DRAFT: November 2008

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Draft proposal for a revised TG 417: Toxicokinetics

INTRODUCTION

1. Studies examining the disposition of a chemical substance are conducted to obtain adequate information on its absorption, distribution, biotransformation (i.e. metabolism) and excretion and to aid in understanding its mechanism of toxicity. Basic toxicokinetic (TK) parameters determined from these studies will also provide information on the potential for accumulation of the test substance in tissues and/or organs and the potential for induction of biotransformation as a result of exposure to the test substance.

2. TK data can be used to assess the adequacy and relevance of the extrapolation of animal toxicity data (particularly chronic toxicity and/or carcinogenicity data) to human hazard and/or risk assessment. Additionally, toxicokinetic studies may provide information useful for addressing issues of dose setting (linearity aspects), route of administration effects, bioavailability (especially with respect to risk assessment issues such as high dose animal to low dose human exposure and route to route extrapolation), and issues related to study design. Specific TK data can be used to develop a physiologically based toxicokinetic (PBTK) model.

3. There are important uses for metabolite/TK data such as suggesting possible toxicities and modes of action and their relation to dose level and route of exposure. In addition, metabolism data can provide information useful for assessing the toxicological significance of exposures to exogenously produced metabolites of the test substance.

DEFINITIONS

4. Definitions used for the purpose of this Test Guideline are provided in Annex.

INITIAL CONSIDERATIONS

5. Countries have different requirements and needs regarding the measurement of endpoints and parameters related to toxicokinetics for different classes of chemicals (e.g. pesticides, biocides, industrial chemicals). Unlike most Test Guidelines, this Test Guideline describes toxicokinetics testing, which involves multiple measurements and endpoints. In the future, several new Test Guidelines may be developed to describe each endpoint separately and in more detail. In the case of this Test Guideline, which test methods or assessments are conducted is specified by the requirements and/or needs of each competent authority.

6. There are numerous studies that might be performed to evaluate the TK behaviour of a chemical for regulatory purposes. However, depending on particular regulatory needs or situations, not all of these possible studies may be necessary for the evaluation of a chemical. In some cases, only a certain set of questions may need to be explored in order to address chemical-associated hazard and risk concerns. For

other situations, additional and/or more extensive TK studies may be necessary, depending on regulatory needs and/or if new questions arise as part of chemical evaluation.

7. All available information on the test article, including data generated by other relevant test methods, should be considered by the testing laboratory prior to conducting the study in order to enhance the quality of the study and minimize animal usage. Physicochemical properties, such as octanol-water partition coefficient (expressed as log P_{OW}), pKa, water solubility, vapor pressure, and molecular weight of a chemical may be useful for study planning and interpretation of results. They can be determined using appropriate methods as described in the relevant OECD Test Guidelines.

ANIMAL WELFARE CONSIDERATIONS

8. Guidance on humane treatment of animals is available in OECD Guidance Document 19 (1). It should be followed for all in vivo studies described in this Test Guideline.

DESCRIPTION OF THE METHODS

Pilot Studies

9. The use of pilot studies is recommended and encouraged for the selection of experimental parameters for the toxicokinetics studies (e.g. metabolism, mass balance, analytical procedures, dose-finding, exhalation of CO_2, etc.). Characterization of some of these parameters may not necessitate the use of radiolabelled substances.

Animal Selection

Species

10. The rat should normally be used for testing because it has been used extensively for toxicokinetic and toxicological studies. The use of other or additional species may be necessary if critical toxicology studies demonstrate evidence of significant toxicity in these species or if their toxicokinetics is shown to be more relevant to humans. Justification should be provided for the use of alternative or additional species.

Age and Strain

11. Young adult rats (normally 6-12 weeks at the time of dosing) should be used. All animals should be of similar age at the outset of the study. The weight variation should not exceed ±20 percent of the mean weight of the test group. Ideally, the strain used should be the same as that used in deriving the toxicological database for the chemical substance.

Number and Sex of Animals

12. A minimum of four animals of one sex should be used for each dose tested. If there are data available that demonstrate substantial differences in toxicity between males and females, the more sensitive sex should be chosen. If there are no such data, then the use of both sexes (four males and four females) is strongly recommended. Justification should be provided for the sex of the animals used.

