no further interactions are possible (i.e., saturation) (Renwick, 1994). Because toxicity studies often use relatively high exposures and may span large dose ranges, it is important to understand whether the pharmacokinetics shift from linear to nonlinear (saturated) kinetics at doses that might be used in toxicity studies. In addition, it is important to understand what processes (e.g., metabolism, renal clearance) are associated with any observed nonlinearity.

Characterizing the possibility of nonlinear kinetics requires that the pharmacokinetics be evaluated over a number of dose levels. This is typically done following a single bolus dose, but may ultimately need to be evaluated following repeated exposures as used in the toxicity study if it is determined that changes in kinetic processes result (e.g., induction of metabolizing enzymes or transporters). The experimental design is compa-

rable to that used to evaluate oral bioavailability, and will include an evaluation of the blood/plasma kinetic time course, the routes and rates of absorption and elimination, the extent of metabolism, and an overall mass balance (particularly if ^{14}C-labeled test material is employed) over the dose range of interest. Data analysis will include the evaluation of blood/plasma AUCs, and the calculation of clearance rates (ml hour^{-1}) and half-lives for both absorption and elimination kinetics. In general, such studies should include at least three dose levels. The low dose used for this evaluation should be within the linear kinetic range, which may require preliminary studies or an iterative study design to identify the appropriate range. Two additional oral dose levels would be selected that ideally range from one-two orders of magnitude higher in dose. These higher doses should not exceed a level that could result in acute toxicological effects following a single-dose administration.

Example E. Dose-dependent kinetics associated with the saturation of renal tubule active secretion (triclopyr and 2,4-D) Triclopyr and the phenoxyacetic acid herbicide 2,4-D are organic acids that are actively secreted in the urine of rats primarily unchanged. In the case of triclopyr, rats were administered a range of doses by iv and oral routes (Timchalk et al., 1990). The time course of ^{14}C-activity in the plasma of rats administered triclopyr at doses ranging from 1 to 100 mg/kg of body weight is illustrated in Figure 3. These data clearly demonstrate that with increasing dose, there is a nonlinear increase in the plasma concentration. Linear kinetics disappear at doses ≥ 5 mg/kg.

A similar analysis was conducted following oral administration of doses of 3 or 60 mg/kg. Based on disproportionate changes in parameters such as peak concentration and AUC in plasma, the data again indicate dose-dependent kinetics for triclopyr.
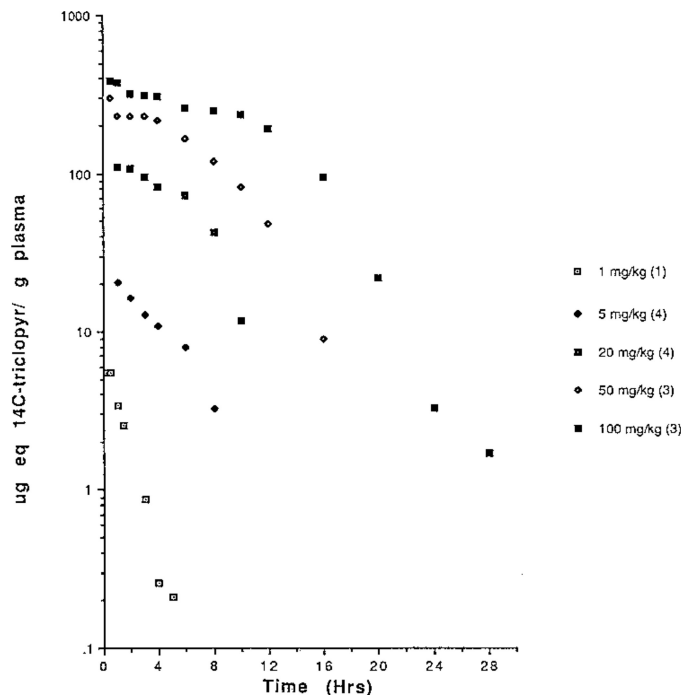
FIG. 3. Time course of radioactivity in rats following iv administration of ^{14}C-triclopyr. Numbers in parentheses are the numbers of animals per group.

A comparison of the overall distribution of radioactivity (Table 5) does not suggest that this dose-dependent response is associated with shifts in the major routes of excretion. The urinary route of excretion predominates regardless of dose. However, the urinary excretion rate is slightly slower following the high oral dose, with a larger percentage of the dose being eliminated in the urine through 24 and 36 hours postdosing relative to the low oral dose. This is consistent with the observed nonlinear plasma kinetics.

Because the previous data were based on measuring total radioactivity, it was necessary to determine if the nonlinear response might be associated with shifts in metabolism by comparing the urinary metabolism profile (Table 6). Based on these data, parent triclopyr

TABLE 5

Distribution of radioactivity recovered 72 hours after male rats were given single oral doses of 3 and 60 mg ^{14}C-triclopyr/kg of body weight (adapted from Timchalk et al., 1990)

| | Percentage of administered dose | |
Samples	3 mg/kg	60 mg/kg
Urine	93.05 ± 0.93	92.91 ± 1.38
Feces	1.98 ± 0.32	2.63 ± 0.40
Cage wash	0.97 ± 0.40	0.25 ± 0.09
CO$_2$ trap	0.07 ± 0.01	0.05 ± 0.03
Tissue and carcass	0.30 ± 0.19	1.51 ± 0.77
Total	96.37	97.35

TABLE 6

The percent distribution of ^{14}C in the urine 0–12 hours postdosing in male rats given single oral doses of 3 and 60 mg ^{14}C-triclopyr/kg of body weight (adapted from Timchalk et al., 1990)

| | Percent radioactivity in urine specimen | |
0–12 hour urine collection	3 mg/kg	60 mg/kg
Peak A	2.17	0.89
Peak B	2.35	0.85
Peak C	4.17	3.19
Peak D	1.55	2.26
Peak E	89.76	92.81

Note. To characterize the extent of metabolism, urine samples from the 0–12 hour and 12–24 hour collection intervals were profiled by HPLC.

(Peak E) still represents the major excretory product. These data were interpreted to suggest that the nonlinearity of the observed pharmacokinetics was primarily due to the saturation of the renal organic acid transport system. This system is responsible for renal clearance of a number of organic acids, including triclopyr and phenoxy herbicides like 2,4-D.

The pharmacokinetic data for the herbicide 2,4-D also were shown to demonstrate a similar dose-dependent nonlinearity. Gorzinski et al. (1987) evaluated the oral pharmacokinetics of 2,4-D in rats. Groups of animals were administered single oral doses ranging from 10 to 150 mg ^{14}C-2,4-D/kg. At 6 and 12 hours postdosing, rats were terminated, and blood, kidneys, and urine were analyzed for ^{14}C activity. Urine specimens were analyzed for 2,4-D by HPLC with radiochemical detector.

Only 2,4-D was detected in the urine, indicating that there was no appreciable metabolism and that the ^{14}C activity was in fact 2,4-D. The time course of ^{14}C excreted in the urine and in the plasma at 6 hours postdosing is presented in Figure 4.

These data clearly illustrate the relationship between plasma concentrations of ^{14}C activity and urinary excretion. At doses greater than 50 mg/kg, nonlinear increases in plasma concentrations are noted due to saturation of urinary excretion of ^{14}C activity. Likewise, as illustrated in Figure 5, the concentration of ^{14}C in the kidney did not increase in proportion to the concentration in the plasma, supporting the conclusion that the dose-dependent kinetics of 2,4-D, like triclopyr, are associated with a saturation of the renal clearance mechanism for these organic acids.

Repeat-Dose Study Design

Although the single-dose studies provide critical perspective on the pharmacokinetics of the chemical of interest, it is also important to understand the impact of repeated daily exposure such as that which occurs in most toxicity studies. Repeated daily exposures will increase the blood concentration over a period of 4–5 half-lives to establish a plateau if the half-life is longer than a few hours (Renwick, 1994). In addition, repeated exposure can contribute to altered pharmacokinetics and toxicological response relative to that seen following a single dose, primarily
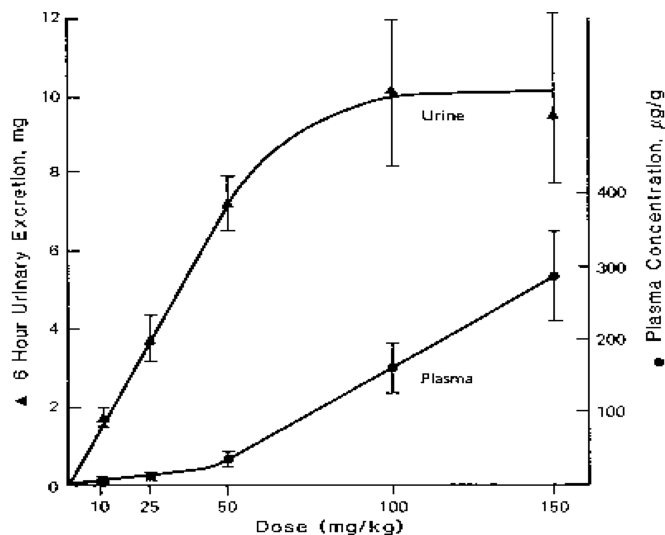
FIG. 4. Plasma ^{14}C concentration and amounts of ^{14}C excreted in the urine six hours after various single oral doses of ^{14}C-2,4-D.

