V B. Metabolism and Pharmacokinetic Studies

Results from animal toxicity studies are used by FDA to determine dose-response characteristics for any effects observed in the evaluation of the safety of food and color additives. Since the delivered dose of a substance to any affected tissue or organ is determined by the pharmacokinetics and metabolism of the substance in the test animal, toxicity studies are more easily interpreted, likely to achieve target doses, and avoid excessive toxicity if data from metabolic and pharmacokinetic studies are available during the planning of short-term, subchronic and/or chronic toxicity studies. Early determination of metabolic pathways and the rates of metabolism in different test species may provide explanations for species differences in any effects which are observed, and suggest biochemical or pharmacologic experiments which might be used to test explanations of such phenomena.

The Agency recommends that petitioners submit data that will enable our scientists to evaluate: 1) the extent of absorption, 2) tissue distribution, 3) pathways and rates of metabolism, and 4) rate(s) of elimination of the parent substance and any metabolites formed for all Concern Level II and III substances (see **Figure 3**). The Agency may recommend submission of additional metabolic and pharmacokinetic data based on the extent to which a chemical is metabolized, the potential toxicity of the metabolites, and the extent to which observed toxic effects seem to correspond to the presence of the parent substance or its metabolites.

1. Considerations in the Design of, Analysis of, and Use of Data from Metabolic and Pharmacokinetic Studies

Pharmacokinetic data can be used to predict plasma concentrations, target tissue doses, and the fate of the administered dose. This information can then help the petitioner and/or the Agency: 1) decide which toxicity studies should be conducted, 2) select doses for chronic toxicity and carcinogenicity studies, 3) determine the mechanism of toxicity and assist in the interpretation of toxicity data, and 4) improve the risk assessment process.

a. Design and Analysis of Metabolic and Pharmacokinetic Studies

Pharmacokinetic studies are most useful when they are performed early in the process of evaluating the toxicity of a chemical. However, additional metabolism and pharmacokinetic studies may be recommended after target organs have been identified in toxicity studies.

Whole animal (oral dosing) studies should be performed to determine gastrointestinal absorption and overall elimination rates for a compound. However, it is often most efficient to perform *in vitro* studies of metabolism before whole animal (oral dosing) studies to determine whether enzyme kinetics may explain known dose response curves or predict non-linear dose response curves. The results of early *in vitro* studies also can be used to optimize the choice of doses in whole animal pharmacokinetic studies.

Additional recommendations concerning the design and analysis of metabolism and pharmacokinetic studies are described below.

i. Test Compound

In selecting the dosage form of a test compound to be administered in metabolic and pharmacokinetic studies, the chemical characteristics of the compound and its route of administration should be considered. The formulation of the test substance used for metabolic and pharmacokinetic studies should exhibit similar patterns of disintegration and/or dissolution as formulations used for toxicity studies. Chemical purity of the test compound should be established; impurities that may affect absorption, distribution, metabolism and excretion of the test compound should be identified. Stability of the compound in its carrier (*i.e.*, food, water, or solvent) also should be determined. Chemical characteristics of the compound (*i.e.*, low solubility, volatility) may make certain routes of administration impossible. It is critical that the dose absorbed into tissues be determined especially in studies where the test substance is added to the feed or water and is ingested *ad libitum*.

Use of radioactive substances facilitates mass balance determinations because radio-labels are relatively easy to detect in samples of tissues and body fluids. Determining the disposition pattern of the radio-label may be adequate for predicting doses that should be used in toxicity studies where the results of a test animal's overall exposure to the substance (parent compound and metabolites) is of concern. The radio-label should not be biologically labile; when a radioactive element is present at more than one position of the test compound, the radio-label should be uniformly distributed in the molecule.