Test Substance

13. A radiolabelled test substance using ^{14}C should be used for all mass-balance and metabolite identification aspects of the study; however, if it can be demonstrated that:

- the mass balance and metabolite identification requirements can be met using the unlabelled test substance,

- the analytical specificity and sensitivity of the method used with non radioactive test substance is equal to or greater than that which could be obtained with the radiolabelled test substance,

then, the radiolabelled compound does not need to be used. Other radioactive and stable isotopes may be used, particularly if the element is responsible for or is a part of the toxic portion of the compound. If possible, the radiolabel should be located in a core portion of the molecule which is metabolically stable (it is not exchangeable, is not removed metabolically as CO_2, and does not become part of the one-carbon pool of the organism). Labelling of multiple sites or specific regions of the molecule may be necessary to follow the metabolic fate of the compound.

14. The radiolabelled and non radiolabelled test substances should be analyzed using appropriate methods to establish purity and identity. The test substance and metabolites (i.e. radiolabelled substances comprising 5% or greater of the administered dose) should be followed until excreted. The radiopurity of the radioactive test substance should be the highest attainable for a particular test substance (ideally it should be greater than 95 percent) and reasonable effort should be made to identify impurities present at or above 2 percent. The purity, along with the identity and proportion of any impurities which have been identified, should be reported. Individual regulatory programs may choose to provide additional guidance to assist in the definition and specifications of test substances composed of mixtures and methods for determination of purity.

Administration of Test Substance

15. The test substance should be dissolved or suspended homogeneously in the vehicle employed for the other oral gavage toxicity studies if such vehicle information is available. Rationale for the choice of vehicle should be provided. The choice of the vehicle and the volume of dosing should be considered in the design of the study. The customary method of administration will be by gavage; however, administration by gelatin capsule or as a dietary mixture may be advantageous in specific situations. Verification of the actual dose administered to each animal should be provided.

16. The maximum volume of liquid that should be administered by oral gavage at one time depends on the size of the test animals, the type of dose vehicle, and whether or not feed is withheld prior to administration. Normally the volume should be kept as low as practical for either aqueous or non-aqueous vehicles. For example, non-aqueous vehicles used for more lipophilic test substances might start at 4 but not exceed 10 mL/kg body weight. For aqueous solutions up to 20 mL/kg body weight may be used. For repeated dosing, when daily fasting would be contraindicated, lower dose volumes (e.g., 2-4 mL/kg body weight) should be considered.

17. Intravenous administration of the test substance and measurement of the test substance in blood and excreta may be used to establish systemic bioavailability. For the intravenous study, a single dose (not to exceed the lower oral dose – see dose selection) of test substance is administered using an appropriate vehicle. This material should be administered in a suitable volume (e.g. 1 mL/kg bw) at the chosen site of administration to at least four rats of the appropriate sex (both sexes might be used, if warranted, see Paragraph 12), or if required by the competent authority.

18. Other routes of administration may be applicable (see paragraphs 72-75). Due consideration should be given to the type of anaesthesia as it may have effects on toxicokinetics.

Dose Selection

19. A single dose is used for the pilot study. The dose should be non-toxic, but high enough to allow for metabolite identification in excreta (see also paragraph 9). For the main studies, in the case of single dose administration, investigation of a minimum of two doses should be preferred compared to the investigation of only one dose level. If AUC is not linear between the two dose levels, this is a strong indication of saturation of one or more of the kinetic processes at the highest dose. The information provided from the provision of two dose group data at this stage should subsequently reduce the number of animals required for later stage toxicokinetic and chronic toxicity testing.

20. In cases where only one dose level is investigated, the dose should be nontoxic, but high enough to allow for metabolite identification in excreta. Where two doses are administered, there should be a low dose at which no toxic effects are observed, but which is high enough to allow identification of metabolites in excreta and a high dose at which there might be changes in toxicokinetic parameters (in order to identify saturation effects or non-linearities in toxicokinetics) and/or overt toxicity. Information from available toxicity data should be considered for dose selection.

21. Dose-setting should be determined by the nature of the experiment and/or the issue being addressed. For example, if an effect of dose on clearance needs to be established, at least one dose should be high enough so as to saturate clearance.