due to induction of metabolizing enzymes, but also due to inhibition of metabolizing enzymes or alterations in transporters. Therefore, the primary objective of these repeat-dose pharmacokinetic studies is to establish whether repeated dosing alters the clearance of the chemical. These data will be used to provide perspective on the design (dose selection) of the longer term studies (90-day, 2-year).
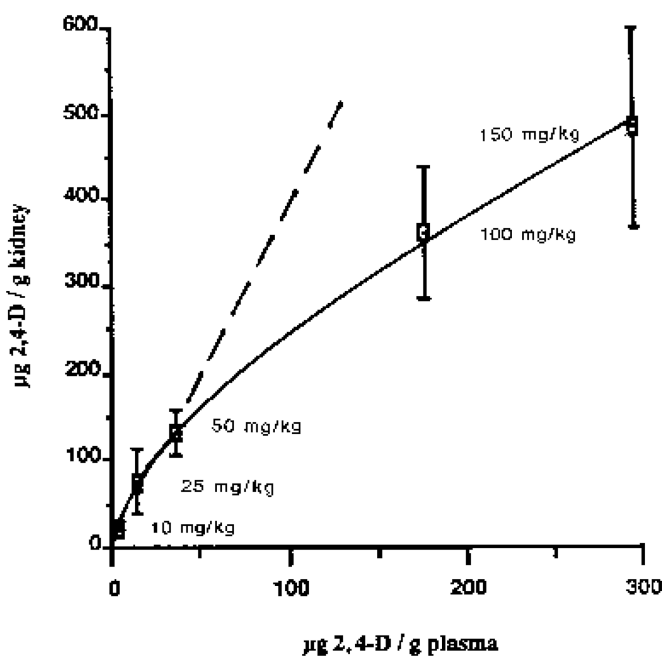

FIG. 5. Kidney concentrations versus plasma concentration of ^{14}C six hours after rats were given various doses of ^{14}C-2,4-D. The dashed line indicates the anticipated kidney concentration if the kinetics of 2, 4-D had remained first order.

A typical experimental design involves daily administration of a non-radioactive dose for a 2-week period, followed by a single ^{14}C-labeled dose on day 15. When the toxicity study involves dietary or drinking water exposures, the repeat dose study may employ these exposures, though generally the radiolabeled dose is given by gavage in order to know precisely the time and amount of exposure prior to collecting blood, tissue, or excreta time-course data. Parent compound and total radioactivity are both quantified; the difference between them is attributed to metabolites, though known metabolites may also be measured directly. The pharmacokinetics are then evaluated against those observed following a comparable single dose. Data analyses include the evaluation of blood/plasma AUCs, the calculation of clearance rates (ml hour^{-1}), and determination of half-lives for both absorption and elimination kinetics. The routes and rates of excretion are determined, as well as the extent of metabolism relative to that observed following a single-dose administration. The repeated dose selected for this study generally will be in the range to be proposed as a high dose in the longer term toxicology studies, though its outcome may result in a modification of the selection of the high dose for the toxicity study.

Example F. Altered kinetics with repeated dose (propylene glycol monomethyl ether, PGME) In studies with PGME, parent compound and metabolite in blood were measured to demonstrate dose-dependent metabolism and its modification by repeated exposures. Circulating levels of parent compound were associated with a sedation effect in the animals that could impact their well-being and survival in a longer duration study, though subchronic toxicity studies showed this effect to be transient due to induction of metabolism. A combination of results from the pharmacokinetic and subchronic toxicity studies was used to establish the high dose for the chronic bioassay (Foran, 1997).

Example G. The impact of repeat-dose kinetics (triclopyr) Timchalk et al. (1990) administered nonradioactive triclopyr for 14 consecutive days. On the fifteenth day, the animals were dosed with a ^{14}C-labeled dose. The dose level selected was within the linear kinetic range and directly comparable to the single-dose kinetics. Although the plasma time course of ^{14}C activity was not determined in this study, the overall distribution, urinary excretion rate, and urinary metabolism were evaluated and compared to the results obtained following a single oral dose. The distributions of ^{14}C activity following either a single or repeated exposure to triclopyr in rats are presented in Table 7, and may suggest a slightly higher tissue/carcass residue (0.4 versus 0.03%) than the single-dose study; overall, however, the distribution of ^{14}C activity is very comparable, with urinary excretion predominating.

The urinary excretion of ^{14}C activity (Table 8) is very comparable between the single and repeated dosing, suggesting that repeated exposure did not appreciably modify the clearance kinetics. Likewise, a comparison of the extent of metabolism (Table 9) indicates that repeated exposure did not modify the metabolism of triclopyr; thus, the single-dose pharmacokinetics can reasonably be used to determine the disposition and clearance of triclopyr following repeated exposures. The lack of alterations with repeated dosing indicates that estimates of internal dose obtained from single dose pharmacokinetic studies should provide reasonable estimates for an internal dosimetry-based dose-response analysis of the repeated-exposure toxicity studies. This can be further strengthened by measuring blood concentrations at selected times in those toxicity studies.

TABLE 7
Distribution of radioactivity recovered 72 hours after male rats were given a single oral or repeated (14-day nonradiolabeled) dose of 3 mg ^{14}C-triclopyr/kg of body weight (adapted from Timchalk et al., 1990)

Samples	Percentage of administered dose	
	3 mg/kg single	3 mg/kg repeat
Urine	94.34 ± 0.98	93.30 ± 1.54
Feces	0.82 ± 0.50	1.92 ± 0.74
Cage wash	0.42 ± 0.35	0.77 ± 0.46
CO_2 trap	0.08 ± 0.01	0.16 ± 0.11
Tissue and carcass	0.03 ± 0.01	0.40 ± 0.24
Total	95.69	96.55

Pharmacokinetics in Support of Reproductive and Developmental Neurotoxicity Studies

The reproductive and developmental studies proposed in the tiered approach for life-stage testing (Cooper et al., 2006) would utilize dietary exposures unless technical problems, such as lack of palatability, resulted in other exposure methods, such as gavage. Pharmacokinetic information can be used to verify exposure or determine the extent of lactational transfer of xenobiotics to help in the design and interpretation of reproductive and developmental toxicology studies. When the critical window for neurodevelopment or other processes is believed to occur in pharmacokinetically different life stages across species (e.g., early postnatal in rats and in utero in humans), this information may be essential for assessing study adequacy and data interpretation (Barton, 2005). However, there are only a few published pharmacokinetic studies that quantitatively evaluate lactational transfer and subsequent dosimetry to the developing neonatal rat pup. Corley et al. (2003) reviewed literature on physiologically-based

TABLE 8
Radioactivity excreted in the urine during the indicated intervals after male rats were given a single oral or repeated (14-day nonradiolabeled) dose of 3 mg ^{14}C-triclopyr/kg of body weight (adapted from Timchalk et al., 1990)

Collection intervals	Percentage of administered dose	
	3 mg/kg single	3 mg/kg repeat
0–12 hours	62.53 ± 5.40	73.46 ± 11.60
12–24 hours	27.01 ± 4.27	17.33 ± 12.03
24–36 hours	2.19 ± 0.76	1.59 ± 0.77
36–48 hours	0.76 ± 0.30	0.45 ± 0.33
48–60 hours	0.35 ± 0.13	0.28 ± 0.20
60–72 hours	0.21 ± 0.03	0.20 ± 0.20
Total	93.05 ± 0.93	93.30 ± 1.54

TABLE 9
Percent distribution of ^{14}C in the urine 0–12 hours postdosing in male rats given a single oral or repeated (14-day nonradiolabeled) dose of 3 mg ^{14}C-triclopyr/kg of body weight (adapted from Timchalk et al., 1990)

0–12 hour urine collection	Percent radioactivity in urine specimen	
	3 mg/kg single	3 mg/kg repeat
Peak A	2.17	0.95
Peak B	2.35	0.75
Peak C	4.17	3.77
Peak D	1.55	2.42
Peak E	89.76	92.11

Note. To characterize the extent of metabolism, urine samples from the 0–12 hour and 12–24 hour collection intervals were profiled by HPLC.

pharmacokinetic (PBPK) models for gestation and lactation, and noted published lactational transfer pharmacokinetic studies for *p,p'*-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), tetrachloroethylene, and trichloroethylene in the rat. The objective of a lactational pharmacokinetic study is to determine quantitatively the extent of transfer of the parent chemical or a potentially toxic metabolite to nursing offspring.

Exposure via the milk can be verified at any time during lactation by verifying the presence of parent compound or principal metabolite(s) in the milk or in both the milk and tissues from the offspring. However, some precautions are needed to rule out other sources of exposure if the milk is not analyzed. Residues in the neonate within the first few days after parturition could potentially reflect carryover from exposure during gestation. Therefore, tissues should be sampled some days later (e.g., postnatal days (PND) 10–21 in rodents), unless kinetic data are available to rule out this possibility. If treatment is via the diet, residues in the offspring could also be from dermal exposure (e.g., crawling in the feeders) or from their consumption of the treated feed. Sampling earlier in lactation (e.g., before PND 14 in rodents) will minimize the opportunity for these alternative sources of exposure. It is difficult to measure dosage (mg/kg/day) from exposure via the milk, because the concentration in the milk and the amount of milk consumed per day varies over the course of lactation, with multiple feedings on each day of lactation. Therefore, measures in the milk and/or pup tissues may only verify that there is some level of exposure via the milk, providing an estimate of the relative magnitude of exposure in the mother and offspring. Finally, the concentration of chemical in tissues from the offspring depends on many time-dependent factors (e.g., the interval between treatment of the mother and suckling; absorption and transfer to the milk; absorption, distribution, and clearance by the offspring). To increase the likelihood of verifying lactational transfer by tissue analysis, it is necessary to manage the time between dose administration and

nursing, and it may be necessary to collect samples at multiple times after nursing.