The radiochemical purity of the test substance (radioactivity actually associated with the compound being tested) is another important consideration. If the test compound is not radiochemically pure and radio-labeled impurities are not identified, and if only the distribution of the radio-label in tissues and body fluids is determined, interpretation of the results may be difficult. For example, for a compound that is 95-96% radioactively pure and minimally absorbed (*i.e.*, approximately 2% absorbed), it is impossible to unequivocally differentiate between 2% absorption of the test compound and 100% absorption of a radioactive impurity present at 2%.

ii. Animals

Metabolic and pharmacokinetic data from two rodent species (usually the rat and mouse) and a non-rodent species (usually the dog) are recommended. If a dose dependency is observed in metabolic and pharmacokinetic or toxicity studies with one species, the same range of doses should be used in metabolic and pharmacokinetic studies with other species. If human metabolism and pharmacokinetic data also are available, this information should be used to help select test species for the full range of toxicity tests, and may help to justify using data from a particular species as a human surrogate in safety assessment and risk assessment. (Human metabolism studies should be conducted according to the guidelines in **Chapter VI B**.)

Metabolism and pharmacokinetic studies have greater relevance when conducted in both sexes of young adult animals of the same species and strain used for other toxicity tests with the test substance. The number of animals used in metabolism and pharmacokinetic studies should be sufficient to reliably estimate population variability (see **Chapter V B 1 e**). A single set of intravenous and oral dosing results from adult animals, when combined with some *in vitro* kinetic results, may provide an adequate data set for the design and interpretation of short-term, subchronic and chronic toxicity studies.

Studies in multiple species may clarify what appear to be contradictory findings in toxicity studies (*i.e.*, equal mg/kg bw doses having less effect in one species than in another). If disposition and metabolite profiles are found to be similar, then differences in responses among species could more reliably be attributed to factors other than differences in metabolism. Studies of the pharmacokinetics and metabolism of a substance in neonatal and adolescent animals provide information about any changes in metabolism associated with tissue differentiation and development. Animals with fetuses of known gestational age should be used for determining the disposition of the test substance in the fetus.

iii. Route of Administration

The most critical parameters required in assessing human exposure and target tissue dose are the gastrointestinal absorption rate and internal elimination rates (renal and hepatic) for the test compound. Without an intravenous (IV) dosing study, it is very difficult to determine what percentage of a chemical is absorbed, because the material excreted in the feces is composed of unabsorbed dose plus biliary and non-biliary (mucosal) elimination.

An intravenous study can provide accurate rates of metabolism-- without interference from intestinal flora--plus rates of renal and biliary elimination, if urine and bile are collected. This route also avoids the variability in delivered dose associated with oral absorption and ensures that the maximum amount of radiolabel is excreted in the urine or bile for purposes of detection. Once IV data and parameters are available, they can be used with plasma concentrations from limited oral studies to compute intestinal absorption via the ratio of Areas Under the (plasma and or urine) Curves or via simulations of absorption with gastrointestinal absorption models.

In single-dose pharmacokinetic studies of oral absorption, the primary concerns are with the extent of absorption and peak plasma or target tissue concentrations of the test substance. If the test vehicle affects gastric emptying, it may be necessary to use both fasted and non-fasted animals for pharmacokinetic studies.

iv. Dosage Regimen

Selection of the dosing regimen for metabolism and pharmacokinetic studies depends on the type of information that is needed. Metabolic and pharmacokinetic parameters are usually determined following a single administration of the test compound. Comparing parameters obtained from studies in which a range of single doses have been administered can be used to determine the doses at which saturation of absorption, distribution, metabolism or excretion occurs. Multiple dosing studies can be used to determine the potential of a compound to induce or inhibit its absorption, distribution, metabolism or excretion. Identification and quantification of the major metabolites following administration of single and multiple doses may indicate whether saturation or induction of a particular biotransformation pathway can occur.

In vitro experiments may be useful in screening for dose dependencies, and provide more accurate descriptions of the enzyme kinetics or other processes underlying dose dependencies observed in the whole animal. *In vitro* studies usually indicate identical metabolic pathways and metabolism rates comparable to those obtained from whole animal studies but require fewer animals to perform and can be completed in less time with fewer resources. [1-3]