22. Single dose toxicokinetic and tissue distribution data may be adequate to determine the potential for accumulation and/or persistence. However in some circumstances repeated dose administration may be needed to address more fully the potential for: i) accumulation and/or persistence or changes in TK (i.e. enzyme induction), or ii) as required by a competent authority. In studies involving repeated dosing, while repeated low dose administration is usually sufficient, under certain circumstances repeated high dose administration may also be necessary (see 6th bullet of paragraph 47).

23. For test substances of low toxicity, typically a maximum dose of 1,000 mg/kg body weight (limit dose) should not be exceeded.

Measurements

Absorption

24. Data (percent of recovery of administered dose from urine, feces, and expired air) should be collected to determine the rate and extent of excretion of test substance, to assist in establishing mass balance, and to estimate the extent of absorption. For investigation of excreta, see paragraphs 35 – 40. If the exact extent of absorption following oral dosing cannot be established from mass balance studies (e.g., where greater than 20 percent of the administered dose is present in faeces), further investigations might be necessary and could comprise either intravenous administration of test material and measurement of test substance in excreta or oral administration of test substance and measurement of test substance in bile.

25. If a biliary excretion study is undertaken, the oral route of administration is typically used. In this study, the bile ducts of at least four rats of the appropriate sex (or of both sexes, if warranted) should be cannulated and a single dose of the test substance should be administered. Following administration of the test substance, excretion of radioactivity/test substance in bile should be monitored as long as necessary to estimate the percentage of the administered dose that is excreted via this route, as follows:

Percent absorption = (amount in bile + urine + expired air + carcass – GI tract contents) / amount administered

26. For the intravenous (IV) study, the disposition of the test substance is compared with the results from dosing via the oral route. In this study, at least four rats should be given the test substance, via intravenous administration in a suitable vehicle. The percentage of administered dose absorbed for the oral route is calculated as follows:

Percent absorption = [$recovery_{oral}$ / $recovery_{IV}$] x [$dose_{iv}$ / $dose_{oral}$]

where recovery = amount of administered dose recovered in urine + expired air + carcass– GI tract contents

27. Plasma/blood kinetics can be determined from the oral and intravenous groups, as described in paragraphs 41-43. The calculation of systemic bioavailability (F) of the test substance or relevant metabolite(s) can then be made as follows:

$F = (AUC_{exp} / AUC_{iv}) \times (Dose_{iv} / Dose_{exp})$

where AUC is the area under the plasma time-course curve, and exp is the experimental route (oral, dermal or via inhalation).

For use in risk assessment of systemic effects, systemic bioavailability of the toxic component is in general a critical parameter and, without further knowledge, preferred over the parameter absorption. This is especially relevant when comparing systemic concentrations from animal studies with analogous biomonitoring data from worker or human volunteer exposure studies. Given that AUC_{exp} and AUC_{iv} are similar (if not, the situation may become more complex due to possible non-linearity), systemic bioavailability (F) is calculated as above. Also, given the prerequisite of similar AUC_{exp} and AUC_{iv}, an important first aim in toxicokinetic screening investigations is to make sure the intended doses are in the linear range.

Tissue Distribution

28. The percent of the total radioactive dose in tissues as well as residual carcass should be determined to establish mass balance at the termination of the excretion experiment. Tissues that should be collected include liver, fat, gastro-intestinal tract, kidney, spleen, whole blood, residual carcass, target organ tissues and any other tissues (e.g., reproductive organs) of potential significance in the toxicological evaluation of the test substance. Analysis of additional tissues at the same time should be considered to maximize utilization of animals and in the event that target organ toxicity is observed in sub-chronic or chronic toxicity studies. The radioactive residue concentration and tissue-to-plasma (blood) ratios should also be reported.

29. A competent authority may also require the evaluation of tissue distribution at additional time points such as the time of peak plasma\blood concentration (e.g. Tmax) or the peak rate of urinary excretion, obtained from the respective plasma\blood kinetic or excretion experiments. This information can be useful for understanding toxicity and the potential for test substance and metabolite accumulation and persistence. Justification for sample selection should be provided; samples for analysis generally should be the same as those above (Paragraph 28).

30. Certain techniques, currently at various stages of development, e.g. quantitative whole-body autoradiography, may prove useful in determining if a test substance concentrates in certain organs or in determining a specific pattern of distribution within a given tissue.