Example H. An experimental design to verify lactational transfer (chlorpyrifos) Mattsson et al. (2000) dosed pregnant Sprague-Dawley rats daily by gavage with chlorpyrifos from gestational day (GD) 6 to PND 10. Concentrations of chlorpyrifos and two metabolites were measured in the milk and in pup tissues (blood) on PNDs 5 and 11. In addition, blood, heart, and brain samples from the pups were assayed to measure cholinesterase inhibition as a biomarker of exposure and toxicity. The study confirmed the presence of chlorpyrifos and chlorpyrifos oxon (the active metabolite for cholinesterase inhibition) in the milk and pup blood, as well as cholinesterase inhibition in the offspring. The results also demonstrated that the levels of exposure and cholinesterase inhibition were considerably lower in the pup than in the dam.

Example I. Lactational transfer for highly lipophilic compounds (DDE) You et al. (1999) dosed dams daily by gavage with 0, 10, or 100 mg DDE/kg of body weight from GD 14 to 18. To determine the contribution of DDE from lactation alone, a cross-fostering design was used. On PND 1, pups were culled and assigned to groups. Pups from naive dams were allowed to nurse from dams that had previously been exposed to DDE (10 or 100 mg/kg), and, after parturition, pups were killed on PND 10, 78, or 200. Blood, liver, and brain samples were collected on PND 10 and analyzed for DDE, whereas only fat was analyzed on PND 78 and 200 for DDE, due to the lipophilic nature of DDE. The results of this study suggest that lactationally exposed pups had DDE concentrations 50 times higher than pups exposed in utero, and indicates that for DDE lactational exposure is important.

Quantitative Pharmacokinetic Analysis for Studies

Utility of Classical Pharmacokinetic Analysis. Pharmacokinetic studies provide time-dependent measurements of concentrations of a chemical and/or its metabolites in various body fluids, such as blood, excreta, or, less frequently, tissues following an exposure. Pharmacokinetic analyses are mathematical analyses to characterize the behavior of the chemical in the body. Classical compartmental or noncompartmental analyses calculate pharmacokinetic parameters, such as T_{max} (time of maximum concentration), C_{max} (maximum concentration), AUC (area under the curve), F (bioavailable fraction of dose), and half-life. For a discussion of pharmacokinetic data interpretations, see Ross et al. (2001), Frantz et al. (1994), Gibaldi and Perrier (1982), O'Flaherty (1981), Renwick (2001), Wilson et al. (1994), and Welling (1986). Computer programs are available for statistically fitting pharmacokinetic data and estimating parameters in compartmental and noncompartmental analyses. These approaches are currently the dominant approaches used with pharmaceuticals.

Classical compartmental analyses can evaluate the ability of a range of mathematical models to fit experimental data. The parameters in the model are often indirectly related to actual anatomy or physiological processes, but they can provide substantial insights into the behaviors of many chemicals (e.g., quantifying dose-dependent changes in clearance). These models have been associated with the development of many

important concepts describing the relationship of blood (whole, serum, or plasma) concentrations to the pharmacological activity or toxicological effects. Various characterizations of blood concentrations are used (e.g., C_{max}, AUC), while changes in blood or body concentrations are described by clearance or half-life. Notable for repeat-dosing toxicity studies is determining whether steady-state conditions are achieved due to exposures occurring frequently enough and for sufficient duration to balance the clearance of the compound.

Classical pharmacokinetic analyses are valuable methods; however, a number of concerns and limitations have been raised—perhaps most notably, questions about the ability to extrapolate across species and relate the mathematical analyses to actual biological processes. When human pharmacokinetic data are collected, as occurs in the initial phases of clinical trials for pharmaceuticals, concerns about cross-species extrapolation are more limited. For environmental chemicals, human data are less frequently available, thus providing one of the major driving forces behind interest in PBPK modeling.

Utility of the PBPK Approach. PBPK models can be used to design experiments, optimize dose selection, test hypotheses about mode of action, and greatly improve the interpretation of animal studies for application to risk assessment. Properly designed animal studies, in turn, provide the information necessary to validate the predictive value of the model. Use of models cannot entirely replace collection of animal data, but can provide refinement. These models use pharmacokinetic data as a strong biological basis for extrapolation across species or dosing patterns (from high to low dose, across temporal dosing patterns or routes of exposure) and to provide estimates of the relevant tissue dose. PBPK modeling is a valuable tool for evaluating data from ADME studies, and often provides a stronger approach for making extrapolations relevant to risk assessment than is possible with classical analyses due to their more explicit modeling of the biological processes.

The use of PBPK modeling can provide a quantitative means for determining the effect of various age- and gender-specific factors on the relationship between the external exposure and the internal target tissue exposure (Clewell et al., 2002). In addition, high- to low-dose extrapolation of tissue dose can be accomplished with PBPK models by accounting for the nonlinear kinetic behavior of chemicals. These models can be very useful in route-to-route extrapolations (Gerrity and Henry, 1990). For instance, the development of an inhalation toxicity value can be derived from an oral exposure study using a PBPK model.

Data from pharmacokinetic and metabolism studies using in vivo animal studies and in vitro methods can be instrumental in the interpretation of animal toxicity with respect to possible human risk. This analysis may be made in the absence or presence of human pharmacokinetic data. In the absence of human data, extrapolation may be by the use of PBPK modeling methods. This approach relies on interspecies allometric scaling with scale-up between animals and humans of such parameters

as tissue volume and blood flow and by their relation to body weight. Assuming that the uptake from blood to tissue (extraction ratio) is a function of the chemical and therefore independent of species, it is possible to derive complex models involving all the major tissues of the body. These models may then be scaled up from known animal data to humans based on the known physiological differences. In addition, in vitro metabolism studies with animal and human tissues can provide information for modeling these processes, rather than assuming allometric scaling of metabolism.

Discussion of Key Uses of Pharmacokinetic Studies in Study Design

Dose Selection Issues When Dose-Dependent Pharmacokinetics Are Observed

A dose-response type of study will determine the possible nonlinearities in kinetics of absorption, distribution, metabolism, and elimination. This type of ADME study can provide clues identifying nonlinearity in the kinetics of the chemical. These clues include the following: Elimination cannot be described by a single exponential process; elimination half-life increases with dose; plasma AUC versus time curve is not proportional to increasing dose; and the composition of excretion products changes quantitatively with increasing dose. Saturation phenomena often produce nonlinear dose-response curves. The proposed number of dose levels in a dose-response ADME study would be at least three, with the dose range spanning a minimum of two orders of magnitude, when 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 produce no or minimal toxicity based on single-dose studies (e.g., $1/10$ LD$_{50}$) or repeat-dose information. Because the dermal route of exposure often results in relatively low blood levels of test chemical, nonlinearity may be better evaluated using the oral or inhalation routes. Depending on the stage of development of a compound and the data available, range-finding studies with limited numbers of animals may be necessary prior to conducting full ADME studies.

Selection of Route of Administration for Reproductive and Developmental Studies

Chemicals are tested in pregnant animals such as mice, rats, or rabbits for their potential to cause malformations and signs of toxicity during fetal development. The pharmacokinetic data from the initial ADME studies that established the extent of oral bioavailability and the dose-dependent kinetics will be used in the study design of the developmental studies. Pregnant females are usually exposed to the test chemical from implantation of the embryo in the uterus (GD 6 in the rodent) to the day before delivery. However, they may be exposed to the chemical throughout pregnancy, and, in some cases, offspring are also exposed following birth. Choosing the route of administration is dependent on a number of factors—most importantly, whether the route

is relevant for potential human exposure. However, this route of potential human exposure may not be feasible. For example, even though agents may be intended for dermal use, dermal absorption may be too limited for a developmental study, as may have been observed in earlier pharmacokinetic route-to-route studies and the bioavailability data. Doses are selected for these reproductive studies so that a no-observed-adverse-effect level (NOAEL) and a lowest-observed-adverse-effect level (LOAEL) can be determined for maternal toxicity and, possibly, fetal toxicity (Corley et al., 2003).

PHARMACOKINETIC STUDIES FOR IMPROVED INTERPRETATION OF TOXICITY STUDY RESULTS

Overview

Information describing the dose of the active form of a chemical reaching the target tissue can be among the most valuable data for interpreting toxicity study results and developing or supporting mode-of-action hypotheses. Example K describes tissue distribution studies in which a specific toxicity is observed in only one of three species. Unusual tissue distribution was demonstrated in the affected species. To obtain such information, data are gathered from a combination of in vivo and in vitro studies. In vitro studies provide data on specific aspects of pharmacokinetics such as metabolism, protein binding, or tissue distribution. A major advantage of in vitro studies is that they can frequently be done for both the species in the toxicity study and in humans, thus facilitating interspecies comparisons. In vivo studies provide an integrated perspective on the relative importance of different processes in the intact biological system for comparison with the results of the toxicity studies. Pharmacokinetic modeling provides a powerful tool for integrating in vivo and in vitro data to provide predictions of tissue dosimetry.

Some of the major objectives of pharmacokinetic studies conducted for purposes of improving the interpretation of toxicity results include the following:

- Describe species differences and qualitative comparisons to humans.
- Support mode-of-action analyses for dose-response assessment.
- Support quantitative extrapolation to humans using internal dose.

Additional uses of the data generated in these studies are to support route-to-route extrapolation through the use of pharmacokinetics, thus minimizing toxicity testing, and to evaluate the potential for changes in pharmacokinetics with life stage (e.g., infants, children, and the aged).

The studies in the previous section were described largely in terms of their utility for informing toxicity study design, but they also play a major role in toxicity study interpretation. Many of the studies identified below would be conducted in an iterative manner following the generation of toxicity study results, particularly from the 28-day adult rat and 90-day dog studies (Doe

et al., 2006). However, in selected cases, some of these studies may also be useful for toxicity study design, particularly for chemicals that are members of a class with known properties. In such cases, those studies might be done prior to the toxicity studies. Finally, virtually all the data from these studies will ultimately contribute to dose-response analyses and other aspects of risk assessment.