v. Sampling

Blood (RBCs, plasma, and serum), urine, and feces are the most commonly collected samples. In addition, a few representative organ and tissue samples should be taken, such as liver, kidney, fat, and suspected target organs. Sampling times should depend on the substance being tested and the route of administration. In general, an equal number of blood samples should be taken in each phase of the concentration-versus-time curve. Intravenous (IV) studies usually require much shorter, and more frequent, sampling than is required for oral dosing. Time spacing of samples will depend on the rates of uptake and elimination. In a typical IV study, blood and tissue samples are taken in a "powers of 2" series, *i.e.* samples at 2, 4, 8, 16, and 30 (32) minutes, 1, 2, 4, 8, and 16 hours. Similar coverage could be obtained with only 7 time points by using a "powers of 3" series: 3, 9, and 30 (27) minutes, 1, 3, 9, and 24 (27) hours. Oral dosing studies usually extend to at least 72 hours, or 5 plasma half-lives, ensuring the excretion of 95% of the absorbed dose. The sampling schedule for an oral dosing experiment might be: 15 minutes, 30 minutes, 1, 2, 4, 8, 24, 48, and 72 hours. Such a sampling scheme would provide data coverage for evaluation of absorption, elimination, enterohepatic recirculation and excretion processes.

Whole Body Autoradiography (WBA) has been used with increasing frequency as a means of identifying tissues which concentrate test substances. This technique allows a small number of animals (5 - 10) to be used for screening purposes with a minimal investment in manual labor. FDA encourages the use of WBA with IV dosing,

as a means of screening and selecting tissues of greatest relevance for later oral dosing studies. Animals used for WBA should be sacrificed during the elimination phase, between 1 and 5 plasma half-lives, since bioaccumulation at steady-state is the primary consideration in selecting specific tissues.

The number of animals used in metabolism and pharmacokinetic studies should be large enough to reliably estimate population variability. In the case of rats and mice, tissue and/or blood sample size is usually the limiting factor: analysis of the substance may require 1 ml or more blood, but it is difficult to obtain multiple blood samples of this size from one animal. As a consequence, a larger number of animals is required (3 - 4 per time point, 7 - 9 time points) when small rodents are used. Such an approach has the advantage of allowing limited sampling of critical tissues (*e.g.* liver, fat) at each time point, an option which is usually unavailable with large animals. The use of humans and large animals generally permits collection of multiple (serial) blood samples. For outcrossing populations like humans and large animals, individual differences in the rates of biotransformation are likely to be greater than those of inbred rodent populations; under these circumstances, more samples/sex/group may be needed to reliably estimate variability.

Individual metabolism cages are recommended for collecting urine and feces in oral dosing studies. Excreta should be collected for at least 5 elimination half-lives of the test substance. When urine concentrations will be used to determine elimination rates, sampling times should be less than one elimination half-life (taken directly from the bladder in IV studies); otherwise, samples should be taken at equal time intervals.

vi. *In Vitro* studies

In Vitro measurements employing enzymes, subcellular organelles, isolated cells and perfused organs may be used to augment the dose response information available from less extensive metabolic and pharmacokinetic studies. Because *in vitro* systems generally are less complex than whole animals, elucidation of a test compound's metabolic pathways and the pathways' kinetic characteristics may be facilitated. Such systems can be used to measure binding, adduct and conjugate formation, transport across cell membranes, enzyme activity, enzyme substrate specificity, and other singular objectives. Biochemical measurements that can be made using *in vitro* systems include: Intrinsic clearances of enzymes in an organ or tissue, kinetic constants for an enzyme, binding constants, and the affinity of the test compound and its metabolites for the target macromolecules. The activity of a hepatic drug-metabolizing enzyme *in vivo* may be approximated by kinetic constants that are calculated from *in vitro* studies; when a first-order approximation is used, the ratio of V_{max} to K_m is equal to the intrinsic clearance of the drug. [4,5] *In vitro* measurements made using readily accessible tissues and body fluids from animals and man may also be useful in elucidating mechanisms of toxicity.

vii. Analysis of Data

Data from all metabolism and pharmacokinetic studies should be analyzed with the same pharmacokinetic model and results should be expressed in the same units. Concentration units are acceptable if the organ or sample size is reported, but percent of dose/organ is usually a more meaningful unit. In general, all samples should be analyzed for metabolites that cumulatively represent more than 1% of the dose.