31. For routes of exposure other than oral, specific tissues should be collected and analyzed, such as lungs in inhalation studies and skin in dermal studies. See discussion below regarding Alternate Routes of Exposure.

Metabolism

32. Excreta should be collected for identification and quantitation of unchanged test substance and metabolites as described under paragraphs 37-40 of the Excretion section of this Test Guideline. Pooling of excreta to facilitate metabolite identification within a given dose group is acceptable. Profiling of metabolites from each time period is recommended. However, if lack of sample and/or radioactivity precludes this, pooling of urine and faeces across several time points is acceptable. Appropriate qualitative and quantitative methods should be used to assay urine, faeces, and expired air from treated animals.

33. Reasonable efforts should be made to identify all metabolites present at 5% or greater of the administered dose and to provide a metabolic scheme for the test substance. Compounds which have been characterized in excreta as comprising 5% or greater of the administered dose should be identified. Identification refers to the exact structural determination of components. Typically, identification is accomplished either by co-chromatography of the metabolite with known standards using two dissimilar systems or by techniques capable of positive structural identification such as MS, NMR, etc. In the case of co-chromatography, chromatographic techniques utilizing the same stationary phase with two different solvent systems are not considered to be an adequate two-method verification of metabolite identity, since the methods are not independent. Identification by co-chromatography should be obtained using two dissimilar, analytically independent systems such as reverse and normal phase thin layer chromatography (TLC) and high performance layer chromatography (HPLC). Provided that the chromatographic separation is of suitable quality, then additional confirmation by spectroscopic means is not necessary. Unambiguous identification can also be obtained using methods providing structural information such as: liquid chromatography/mass spectrometry (LC-MS), or liquid chromatography/tandem mass spectrometry (LC-MS/MS), gas chromatography/mass spectrometry (GC-MS), and NMR.

34. If identification at 5% or greater of the administered dose is not possible, a justification/explanation should be provided in the final report. It might be appropriate to identify metabolites representing less than 5% of the administered dose to gain a better understanding of the metabolic pathway for hazard and/or risk assessment of the test substance. Structural confirmation should be provided whenever possible. This may include profiling in plasma or blood or other tissues.

Excretion

35. Data (percent recovery of administered dose from urine, faeces, and expired air) should be collected to determine the rate and extent of excretion of test substance, to assist in establishing mass balance, and to estimate the extent of absorption. Generally, total recoveries of administered test substance (radioactivity) in the order of >90% are considered to be adequate. The quantities of test substance (radioactivity) eliminated in the urine, faeces, and expired air should be determined at appropriate time intervals (discussed below). Repeat dose experiments should be designed to allow for collection of excretion data. This will allow for comparison to single dose experiments.

36. If a pilot study has shown that no significant amount of test substance (radioactivity) (according to Paragraph 40) is excreted in expired air, then expired air does not need to be collected in the definitive study.

37. Each animal is to be placed in a separate metabolic unit for collection of excreta (urine, faeces and expired air). At the end of each collection period (see below), the metabolic units should be rinsed with appropriate solvent to ensure maximum recovery of the test substance (radioactivity). Collection of excreta should be terminated at 7 days, or after at least 90 percent of the administered dose has been recovered, whichever occurs first.

38. The total quantities of substance (radioactivity) in urine are to be determined for at least two time points on day 1 of collection, one of which should be at 24h post dosing, and daily thereafter until study termination, unless pilot studies suggest alternate or additional time points for collection.

39. The total quantities of test substance (radioactivity) in faeces should be determined on a daily basis beginning at 24 h post-dosing until study termination, unless pilot studies suggest alternate or additional time points for collection. A rationale should be provided for alternative collection schedules.

40. The collection of CO_2 and other volatile materials may be discontinued in a given study experiment when less than 1 percent of the administered dose is found in the exhaled air during a 24-h collection period.

Time Course Studies

Plasma/Blood Kinetics

41. The purpose of these studies is to obtain estimates of basic TK parameters [e.g. half-life ($t_{1/2}$), area under the curve (AUC)] for the test substance. These studies may be conducted at one dose or, more likely, at two or more doses. Dose setting should be determined by the nature of the experiment and/or the issue being addressed. Kinetic data may be needed to resolve issues such as substance bioavailablity and/or to clarify the effect of dose on clearance (e.g. to clarify whether clearance is saturated in a dose-dependent fashion).