Pharmacokinetic Study Designs for Toxicity Data Interpretation

Metabolite Identification

Metabolite identification is required under the U.S. EPA OPPTS 870.7485 Guidelines for Metabolism and Pharmacokinetics (U.S. EPA, 1998). These guidelines indicate that metabolites in excreta accounting for 5% or more of the administered dose should be structurally characterized.

Metabolite identification provides information for several purposes in the interpretation and use of toxicity study results for risk assessment. Structure–activity relationships may identify toxicophores that could serve as structural alerts predictive of toxicity from the metabolites (e.g., Green, 2002). Comparisons of metabolites across species can inform interspecies extrapolation. Evidence that toxicological test species form metabolites that are the same as plant metabolites or other environmental residues is also used to evaluate the need for additional testing. If novel metabolites formed in the environment might be consumed as residues, then toxicological testing may be required. If the metabolites were formed in the test species, additional testing may not be required. A quantitative pharmacokinetic modeling approach to limit toxicity testing for compounds produced as metabolites of other compounds has been described (Barton et al., 2000).

> *Example J. Metabolite identification for data interpretation* In a metabolism study, groups of 10 male mice were given single oral doses (25 or 250 mg/kg) of Chemical B. Additionally, two groups of three male mice were given either the low or high dose to assess acute toxicity, and another group was given no test material (controls) to assess toxicity (i.e., not for pharmacokinetic purposes).
>
> No overt signs of toxicity or histopathologic changes were observed following the single oral dose. Dose confirmation indicated that the administered doses represented 95–103% of the nominal radioactivity and 102–106% of the nominal parent chemical. Mean total recovery of administered radioactivity (percent of dose) was 99% and 101%, respectively, for the low- and high-dose groups. Total absorption over a 72-hour period, implied from urinary excretion/cage wash and tissue/carcass burden data, was 77% and 83% for the low- and high-dose groups, respectively. Urinary excretion assessed over a 72-hour period accounted for 76% and 82%, respectively, of the low and high dose, most of which occurred within 36 hours of dosing. Fecal excretion over the 72-hour period accounted for 22% and 16%, respectively, of the low and high dose. Under the conditions of this study, neither absorption nor excretion of Chemical B appeared to be saturated. Tissue/carcass burden at 72 hours postdosing was minimal, and accounted for only 0.8% (low dose) and 0.6% (high dose) of the administered radioactivity (1.8 and 9.7 μg eq/g, respectively, for the low and high dose). At the doses tested, Chemical B

did not exhibit potential for tissue accumulation following a single oral dose.

The urinary metabolite profile in mice included four distinct peaks as determined by high-performance liquid chromatography (HPLC) analysis: an acid metabolite, a glycine conjugate of the acid metabolite, a taurine conjugate of the acid metabolite, and the parent compound. All four components were detected in the urine of high-dose mice, but no parent compound was detected in the urine of the low-dose mice. This might indicate that the high dose was close to saturation of metabolism, given appropriate detection limits for the parent compound. Over the 72-hour period, the glycine conjugate of the acid metabolite represented 71% of the administered dose in the low-dose group and 70% in the high-dose group. These results indicate the doses spanned an appropriate range in metabolic capacity, with the high dose in the lower end of the range where metabolic saturation would become apparent. Saturable phenomenon are often incorrectly described as if there is an absolute cutoff above which they occur. A process described by a rectangular hyperbola such as the Michaelis–Menten enzyme kinetics equation, saturable transport kinetics, or reversible binding of a ligand to a protein exhibits 83% maximal activity at concentrations 5× that giving half-maximal activity, 91% maximal at 10× half-maximal, and 98% maximal at 50× half maximal. Thus, saturation increasingly occurs over an order of magnitude (log unit) change in concentration. The limited breakthrough of parent compound and the acid metabolite representing essentially the same fraction at the low and high dose indicate that the high dose is just beginning to enter the range for saturation of metabolism. These data were used to support evidence that the high dose used in carcinogenicity studies was not excessive. The data demonstrating that Chemical B can be metabolized to an acid metabolite known to be toxic support the inclusion of the metabolite as a residue to be assayed in food crops and included in the tolerance (unpublished data summarized by U.S. EPA).

Pharmacokinetic Sampling During the Conduct of Toxicity Testing

Assessment of systemic exposure (i.e., internal concentrations of chemical) in toxicity studies is a fundamental aspect of toxicity studies for pharmaceuticals (Hawkins and Chasseaud, 1985), but has not been common for toxicity testing of agricultural or industrial chemicals (Saghir et al., 2005). In part, this reflects the fundamental understanding in the pharmaceutical area that the blood concentration of the drug is an essential indicator of its presence at a therapeutically useful level to which the blood levels in the toxicity studies can be related. This contrasts with the continued focus on exposure doses for agricultural and industrial chemicals. Nonetheless, measures of internal dose are extremely valuable for interpreting toxicity studies and informing the numerous extrapolations (e.g., cross-species, cross-exposure regimens) that arise in chemical risk assessment, making measurement of internal concentrations in toxicity studies desirable.

As indicated in the U.S. FDA "Toxicokinetics: Guidance on the Assessment of Systemic Exposure in Toxicity Studies" (U.S. FDA, 1995), internal concentrations may be determined in animals in the toxicity study or satellite groups. Satellite groups are more commonly used for rodents, while studies with larger animals can readily accommodate blood sampling from the animals in the toxicity studies. Infrequent sampling in rats is possible,

though it may be desirable to sample the controls as well to ensure that all animals receive the same handling. Sampling of blood at terminal sacrifice is useful for mice, rats, or other species. The timing in relation to dosing and the half-life of the compound need to be considered because little chemical will be present after five half-lives, thus potentially making detection difficult.

Sampling animals in the toxicity study provides information about animals under conditions of repeated dosing that are rarely addressed in pharmacokinetic studies (though a shorter duration repeat-dosing pharmacokinetic study was recommended earlier in this article). Such sampling during the conduct of toxicity testing also incorporates the route and matrix of the study. This can be very valuable information for comparison with shorter term pharmacokinetic studies and for validation of pharmacokinetic models for risk assessments based upon internal dosimetry. Furthermore, when the animals in the toxicity study itself are sampled, insights are gained into variations in response within a dose group (e.g., higher responses in animals with higher levels of chemical in blood).

In Vitro Metabolism Studies in Rodents, Humans, and Other Species

Metabolism is frequently one of the most important pharmacokinetic processes for toxification of a compound (i.e., formation of a toxic metabolite) and clearance. Thus, in vitro metabolism studies can be invaluable for predicting differences in metabolism and pharmacokinetics across species, gender (Gleiter and Gundert-Remy, 1996), and ages. These metabolic differences may be quantitative (i.e., different rates of metabolism) or qualitative (e.g., formation of different metabolites). In vitro metabolism studies have not been required for registration of agricultural chemicals. However, they may provide one of the most valuable ways to limit animal use by assisting in pharmacokinetic modeling for toxicity study design, interpretation, and use in risk assessment. They can provide valuable information for selecting a test species with similarities to humans or avoiding a species with significant differences (as illustrated in Example B).

In vitro studies have been commonly used to characterize metabolism, but are increasingly used to predict in vivo metabolic rates. Estimates of quantitative differences across species or life stages can be incorporated into pharmacokinetic models to provide a basis for cross-species extrapolations (Pritchett et al., 2002; Sweeney et al., 2003) or life-stage extrapolations (Timchalk et al., 2002a; Clewell et al., 2002; Gentry et al., 2003; Sarangapani et al., 2003) early in product development for purposes of toxicity study design or later for interpretation of toxicity studies and application in risk assessment. In addition, when predictions of target tissue concentrations of reactive intermediates are desired, in vitro metabolism studies generally present the best approach for estimating metabolic activities in different tissues (Clewell, 1995). In vivo pharmacokinetic

studies generally only determine the rates of total metabolic clearance rather than the contributions of individual tissues. When extrahepatic metabolism is large, the total metabolism may be so great that it is apparent other tissues are involved, but their identities will be undetermined. In the pharmaceutical industry, individually expressed cytochrome P-450 enzymes are used to determine which forms are important contributors to the metabolism (Salonen et al., 2003). This information can be used to predict potential interactions of drugs (e.g., inhibition or, perhaps, induction) and to determine whether the important enzymes are ones that are expressed polymorphically (i.e., different genetic variants with different activities are know in human populations) or with a distinct development (i.e., age-dependent) profile. These kinds of information can also be applied to decision making about agricultural chemicals (Gentry et al., 2002).

In vitro metabolism studies can be carried out using experimental systems that include subcellular fractions, isolated cells such as hepatocytes, or precision-cut tissue slices (Brandon et al., 2003; Salonen et al., 2003). The most commonly used subcellular fractions are microsomes (preparations of the endoplasmic reticulum with membrane-bound enzymes, notably the cytochromes P-450) and cytosol (soluble cytoplasmic enzymes), although isolated mitochondria or other subcellular fractions are sometimes important. For compounds metabolized in blood, plasma, or serum, red blood cell lysates can be used. The major strength as well as limitation of studies with subcellular fractions is that they examine parts of metabolic pathways. Frequently, pathways involve sequential metabolism by membrane-bound and soluble enzymes (e.g., hydroxylation by a P-450 followed by conjugation by a sulfotransferase). Because of this, metabolites may be formed by pathways that are poorly operative in intact, integrated systems. By contrast, isolated hepatocytes and tissue slices maintain cellular structure and tissue structure in the latter case. These systems can better mimic the in vivo metabolic pathways, although there are substantial experimental concerns that must be carefully controlled (e.g., changes in the expression of enzymes, notably cytochromes P-450 with culture conditions and time).