A variety of rate constants and other parameters can be obtained from IV and oral dosing data sets, provided that good coverage of the distribution, elimination, and absorption (oral dose) phases is available. Typical parameters calculated to characterize the disposition of a test substance are: half-lives of elimination and absorption; area under the concentration-versus-time curve (AUC) for blood; total body, renal and metabolic clearances (Cl); volume of distribution (V_d); bioavailability (F); and mean residence and absorption times (MAT, MRT). Some of these parameters, such as half-lives and elimination rates, are easily computed from one another; the half-life is more easily visualized than the rate constant. [6,7]

Computation of oral absorption (k_a) and elimination (E) rates is often complicated by the "flip-flop" of the absorption and elimination phases when they differ by less than a factor of 3. [8] Because of these analysis problems, computation of absorption and elimination rates should not be attempted on the basis of oral dosing

results alone.

Blood-tissue uptake rates (k_{j1}) can often be approximated from data at early ($t < 10$ minutes) time points in IV studies, provided that the blood has been washed from the organ (*e.g.* liver) or the contribution from blood to the tissue residue is subtracted (fat). High accuracy is not usually required since these parameters can be optimized to fit the data when they are used in more complex models. Tissue-blood recycling rates (k_{1j}) and residence times can be computed from partition coefficients if estimates of uptake rates are available.

Tissue/blood partition coefficients (R_{j1}) should be determined when steady-state has been achieved. Estimates based on samples obtained during the elimination phase following a single dose of the test substance may lead to underestimates of this ratio in both eliminating and non-eliminating tissues unless its half-life is very long. Correction of these values for elimination has been described by several authors. [9,10]

It may be important to determine the degree of plasma protein and red blood cell binding of the test substance; calculation of blood clearance rates using plasma or serum concentrations of the substance that have not been adjusted for the degree of binding may under- or over-estimate the true rate of clearance of the test substance from the blood. This is usually done through experiments *in vitro*.

Two classical methods used in the analysis of pharmacokinetic data are the fitting of sums of exponential functions (2- and 3-compartment mammillary models) to plasma and/or tissue data, and less frequently, the fitting of arbitrary polynomial functions to the data (non-compartmental analysis).[8,11,12]

Non-compartmental analysis is limited in that it is not descriptive or predictive; concentrations must be interpolated from data. The appeal of non-compartmental analysis is that the shape of the blood concentration-versus-time curve is not assumed to be represented by an exponential function and, therefore, estimates of metabolic and pharmacokinetic parameters are not biased by this assumption. In order to minimize errors in parameter estimates that are introduced by interpolation, a large number of data points that adequately define the concentration-versus-time curve are needed.

Analysis of data using simple mammillary, compartmental models allows the estimation of all of the basic parameters mentioned above, if data for individual tissues are analyzed with 1 or 2 compartment models, and combined with results from 2 - 3 compartment analyses of blood data. "Curve Stripping" analysis can be applied to such simple models through the use of common spreadsheet programs (*i.e.* LOTUS 1-2-3), as long as a linear regression function is provided in the program. Optimization of the coefficients and exponents estimated may require the use of more sophisticated software: a number of scientific data analysis packages such as RS/1 and SigmaPlot have the necessary capabilities. Specialized programs such as NONLIN [13], CONSAM,[14] or SIMUSOLV [15] will be needed when more complex models must be analyzed. Coefficients and exponents from mammillary models can be used to calculate other parameters; however, they should not be taken too literally, since mammillary models assume that all inputs are to a central pool (blood), which communicates without limitation into other compartments.[16,17] This approach does not include details such as blood flow limitations, anatomical volumes or other physiological limits in the animal.