Two basic approaches can be used in designing in vitro metabolism studies: chemical-specific studies or identification of the enzymes involved. As xenobiotic-metabolizing enzymes and their genes have been characterized, methods have been developed for specifically expressing or characterizing the involvement of different related members of multienzyme families (e.g., cytochromes P-450, glucuronyl and glutathione transferases) (Hodgson, 2003). Increasingly, quantitative data describe the expression of different isoforms across species or at different ages permitting extrapolations across species, to different ages, or among the human population (Timchalk et al., 2002b; Gentry et al., 2002; Jonsson and Johanson, 2001; Dorne et al., 2002, 2004). However, many chemicals are metabolized by multiple isoforms, and some xenobiotic-metabolizing enzymes are not well-conserved across species (e.g., many cytochromes [CYPs]). Thus, studies of the metabolism of the

chemical of interest using subcellular fractions from different tissues, species, age groups, or human populations comprise a valuable tool for obtaining qualitative and quantitative metabolic information for extrapolations.

Tissue Distribution

Differences in tissue distribution characteristics of a compound can be an important determinant of its potential to cause toxicity in those tissues to which it distributes. In addition, tissue distribution may be an important determinant of the ability of a compound to accumulate, although this is substantially modified by the rate at which the compound is cleared.

The persistence of compounds and their accumulation have been major environmental concerns. The U.S. EPA OPPTS 870.7485 Guidelines for Metabolism and Pharmacokinetics (U.S. EPA, 1998) indicate that the total radioactive dose in tissues taken at terminal sacrifice in the Tier 1 study will be evaluated when a significant amount of the administered dose is unaccounted for in excreta (i.e., urine, feces, exhaled air). There is very limited value in collecting tissue distribution data for long periods after dosing when the vast majority of the dose has been eliminated. Potential accumulation in fat is reasonably well predicted by physicochemical properties, notably the octanol:water partition coefficient (K_{ow} or log P); consequently, measurements in fat for long periods after dosing will largely be confirmatory. Another major limitation of much currently collected terminal tissue distribution data is that measurements of total radioactivity do not determine if it is parent compound or metabolite that remains in the body.

Correlation of tissue distribution with target tissues in toxicity studies is better accomplished while substantial amounts of the chemical remain present in the body, for example, at one or more times around the peak blood concentration following oral absorption. Such data should quantify parent compound and metabolites, to the extent feasible. If the metabolites are unknown or difficult to quantify, subtracting parent compound from total radioactivity will provide an estimate of the behavior of the total metabolites formed.

Example K. The use of tissue distribution data to interpret toxicity studies (metosulam and paraquat) Unusual species differences in target organ toxicity are observed for the triazolopyrimidine sulfoanilide herbicide, metosulam. The beagle dog was the only species in which the eye was a target organ. A comparative pharmacokinetic, target organ distribution, and metabolism study was conducted in rats, mice, and dogs to provide perspective on the observed species differences. Analysis of the tissue distribution was based on quantification of radioactivity in key tissues and, in the case of the eye, histoautoradiography of frozen tissue sections. Analysis of radioactivity and histoautoradiography of the dog eyes indicated that the retina, a target for toxicity in the dog, did exhibit affinity for the radiotracer, whereas there was no evidence of radioactivity localization in the eyes of rats (Timchalk et al., 1997). Comparisons of the lung toxicity observed with paraquat but not diquat were explained by the much greater distribution to lung of the former compound

(Sharp et al., 1972). These data provide powerful information for interpreting toxicity study results to provide a basis for selecting appropriate species and internal dose metrics for risk assessment.

Example L. The use of tissue distribution data to interpret toxicity studies (diethanolamine) The carcinogenicity of diethanolamine is another example of the importance of tissue distribution (Lehman-McKeeman, 2002). At high concentrations, it leads to choline-deficiency-induced liver cancer in mice, but not in rats. Diethanolamine specifically accumulates in the liver apparently via a transporter for choline, blocking choline uptake. Choline is incorporated into lipids, which are largely exported out of the liver, but diethanolamine is a poor substrate for incorporation into lipid and for methylation, so it only slowly exits the tissue. Tissue distribution studies demonstrating the specific accumulation in liver were a key piece of information characterizing the mode of action for this compound in mice (Mathews et al., 1997).

Nonrodent Laboratory Animal Pharmacokinetics

Most mammalian toxicity tests for pesticides are conducted using rodent species, that is, the rat or mouse. However, nonrodent tests are also required. The beagle dog is the second species most often used, particularly for subchronic and chronic toxicity studies. Distinct differences in toxicity are sometimes observed between rodent and nonrodent species. Tibbits (2003) reviewed species-specific differences related to the pharmacokinetics and metabolism of the dog. The author concluded that physiologic characteristics unique to the dog could affect pharmacokinetics, making comparisons between dogs and other test species occasionally difficult. Some characteristics that differ in dogs compared to other species include gastrointestinal function (gastric pH, gastric mobility, and intestinal mobility), hepatic metabolism (*N*-acetylation, CYP450 substrate specificity), renal function, and plasma protein binding. These differences may produce differences in pharmacokinetic parameters such as bioavailability, AUC, or clearance in dogs, compared to rats or other species. When differences are noted in toxicology tests across species, pharmacokinetics and metabolism studies in the dog may help explain why they occur, and aid in determining the relevance to humans (Timchalk, 2004).

Example M. Species differences in bioavailability (atomoxetine hydrochloride) Mattiuz et al. (2003) evaluated the pharmacokinetics and metabolism of atomoxetine hydrochloride. Fischer 344 rats and beagle dogs were given either single iv or oral doses of atomoxetine hydrochloride. Urine and feces were collected for up to 96 hours, and blood samples were taken for up to 48 hours. The test material was well absorbed orally in both species. However, distinct differences were noted in bioavailability, that is, only 4% in rats but 74% in dogs. The lower bioavailability in the rat appeared to be due to a more efficient first pass clearance of the parent molecule, lowering the overall systemic exposure to this molecule, but increasing exposure to the metabolites as compared to the dog. If there are differences in the inherent toxicity of the parent and a metabolite, this difference in bioavailability could dramatically affect the toxicity results observed between rats and dogs. The large difference in bioavailability for the same exposure dose would make it essential to utilize internal dosimetry for the dose-response analysis. In vitro data on metabolism in rats, dogs, and humans

could be combined with these in vivo data to better predict human risks.

Example N. Species differences in blood kinetics (itraconazole) The blood kinetics of itraconazole following oral administration was studied in Sprague-Dawley rats, New Zealand white rabbits, and beagle dogs (Yoo et al., 2002). The half-life of the parent compound was similar in the rat (5.2 hours) and the rabbit (9.4 hours), but considerably longer (28 hours) in the dog. The half-life of the primary metabolite was also longer in the dog (57.5 hours) compared to the rat (11.8 hours) and the rabbit (12.4 hours). Interestingly, human data are also available for itraconazole, and the dog data more closely match the human blood kinetics. Clearance across species is often thought to scale as a function of body weight raised to the $3/4$ power (O'Flaherty, 1989), but in this example, the dog appears to have significantly slower clearance than would be expected based on scaling.

Example O. Species differences in pharmacokinetics leading to differences in toxicity (triclopyr) Pharmacokinetic studies have been conducted in multiple species, including the dog, monkey, rat, and human (Timchalk and Nolan, 1997; Timchalk et al., 1990; Carmichael et al., 1989). Triclopyr is an organic acid that causes kidney toxicity at higher doses in the form of degeneration of the descending tubules. The NOAEL in the rat is 20 mg/kg body weight/day; however, in the dog, the NOAEL is only 0.5 mg/kg body weight/day. It was hypothesized that this difference is due to dogs having a lower capacity to excrete organic acids. A specific pharmacokinetic study with triclopyr was conducted in dogs and monkeys to compare to existing data in rats.

Three male beagle dogs were used for a pharmacokinetic analysis following an oral exposure to unlabeled test material at doses of 0.5, 5, and 20 mg/kg body weight triclopyr. Blood samples were collected for 24 hours, and urine was collected for up to 168 hours after dosing. An additional three dogs were included in an intravenous-exposure renal clearance study using both unlabeled and ^{14}C-labeled triclopyr. The concentration of triclopyr in the blood was gradually increased until active secretion into the urine was saturated. A single male rhesus monkey was also included in an intravenous-exposure renal clearance study. A single dose of 30 mg/kg body weight was used, and the same animal was retested at the same dose 10 days after the initial dosing. Blood and urine were analyzed in both the dog and monkey clearance studies.

Triclopyr was shown to be rapidly absorbed in dogs (as in all other species tested) following oral administration of doses from 0.5 to 20 mg/kg body weight. The terminal-phase half-life in dogs was 25 hours and in the monkeys approximately 6 hours. In rats, the half-life (3.6 hours) is considerably shorter than in the dog and closer to the monkey. The half-life in humans at approximately 5 hours is much closer to that of the rat and monkey than that of the dog. At 0.5 and 5 mg/kg body weight doses in dogs, the mean AUC and clearance were essentially proportional to dose. However, at 20 mg/kg body weight, the AUC was 50-fold greater than that at 0.5 mg/kg body weight, at which a 40-fold increase would have been predicted by the dose increase. The clearance was also affected at 20 mg/kg body weight, showing a decreasing trend. This modestly nonlinear relationship was shown to be due to saturation of the renal excretion process. The pharmacokinetic data for triclopyr are also nonlinear in the rat; however, this occurs at higher doses. Overall, it was demonstrated that the dog has a lower capacity to excrete triclopyr than the rat, monkey, or human, and a greater susceptibility to toxicity would be expected. The results of the dog pharmacokinetic study calls into question the relevance of using triclopyr toxicity data in dogs for human health risk evaluation, particularly absent appropriate pharmacokinetic adjustments.