Physiologically based pharmacokinetic models (PB-PK) were developed to overcome the limitations of simple mammillary models. Physiologically based models describe the disposition of test substances via compartmental models which incorporate anatomical, biochemical and physiological features of specific tissues in the whole animal. The types of information added include organ-specific blood flows, volumes, growth models and metabolism rates. Metabolic parameters often are obtained from *in vitro* studies (*i.e.*, enzyme reaction rates in cultured hepatocytes, plasma protein binding, *etc.*), while other parameters are becoming available as standard parameters in the literature. Parameters from mammillary models can be used to compute the value of parameters used in physiological pharmacokinetic models, using tissue-specific blood flows, anatomical volumes, and other information (literature values). Estimation of parameters for a simple mammillary model is often the first data reduction step in creating a physiological model.[5,18]

Because PB-PK models are based on physiological and anatomical measurements and all mammals are inherently similar, they provide a rational basis for relating data obtained from animals to humans. Estimates of

predicted disposition patterns for test substances in humans may be obtained by adjusting biochemical parameters in models validated for animals; adjustments are based on experimental results of animal and human *in vitro* tests and by substituting appropriate human tissue sizes and blood flows. Development of these models requires special software capable of simultaneously solving multiple (often very complex) differential equations, some of which were mentioned above. Several detailed descriptions of data analysis have been reported.[7,19]

b. Use of Data from Metabolism and Pharmacokinetic Studies

Information from metabolism and pharmacokinetic studies can be used in the design and analysis of data from other toxicity studies. Some examples are described below.

Design of Toxicity Studies: The concentration-versus-time curve, peak, and steady-state concentrations of the test substance in blood or plasma provide information on the distribution and persistence of the substance in the animal which may suggest essential elements in the design of toxicity studies. For example, when metabolic and pharmacokinetic studies indicate that the test compound accumulates in the bone marrow, long-term toxicity tests should include evaluation of the test compound's effect on hematopoietic function and morphology. If a test compound is found to accumulate in milk, an investigator should plan to perform reproductive toxicity studies with *in utero* exposure and a nursing phase (cross-fostering study; see **Chapter IV C 8**). In addition, information from metabolic and pharmacokinetic studies can be used to predict the amount of test compound that enters biological compartments (tissues, organs, *etc.*) that may not suffer a toxic insult but may serve as depots for indirect or secondary exposure.

Setting Dose Levels: There is considerable debate about the use of metabolic and pharmacokinetic data in setting doses to be used in toxicity studies, particularly chronic toxicity and carcinogenicity studies. Current NTP policy for selecting the highest dose in carcinogenicity bioassays is described in **Chapter IV C 6 b**. In 1984, the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation also recommended that pharmacokinetic data be considered along with subchronic toxicity data in setting all dose levels except the maximum-tolerated dose (MTD) in the carcinogenesis bioassay of chemicals.[20] FDA agrees with these statements and recommends that pharmacokinetic data be used in conjunction with the results of short-term and subchronic toxicity studies to set appropriate dose levels for chronic toxicity, reproduction and teratology studies, and for setting dose levels below the MTD (highest dose) in carcinogenicity studies.

Determining Mechanisms of Toxicity: Information from metabolic and pharmacokinetic studies can be used to supplement conventional toxicology data in elucidating mechanisms of toxicity. Metabolites identified by a pharmacokinetic study can suggest mechanisms underlying a toxic response. Biologically reactive intermediates are often implicated in a toxic response; however, such metabolites are usually short-lived, reacting in the vicinity of their formation. The presence of potentially reactive intermediates can be deduced indirectly by measuring the formation of characteristic macromolecular (DNA, RNA, protein) adducts and metabolic conjugates. Measurement of metabolic conjugate vs adduct formation and the affinity of a compound and/or its metabolites for the target molecule may help identify mechanisms of toxicity and effective routes of detoxification.

Information from *in vitro* test systems concerning the formation of critically reactive metabolites may be used to establish the relationship between the formation of the reactive metabolite *in vivo* and duration of exposure to the test compound. This relationship is important in circumstances where critically reactive metabolites are only formed when the capacities of normal metabolic and other defensive or adaptive mechanisms are exceeded. Determining the concentrations of the test substance at which saturation of binding occurs may indicate at what concentration a compound is likely to deplete detoxifying conjugation pools and become available to react with target macromolecules.