Serum Protein Binding Evaluation

Albumin, sex-hormone-binding globulin, α1-acid glycoprotein, and α-fetoprotein are some of the major serum proteins involved in noncovalent binding of endogenous and exogenous compounds. Serum binding influences the free concentration of chemical in blood that is available to tissues to induce toxicity (Mendel, 1992; Levy and Moreland, 1984). Due to differences across species, gender, life stages (e.g., pregnancy, fetal, and early postnatal periods), and disease states in binding proteins and their capacities to bind different chemicals, it can be important to account for serum binding proteins when interpreting toxicity studies and extrapolating to other species, sexes, or life stages (Teeguarden and Barton, 2004; Hill and Abramson, 1988).

Albumin is a major binding protein with high capacity (i.e., high concentration, 0.5 mM in adult humans) but general low affinity for acidic drugs and other xenobiotics. α1-Acid glycoprotein is considered the major binding protein for basic drugs. Sex-hormone-binding globulin is a primate protein (not found in rodents) involved in high-affinity binding for estradiol, testosterone, and other endogenous hormones; it also binds exogenous compounds, though typically with lower affinities. Humans and rodents express α-fetoprotein at highest levels in utero, with concentrations dropping dramatically after birth. Rodent α-fetoprotein binds estradiol with high affinity, limiting its availability and apparently playing a role in the timing of puberty as its concentration decreases after birth (Greenstein, 1992). However, the human form does not bind estradiol; therefore, there is a major species difference in this activity.

Binding proteins typically limit the availability of the chemicals bound to them to distribute to tissues. But the concentration available to tissues in which a compound is highly cleared (e.g., metabolism) is generally greater than the free concentration, and may be as high as the total concentration in blood (Mendel, 1992). This is due to the concentration gradient created by clearance during passage through the tissue, leading to dissociation of chemical from the binding protein. For purposes of interpreting results of toxicity studies in different species and extrapolating them to humans, it can then be important to evaluate whether the free concentration of agricultural chemicals in serum should be measured using approaches similar to those considered for pharmaceuticals (Levy and Moreland, 1984).

Biliary Excretion

Standard ADME guideline studies, that is, U.S. EPA OPPTS 870.7485 (US EPA, 1998), require a determination of urine and fecal excretion. However, it is difficult to determine from these data whether the amount recovered in the feces is due to nonabsorption of the test material or the result of biliary excretion. For test materials with significant amounts recovered in the feces, it may be important to determine this distinction. The US EPA OPPTS harmonized test guideline 870.7485 states: "If the extent of [oral] absorption cannot be established from Tier 1

studies, or where greater than 20 percent of the administered dose is in feces, a study to determine the extent of absorption is required." This is most often accomplished by conducting a separate biliary excretion study. By knowing the amount of biliary excretion, one can determine the percentage of oral absorption by adding the amounts of urinary excretion and biliary excretion. This will allow the calculation of systemic exposure from the oral route, which can then be used in risk assessments to compare systemic exposure following different routes of exposure (i.e., oral, dermal, inhalation).

For a biliary excretion study, U.S. EPA OPPTS test guidelines suggest the use of at least three rats of one sex. Both sexes can be used if there is any evidence of a sex difference in fecal elimination. The bile ducts are cannulated, and a single oral dose of ^{14}C-labeled test material is administered. Excretion of radioactivity in bile is monitored as long as necessary to determine if a significant portion of the administered material is excreted via this route. Typically, a 48-hour time period is sufficient.

Klaassen (1996) states: "The biliary route of elimination is perhaps the most important contributing source to the fecal excretion of xenobiotics and is even more important for the excretion of their metabolites." Biliary excretion can occur by either passive diffusion or active secretion; however, the latter is typically more important. Specific transport systems exist for certain types of substances. Typically, low-molecular-weight compounds are only poorly excreted via bile, whereas compounds with high molecular weights or conjugates with molecular weights greater than 300 are excreted into bile more readily. However, there are important species differences in this threshold. In humans, the threshold occurs around molecular weights of 450–500.

Because a significant portion of biliary excretion occurs via an active process, it can theoretically become saturated at high doses. This has been demonstrated for several substances including dimethomorph (Dijk, 1990), NBQX (Hansen, 1995), ampicillin (Mesnard-Ricci and White, 1998), and Rose Bengal (Wang et al., 1992). As with saturation of renal excretion, excessively high doses of chemicals that saturate biliary excretion could lead to blood levels that are not proportionate to dose. As a result, altered physiological conditions can be produced that lead to secondary toxicity and/or liver injury that is not relevant to lower dose levels. This should be considered when selecting dose levels for chronic toxicity studies.

Saturation of biliary excretion is not typically determined in the ADME studies; consequently, a follow-up study may be necessary. The published literature on biliary excretion saturation includes a variety of protocols including in vivo perfusion and in vitro techniques. However, a protocol specific to helping in dose level selection for oral pesticide toxicity studies could not be found. Nevertheless, in theory, a protocol could be employed similar to that used for monitoring renal excretion saturation by monitoring the AUC for radiolabel or parent and metabolites in serum in groups of rats at multiple dose levels given a single radiolabeled dose following a pretreatment period with

TABLE 10
Percentage of ^{14}C-dimethomorph administered dose in male rats (Dijk, 1990)

Compartment	10 mg/kg body weight	500 mg/kg body weight
Total recovery	110.8	91
Unabsorbed fraction		
Feces	7.6	21.8
Digestive tract	0.4	3.3
Total unabsorbed	8.0	25.1
Absorbed fraction		
Urine	6.6	14.8
Bile	95.1	49.1
Cage wash	0.5	0.4
Residual carcass	0.6	1.6
Total absorbed (urine, bile, wash, carcass)	102.8	65.9

unlabeled material. Blood levels greater than those predicted by the increase in dose (nonlinearity) would indicate saturation of excretion. A confirmatory test is necessary to demonstrate that the nonlinear blood levels are due to saturation of bile excretion using bile duct-cannulated rats at the various dose levels.

Example P. Biliary excretion (dimethomorph) An ADME guideline study with dimethomorph demonstrated a significant portion of radioactivity was being eliminated in the feces. It was uncertain if this was due to biliary excretion or nonabsorption. A follow-up biliary excretion study was conducted (Dijk, 1990). Groups of 3 male and 3 female bile-duct-cannulated Sprague-Dawley rats were administered ^{14}C-dimethomorph via gavage at a dose of 10 mg/kg body weight or 500 mg/kg body weight. Bile, urine, and feces were collected for 48 hours after dosing. Animals were sacrificed at 48 hours, and the digestive tract and residual carcasses were analyzed. A cage wash and analysis was also performed at 48 hours. Radioactivity was determined using a liquid scintillation counter. For brevity, male data are reported only. Results for the low and high doses are given in Table 10.

At the low dose, a large portion of the dose is excreted via the bile. Now that the amount in bile is known, it can be added to the amounts in urine, cage wash, and residual carcass to get an estimate of oral absorption. Taking this into account, it can be determined that the absorption, and subsequently the systemic exposure, of dimethomorph is quite high following oral exposure.

At the high dose, oral absorption is less, that is, approximately 66% of the administered dose compared to 96% at the low dose. This indicates a saturation of absorption at higher doses. Biliary excretion also appears to be saturated at the high dose. At the low dose, approximately 95% of the absorbed dose is excreted via bile; however, at the high dose, a lower amount, approximately 75% of the absorbed dose, is excreted via bile. This is confirmed by the biliary excretion rate data. At the low dose, radioactivity was rapidly eliminated via bile, with an approximate half-life of 2.9 hours as calculated by means of first-order rate kinetics for the time interval 6 to 24 hours. At the high dose, the biliary excretion half-life was increased to 11 hours. Such saturation of biliary excretion should be considered when selecting dose levels for toxicology studies. Prior to

dose selection, follow-up work would be needed to include a blood concentration test as described above with more dose levels. Blood AUC results would most likely demonstrate that blood levels do not increase linearly with dose. Doses above the saturation point may involve physiological and pharmacological changes that are not relevant at much lower dose levels to which humans would most likely be exposed. These kinds of data would also be critical for a dose-response analysis based upon internal dosimetry because of the nonlinearity with dose.

Enterohepatic Recirculation

Enterohepatic recirculation is the process whereby a chemical is reabsorbed in the gastrointestinal tract following biliary excretion. Glucuronide conjugates are the principal compounds involved in this process, but parent chemicals that are eliminated in bile can also be reabsorbed. Glucuronidation is a major pathway of chemical biotransformation and ultimately excretion. The size of the aglycone (parent compound) primarily determines whether the glucuronides are excreted from the body in bile or urine. In the rat, glucuronides are preferentially excreted in bile if the molecular weight is >350. The molecular weight cutoff points vary among mammalian species (Klaassen, 1996). Chemicals conjugated with glucuronic acid are substrates for β-glucuronidase. Intestinal microflora possess this enzyme, and the aglycone can be formed in the intestine and become available for reabsorption starting the cycle of enterohepatic recirculation.

> *Example Q. The impact on toxicity of differing degrees of enterohepatic recirculation (methapyrilen)*   Methapyrilen, an H1-receptor antihistamine, causes periportal necrosis in rats. The degree of the effect was shown to be linked to glucuronide conjugation and enterohepatic recirculation (Ratra et al., 2000). Rats were treated for three consecutive days with the chemical. In bile-cannulated rats, cumulative excretion of the chemical in the bile considerably exceeded that observed in the feces at all time points, indicating that enterohepatic recirculation occurred. In one group of rats, enterohepatic recirculation was interrupted by cannulating the bile duct. The degree of liver toxicity in these rats was comparably less than that seen in rats that did not receive this procedure. It was proposed that biliary recycling led to higher systemic levels of toxic metabolites.