Improving the Risk Assessment Process: Information from metabolic and pharmacokinetic studies increasingly is being incorporated into risk assessments. Conventional risk assessments typically involve

linear extrapolation of external dose and an inter-species scale factor based on body weight or body surface area. Risks calculated by this approach may be under- or over-estimated. Many of the biological processes involved in the absorption, distribution, metabolism and excretion of a compound are dose dependent and, therefore, the toxicity observed may not be a simple function of administered dose. Development of appropriate pharmacokinetic models may enhance our ability to use metabolic and pharmacokinetic information in risk assessment.

2. Recommended Metabolism and Pharmacokinetic Studies

FDA believes that data from studies on the absorption, distribution, metabolism, and excretion of a chemical can provide insight into mechanisms of toxicity of chemicals and are essential in the design and evaluation of results from other toxicity studies. FDA believes that a set of basic pharmacokinetic and metabolism studies should be performed for all Concern Level II and III substances, but that additional studies may be recommended for a particular additive. Recommended studies should be performed with two rodent species (usually the rat and mouse) and one non-rodent species (usually the dog). In general, what constitutes an appropriate set of metabolism and pharmacokinetic studies will depend on the anticipated degree and type of toxic response to a test compound and by the estimated magnitude of human exposure to the compound. The recommended set of basic studies are:

- Intravenous studies using a tracer level dose should be conducted in adult male and female animals of species in which toxicity studies have already been conducted or in which chronic toxicity studies are contemplated. Blood, liver, and fat samples should be taken at all time points. The size and timing of urine and bile samples will depend on the dose of tracer and rate of excretion by each of these routes. Samples taken over periods of 30 min to 2 hours, at 2 or 3 time points, should be sufficient for computation of the cumulative excretion by these routes. Plasma, urine and bile should be analyzed for metabolites of the test substance that cumulatively represent more than 1% of the dose. Estimates of uptake and elimination rates should be made for each tissue sampled, using 2-compartment models.

- Studies of the rate of metabolism (of the parent compound) as a function of dose (or concentration) should be conducted *in vivo* or *in vitro*, guided by results of metabolite analyses from the intravenous studies and available toxicology information. Hepatocytes or perfused livers will normally be used for such studies, but an examination of the distribution of metabolites between the plasma, bile and urine after IV dosing may indicate that the kidney is important in the metabolism of some chemicals. Enzyme kinetic parameters resulting from *in vitro* studies may be scaled up to whole organ rates and used to predict rates of metabolism in the whole animal as a function of dose.

- Oral dosing studies should be conducted in *ad libitum* fed animals, to determine the rate and cumulative absorption of the substance. Dosage and sampling times should be selected on the basis of results from toxicity tests, metabolic dose response data (**ii**, above), and elimination rates determined from IV dosing studies. Bioaccumulative tissues should be sampled in addition to blood, urine and feces. A tissue that does not accumulate the substance should also be included for reference purposes. Whole Body Autoradiographic studies are recommended as a method for identifying bioaccumulative tissues prior to the initiation of oral dosing studies.

3. Additional Studies

Studies of enzyme induction and potential pharmacological adaptation should be conducted whenever chronic studies are recommended. The resulting information can be incorporated into multiple or continuous dosing models to simulate the plasma and tissue levels of test substance expected for a variety of doses in chronic studies being planned.

In cases where reproductive studies are recommended, pharmacokinetic experiments evaluating the

distribution of the substance in the fetus, mother's milk, and neonates should be performed as an aid in selecting doses and designing reproductive toxicity studies. If the metabolic potential of the fetal and/or neonatal liver can be assessed in a preliminary *in vitro* study, this step is highly recommended.

Assuming that IV and oral dosing studies have already been completed for both male and female adult animals prior to the reproductive pharmacokinetic studies, sampling can be more limited, ie. excretion studies combined with limited sampling of maternal blood, fetuses, milk, and neonatal tissues may be sufficient for characterization of the metabolic and pharmacokinetic processes of interest in pregnancy.

Depending on the types of toxic effects observed and the importance of understanding the mechanisms of these effects to the safety assessment of a direct food or color additive used in food, additional biochemical or *in vitro* experiments may be submitted by the petitioner in support of any mechanism proposed. Such studies should be substance-specific, and should be based on consultation with CFSAN, as appropriate.

Chapter V B: Metabolism and Pharmacokinetic Studies

1. Bäärnhielm, C., Dahlbäck, H. and Skånberg, I. (1986) *In vivo* pharmacokinetics of felopidine predicted from *in vitro* studies in rat, dog, and man. *Acta Pharmacol.Toxicol. 59:113-122.*

2. Green, C.E., LeValley, S.E. and Tyson, C.A. (1986) Comparison of amphetamine metabolism using isolated hepatocytes from five species including human. *J.Pharmacol.Exp.Ther. 237(3):931-936.*

3. Lin, J.H., Sugiyama, Y., Awazu, S. and Hanano, M. (1982) *In vitro* and *in vivo* evaluation of the tissue-to-blood partition coefficient for physiological pharmacokinetic models. *J.Pharmacokin.Biopharm. 10:637-647.*

4. Rane, A., Wilkinson, G.R. and Shand, D.G. (1977) Prediction of hepatic extraction ratio from *in vitro* measurement of intrinsic clearance. *J.Pharmacol.Exp.Ther. 200:420-424.*

5. Gillette, J.R. (1986) On the role of pharmacokinetics in integrating results from *in vivo* and *in vitro* studies. *Fd.Chem.Toxicol. 24:711-720.*

6. Wagner, J.G. and Nelson, E. (1963) Percent absorbed time plots derived from blood level and/or urinary excretion data. *J.Pharm.Sci. 52:610-611.*

7. Gibaldi, M., and Perrier, D., eds. (1982) In, Pharmacokinetics. 2nd edition. Marcel Dekker Publishing Co. New York, N.Y.

8. Notari, R.E. (1987) In, Biopharmaceutics and Clinical Pharmacokinetics. Marcel Dekker Publishers, Inc. New York, N.Y. *Chapter 4.*

9. Chen, H.S. and Gross, J.F. (1979) Estimation of tissue-to-plasma partition coefficients used in physiological pharmacokinetic models. *J.Pharmacokin.Biopharm. 7(1):117-125.*

10. Lam, G., Chen, M-L. and Chiou, W.L. (1982) Determination of tissue-to-blood partition coefficients in physiologically-based pharmacokinetic studies. *J.Pharm.Sci. 71:454-456.*

11. Benet, L.Z. (1972) General treatment of linear mammillary models with elimination from any compartment as used in pharmacokinetics. *J.Pharm.Sci. 61:536-541.*

12. Vaughan, D.P. and Trainor, A. (1975) Derivation of general equations for linear mammillary models when the drug is administered by different routes. *J.Pharmacokin.Biopharm. 3:203-218.*

13. Pedersen, P.V. (1977) Curve fitting and modeling in pharmacokinetics and some practical experiences with NONLIN and a new program FUNFIT. *J.Pharmacokin.Biopharm. 5:513-531.*

14. Boston, R.C., Palmer, M.S., Golden, P.D. and Ramberg, C.F. (1988) Users notes for enhancement to SAAM/CONSAM Version 30 resource facilities for kinetic annalist. Contact the authors at the National Institute of Health, Building 10, Bethesda, Maryland.

15. Steiner, E.C., Rey, T.D., McCroskey, P.S. (1990) Simusolv modeling and simulation software reference. Dow Chemical Co. Midland, Michigan.

16. Rescigno, A. and Segre, G. (1966) In, Drug and Tracer Kinetics. 1st American edition. Blaisdale Publishing Co. Waltham, MA. *p. 91.*

17. Fagarasan, J.T. and DiStefano, J.J. (1989) Hidden oscillations in generalized linear mammillary compartmental models. *Math.Biosci. 93:79-95*.

18. O'Flaherty, E.J. (1989) Interspecies conversion of kinetically equivalent doses. *Risk Anal. 9:587-598.*

19. Gerlowski, L.E. and Jain, R.K. (1983) Physiologically based pharmacokinetic modeling: principals and applications. *J.Pharmacol.Sci. 72(10):1103-1127*.

20. National Toxicology Program Ad Hoc Panel (1984) In, report of the National Toxicology Program Ad Hoc Panel on Chemical Carcinogenesis testing and evaluation. *pp.125-127.*