> *Example R. Impact on toxicity of differing amounts of enterohepatic recirculation among species (indomethacin)*   The intestinal toxicity of indomethacin is directly related to the accumulated exposure of that tissue, which in turn is related to enterohepatic recirculation (Duggan et al., 1975). Cumulative exposure of the intestinal mucosa as a percentage of dose was species dependent. Cumulative biliary elimination in dogs and rats was greater than the original administered dose ($3.6\times$ and $1.3\times$, respectively) due to enterohepatic recirculation, while biliary elimination in rhesus monkeys, rabbits, and humans was much lower. The dog was the most sensitive species in toxicology studies, directly corresponding to cumulative dose to the intestinal tissue, which was approximately 40-fold greater than in humans. These data could be used to make choices of the most appropriate species for risk assessment or to support use of an internal dosimetry-based approach that would appropriately account for the difference in intestinal dose across species for the same exposure dose.

> *Example S. Species differences in carcinogenicity with differences in enterohepatic recirculation (oxazepam)*   Oxazepam, a

benzodiazepine drug (summary of various studies by Cunningham and Bucher, 1998), was shown to produce liver tumors in two different strains of mice, but not in rats. Oxazepam and its metabolites are excreted primarily in the feces in both rats and mice; however, the metabolites differ significantly between the two species. Rats do not eliminate a significant amount of oxazepam as the glucuronide conjugate; however, this is a significant portion of the biliary excretion in mice. In mice, the glucuronide metabolite is acted upon by the gut microflora glucuronidases, releasing parent oxazepam, which is available for enterohepatic recirculation. This results in higher serum (and subsequently liver) levels in mice. Humans have been shown to be more like rats, and do not excrete a significant amount of oxazepam glucuronide in the feces. It is proposed that the rat is a better model for human carcinogenic potential of oxazepam than the mouse.

Pharmacokinetics Linked to Pharmacodynamic Studies

The modes of action of pesticides involve biochemical events associated with pharmacodynamic (PD) processes that lead to the final toxic outcome. A particularly well studied example of this is inhibition of cholinesterase enzymes by organophosphates and carbamates. It is possible to obtain pharmacokinetic measures of the levels of chemical in blood or other tissues, as well as some PD measures or biomarkers of potential effects, such as inhibition of serum or brain cholinesterase activity. These biochemical effects can be included in PBPK models to create an integrated pharmacokinetic and PD model (Timchalk et al., 2002a, 2002b). Such an integrated model could be used in risk assessment and would represent one form of a biologically-based dose-response model.

IMPROVING HUMAN HEALTH RISK ANALYSIS THROUGH APPLIED PHARMACOKINETICS
Introduction

The ultimate goal of carrying out a program of toxicology and pharmacokinetic studies is to provide estimates of potential risks for humans and exposure conditions that will be protective of human health. All of the previously described studies will contribute to this effort. However, additional studies exist that can be particularly informative for risk assessment. These studies are described here, and can inform hypotheses regarding total potential systemic dose, as a function of toxicologically meaningful time periods from all possible routes of exposure. In this regard, implementation of the Food Quality Protection Act (FQPA; http://www.epa.gov/oppfead1/fqpa/backgrnd.htm) in the United States has resulted in a new risk analysis paradigm that requires consideration of potential aggregate exposures (i.e., multiple exposure routes) and cumulative exposures (i.e., multiple chemicals) and health risks using probabilistic models such as Calendex (http://www.exponent.com/practices/foodchemical/), CARES (http://cares.ilsi.org/), and Lifeline (http://www.thelifelinegroup.org). While these models are publicly available and have been evaluated by the U.S. EPA FIFRA Scientific Advisory Panel (SAP; http://www.epa.gov/scipoly/sap/index.htm), it is important to acknowledge the significant uncertainties

associated with population-based, stochastic models and associated exposure and risk analyses. Aggregate and cumulative assessments endeavor to address demographic, geographic, and temporal specificity associated with longitudinal patterns of exposures to chemicals such as pesticides. These assessments are intended to result in an integrated, multisource (dietary, drinking water, and residential), and multiroute (direct and incidental ingestion/oral, dermal, and inhalation) exposure analyses and associated total absorbed dose distributions. Exemplary analyses have been presented by the U.S. EPA Office of Pesticide Programs (e.g., http://www.epa.gov/oppsrrd1/cumulative/carbamate_cumulative_factsheet.htm).

Objectives of ADME Studies to Assist in Risk Assessment

All of the ADME studies described thus far have potential application to risk assessments for improved cross-species, high-to-low dose, and other extrapolations. Additional risk assessment applications are described here, as well as the design of studies for several of these applications. Route-to-route extrapolation is one of the major areas addressed because, in general, toxicity studies have been conducted via a single route. For many pesticides, extrapolation of oral toxicity studies to assess risks from dermal exposure is particularly important. Similarly, pharmacokinetically-based approaches could be used to extrapolate for inhalation exposures, but this is not further described here. Human biomonitoring and human clinical pharmacokinetic studies can provide valuable information for risk assessment by bounding human exposure estimates and verifying how pharmacokinetic predictions from animals apply to humans.

Dermal Pharmacokinetics and Route Extrapolation

With the exception of highly volatile chemicals such as fumigants, the dermal route is the primary route of exposure to most pesticides for professional and consumer "operators" (mixers, loaders, and applicators) (Wolfe, 1976) and in reentry situations for both workers (Fenske et al., 1989) and residents (Zartarian and Leckie, 1998; Zartarian et al., 1998, 2000). Because the majority of toxicity studies for these chemicals use oral exposures, it is necessary to relate the bioavailability of an oral dose to these human dermal exposures or carry out dermal toxicity studies. In addition to extrapolation across routes, there is the need to extrapolate across species and from experimental exposure regimens to human exposure conditions.

Route extrapolation is based on the premise that equivalent target tissue dose would produce the same toxicity outcome regardless of the exposure route. Generally, this applies to systemic effects. Contact site effects (e.g., dermal sensitization or irritation) require studies by the relevant exposure route. The target tissue dose metric used for route extrapolation should be closely related to a key event in the mode of action resulting in the toxicity. Often, relatively simple measures of dose are used, such as the AUC in blood, the peak concentration, or the average daily

absorbed dose, because a detailed mode of action is not known; however, in cases for which the mode of action is known, the appropriate metric should be selected. Typically, dermal absorption is a relatively slow and inefficient process, resulting in lower doses with a longer time to maximum concentration compared to generally well-absorbed oral bolus dosing. Dietary exposures also occur more slowly over a period of hours as animals eat and thus are somewhat more similar to dermal exposures than are bolus doses. Compounds with very short half-lives (e.g., carbamates) require careful consideration because acute effects can be associated with peak concentrations; therefore, divided doses spaced at sufficiently long intervals would not produce the toxicity occurring with a single large dose (Vandekar et al., 1971). Approaches to estimating these dose metrics range from relatively simple adjustments for absorbed dose (Example T) to use of PBPK models as discussed earlier.

Use of laboratory animals, particularly rats, for either dermal penetration studies or dermal toxicity studies has the significant limitation that rat skin appears to be more permeable than human skin (Ross et al., 2001). Use of this model may, in general, be health protective (i.e., human exposures would generally be less than predicted from rats). However, better risk estimates would be obtained by incorporating improved science that quantifies human dermal absorption. The extent of this overestimate for a number of pesticides ranges from 1.5- to 14-fold, with a median of 4-fold excess absorption in rats compared to humans (Table 11; Ross et al., 2001).

Two major approaches exist for obtaining estimates of human dermal absorption: an in vivo/in vitro rat/human parallelogram approach, and controlled dermal absorption studies in humans. The parallelogram approach, which has been applied in some regulatory settings including in Europe, is described here. Human dermal absorption studies are discussed later, as well as other human clinical pharmacokinetic studies.

The ratio of human to rat in vitro dermal absorption can be used as a means to estimate in vivo human dermal absorption from in vivo rat dermal absorption data, that is,

$$\text{In vivo}_{\text{human}} = (\text{in vitro}_{\text{human}}/\text{in vitro}_{\text{rat}})(\text{in vivo}_{\text{rat}})$$

While this approach was evaluated by an expert OECD panel which determined that there was currently insufficient published data to validate it, compilation of unpublished in vitro and in vivo data for a variety of chemicals obtained from rats and humans under comparable experimental conditions (Ross et al., 2001) could serve this purpose.

Other outstanding issues that can alter estimates for dermal absorption include the impact of dose density (Wester and Maibach, 1976), nonuniform distribution on the body (Krieger, 1995), consideration of systemic bioavailability (i.e., absorption into the body) versus presence in skin, and the comparability of in vivo or in vitro rodent exposures to the exposure kinetics of humans (e.g., workers are exposed throughout the course of a day while experimental studies place the entire dose on the skin at the start of the study). Further discussion of these

TABLE 11
In vivo dermal absorption of pesticides in rats and humans[a]

Pesticide	Human absorption (percent)	Rat absorption (percent)	Ratio: rat/human
Atrazine	3	8 [b]	2.7
Azinphosmethyl	16	44[c]	2.8
Chlorpyrifos	10[d]	66[b]	6.6
DEET	7[e]	32[f]	4.6
Diquat	1[g]	3[h]	3.0
Isofenphos	4[i]	16[j]	4.0
KBR 3023	4[k]	23	5.8
Lindane	9	31	3.4
Malathion	6	28[l]	4.7
Orthophenyl phenol	43[m]	65[n]	1.5
Parathion	10	95	9.5
Permethrin	2[o]	28[p]	14.0
Propoxur	20[h]	50[q]	2.3
Grand mean ± SD	10 ± 11	38 ± 26	5.0 ± 3.4

[a] Adapted from data compiled by Wester and Maibach (1993), except as otherwise noted.
[b] Shah and Guthrie (1983).
[c] Thongsinthusak et al. (1999).
[d] Thongsinthusak and Krieger (1991).
[e] Selim et al. (1995).
[f] Snodgrass et al. (1982).
[g] Formoli. (1995).
[h] Feldmann and Maibach (1974).
[i] Wester et al. (1992).
[j] Brodberg (1990).
[k] Sangha et al. (1998).
[l] Dary et al. (1994).
[m] Timchalk et al. (1998).
[n] Hughes and Hall, (1995; phenol compared to orthophenyl phenol).
[o] Formoli (1991).
[p] Thongsinthusak and Ross (1999).
[q] Sanborn (1994).

topics is found in published literature (e.g., Ross et al., 2001, and references therein).

Example T. Dermal absorption This example illustrates the use of the in vitro rat to human dermal absorption comparison to back-correct the values obtained from the in vivo rat study (Table 12). The dermal absorption value obtained from this exercise is subsequently used in an operator and bystander risk assessment to predict systemic dose from potential dermal dose.

The total amount of applied radioactivity absorbed in the in vitro studies by 24 hours was three times greater for rat skin than human skin following application of the high-dose level formulation (600 g/L), and six times greater for rat skin than human skin following the low-dose application (1.5 g/L). For risk assessment, adjusting the rat in vivo dermal absorption (3% and 35%) by these factors would estimate human dermal absorption values of 1% and 6% for mixer/loaders and applicators, respectively (Bayer, unpublished data).

TABLE 12
Comparison of percent dermal absorption in rat and human test systems (Bayer, unpublished data)

Compound F	Percent absorption concentrate formulation (mixing and loading)	Percent absorption diluted (application)
In vivo rat	3	35
In vitro rat	0.52	39.0
In vitro human	0.17	6.5

Human Biological Monitoring Studies

Human biological monitoring and biological marker measurement studies provide an important and credible means for establishing aggregate and/or cumulative absorbed doses to chemicals such as pesticides following specific situations or exposure scenarios (e.g., lawn care product application at a residence) or for establishing baseline, population-based background levels (Woollen, 1993). The results from these studies, such as temporal situational biological monitoring, provide a realistic description of human dose levels. They provide a basis for evaluating predictive toxicokinetic models or the use of specific toxicokinetic data for purposes of "higher tier" or refined exposure/dose assessments using methods recommended by regulatory agencies (U.S. EPA, 1997).

Monitoring has several definitions, depending on the nature of the sampling. Environmental or ambient monitoring is the measurement of pesticides on surfaces, in the air, or coupled to other media. Biological monitoring is the routine analysis of human tissues, expired air, or excreta for direct or indirect evidence of human exposures. Surveillance strategies may include field studies under prescribed workplace conditions, situational (opportunistic) studies of human exposures under normal conditions of use (Krieger et al., 2001), or part of regularly scheduled or medical exams. These data can provide valuable, though limited, information concerning human ADME, particularly if both ambient and biological monitoring data are available.

Biomonitoring, the routine analysis of human tissues or excreta for direct or indirect evidence of human exposures to pesticides, can provide unique insights into the relationship between dose and putative toxicity thresholds established in experimental animals, usually rats. Pioneering research by Elkins (1954) on the relationship between concentrations of chemicals in the workplace and their concentrations in body fluids helped to establish the Biological Exposure Index (ACGIH, 2002). Urine is the most frequently used biological specimen, due to its noninvasive nature and ease of collection and its importance as a route of excretion for most analytes. Although introduction of parathion and other organophosphate insecticides prompted analysis of *p*-nitrophenol and cholinesterase activity as a means to manage uncertain worker exposure, pesticide biomonitoring has been underutilized.

Example U. Human biomonitoring (carbaryl) Exposure to carbaryl has been monitored using urinary 1-naphthol and plasma pseudocholinesterase activity as biomarkers (Comer et al., 1975). A relationship between 1-naphthol and pseudocholinesterase was not established. The rapid reactivation of cholinesterase inhibited by carbamates requires prompt sampling and analysis following exposure (<4 hours) (Lauwerys and Hoet, 1993). This illustrates the importance of understanding the dose-response time domain and associated kinetics for a given chemical of interest and the relevance to biomarker measurement programs. This example suggests that urinary biological monitoring for carbaryl (1-napthol urinary biomarker) requires collection of daily (24 hour) urinary samples before and immediately after (for multiple, sequential days) a well-characterized exposure event (Shealy et al., 1997).

Controlled Human Exposure Studies

Pharmacokinetic or biomonitoring studies of controlled human exposures can play a role by improving interspecies extrapolations when carried out under appropriate scientific and ethical standards (NRC, 2004). Controlled biomonitoring studies may include standardized activities in a treated environment to better evaluate the relationship between external exposure and biomarkers, but are otherwise similar to biomonitoring studies described in the previous section (Krieger et al., 2001).

Controlled human exposure studies are frequently clinical pharmacokinetic studies in which chemical concentrations in blood, urine, exhaled breath, or other biological media are measured following dermal, oral, or inhalation exposures. These studies are similar in nature to those carried out by the pharmaceutical industry in healthy humans to determine human pharmacokinetics. Such studies are valuable for improved interspecies extrapolations of pharmacokinetics and improved route extrapolation, particularly for dermal exposures. As mentioned previously, additional work may be necessary to validate the parallelogram or ratio approach for in vivo/in vitro rat/human dermal extrapolation, which would necessarily require controlled human dermal exposures. The scientific and ethical justifications for such an effort that would validate in vitro methods for future applications are demonstrable. Improved analytical chemistry methods, such as those capable of detecting low levels of compounds in biomonitoring studies of the general population, make it feasible to carry out controlled human pharmacokinetic studies with very low doses, far below levels that might produce effects.

Clinical pharmacokinetic studies are more strongly justified and planned when coupled with prior in vitro human metabolism studies (assuming metabolism is a major clearance process) and predictive pharmacokinetic modeling. These studies provide critical information for selecting parameters for human PBPK models, which can then be validated from the in vivo data. This will enable the further application of PBPK modeling for improving risk assessments of a wide range of chemicals.

CONCLUSIONS AND TIERED APPROACH

A wide range of ADME studies has been described that can improve the safety assessment of agricultural chemicals and
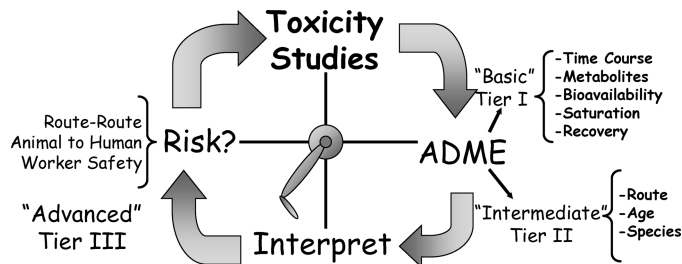

FIG. 6. The iterative process for collecting ADME and toxicity data for use in risk assessment, illustrating the three tiers for ADME data collection. Collecting basic toxicity and ADME data is followed by initial efforts to interpret the results and their implications for human risk assessment. These initial results are used to plan additional toxicity and pharmacokinetic studies and proceed through the cycle as needed to obtain a substantially complete perspective.

other pesticides. The focus has been on the uses of the information, rather than on prescribed studies. Design of appropriate ADME studies must reflect the physical/chemical properties of the compound under study, the toxicity endpoints of concern, and the anticipated human exposures for which risks need to be evaluated. Thus, these studies involve an iterative process as additional information becomes available; no single set of studies can provide the necessary and correct information for every compound (Figure 6). The progression of pharmacokinetic studies generally begins with studies using exposures that are relatively artificial (e.g., single intravenous or gavage dose) that simplify the interpretation of the data, and move on to studies that increasingly mimic the repeated, dietary studies that are used in toxicity studies. The latter conditions are clearly the most important—hence, the recommendation for measurements of chemicals in the blood of animals (or satellite groups) in the toxicity study. But the toxicity studies involve exposures that are less easily characterized in terms of amounts and timing of exposure and pharmacokinetics that may have changed with exposure dose, duration, or age of the animal. These variables further complicate the interpretation of the toxicity data. Thus, there is the need for the organized progression of data collection.

The tiered approach to ADME data development is based on the acquisition of data in three general categories:

- Basic: Data that can be useful for dose selection and toxicity study design, such as half-life determination. For a new chemical, these studies would provide basic data on which to build further studies.
- Intermediate: Data needed for study interpretation, absorbed dose estimates, and duration extrapolations.
- Advanced: Data to support better understanding of a compound's mode of action, allow the evaluation of pharmacodynamic concordance, and develop other risk assessment applications, particularly route and interspecies extrapolations.

A goal of the HESI ACSA Technical Committee is to recommend tiered studies that will provide a useful, but flexible, ordering of data collection. This paper describes a range of studies that would be useful for improving toxicity study design, study interpretation, and risk assessment. It does not specify final data requirements. The logic of when and how to collect the data will vary for each compound under consideration, but is generally expected to follow the tiers described in this paper.

ABOUT HESI

The ILSI Health and Environmental Sciences Institute (HESI) is a global branch of the International Life Sciences Institute, a public, nonprofit scientific foundation with branches throughout the world. HESI provides an international forum to advance the understanding and application of scientific issues related to human health, toxicology, risk assessment and the environment. HESI is widely recognized among scientists from government, industry, and academia as an objective, science-based organization within which important issues of mutual concern can be discussed and resolved in the interest of improving public health. As part of its public benefit mandate, HESI's activities are carried out in the public domain, generating data and other information for broad scientific use and application. Further information about HESI can be found at http://www.hesiglobal.org.

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