42. For these studies a minimum of four animals per dose group is recommended.

43. Following administration of the test substance (radiolabelled), blood samples should be obtained from each animal at suitable time points using appropriate sampling methodology. The number of blood samples which can be obtained per animal might be limited by the sensitivity of the analytical method. In some instances, compromises might be required: it might be necessary to pool data from more than one animal. If a radiolabelled substance is used, analysis of total radioactivity present might be adequate. If so, total radioactivity should be analyzed in whole blood and plasma or plasma and red blood cells to allow calculation of the blood/plasma ratio. In other circumstances, more specific investigations requiring the identification of parent compound and/or metabolites might be necessary.

Other Tissue Kinetics

44. The purpose of these studies is to obtain time course information to address questions related to issues such as toxic mode of action, bioaccumulation and biopersistence via determination of levels of test substance in various tissues. The selection of tissues and the number of time points evaluated will depend on the issue to be addressed and the toxicological database for the chemical substance. These studies might involve single or repeated dosing. A detailed rationale for the approach used should be provided.

45. Reasons for performing these studies might include:

- Evidence of extended blood half-life, suggesting possible accumulation of test substance in various tissues or

- interest in seeing if a steady state level has been achieved in specific tissues (e.g. in repeated dosing studies, even though blood steady state levels of test substance may have been achieved, there may be interest in ascertaining that a steady state level has also been attained in target tissues);

46. For these types of time-course studies, an appropriate oral dose of test substance should be administered to a minimum of four rats per dose per time point and the time course of distribution monitored in selected tissues. Only one sex may be required, unless gender specific toxicity is observed. Whether total radioactivity or parent substance and/or metabolites are analyzed will also depend on the issue being addressed. Assessment of tissue distribution should be made using appropriate techniques.

Enzyme Induction/Inhibition

47. Studies addressing possible test substance-related changes in metabolism may be needed under one or more of the following cases:

1. Available evidence indicates a relationship between metabolism and enhanced toxicity;

2. The available toxicity data indicate a nonlinear relationship between dose and metabolism;

3. The results of metabolite identification studies show identification of a potentially toxic metabolite that might have been produced by an enzyme pathway induced by the test material;

4. In explaining effects which are postulated to be linked to enzyme induction phenomena (e.g. where induction can plausibly be invoked as a factor in the production of effects - the occurrence of which may depend on the level of inducible enzymes present);

5. If toxicologically significant alterations in the metabolic profile of the test substance are observed through either *in vitro* or *in vivo* experiments with different species or conditions, characterization of the enzyme(s) involved may be needed (e.g. Phase I enzymes such as isoenzymes of the Cytochrome P450-dependent mono-oxygenase system, Phase II enzymes such as isoenzymes of sulfotransferase or uridine diphosphate glucuronosyl transferase, or any other relevant enzymes). This information might be used to evaluate the pertinence of species to species extrapolations.

6. Appropriate study protocols to evaluate test substance related changes in TK, suitably validated and justified should be used. Example study designs consist of repeat dosing with unlabeled test substance, followed by a single radiolabelled dose on day 14, or repeated dosing with radiolabelled test material and sampling at days 1, 7 and 14 for determination of metabolite profiles. Repeated dosing with radiolabelled test substance may also provide information on bioaccumulation (above).

SUPPLEMENTAL APPROACHES

Use of in vitro information

48. Several questions concerning the metabolism of the substance may be addressed in *in vitro* studies such as dermal absorption (2) using appropriate test systems. Subcellular fractions e.g. microsomes from liver may be used to study possible systemic metabolites. Local metabolism in the target organ, e.g. lung may be of interest for risk assessment. For these purposes, microsomal fractions of target tissues may be useful. Studies with microsomes may be useful to address potential species and life-stage differences and characterize enzyme parameters (K_m and V_{max}) which can aid in the assessment of dose dependency of metabolism in relation to exposure levels. In addition microsomes may be useful to identify the specific microsomal enzymes involved in the metabolism of the substance which can be relevant in species extrapolation. The potential for induction of biotransformation can also be addressed by using liver microsomes of pretreated animals. The results from *in vitro* investigations can also be used in the development of PBTK models (3).

49. PBPK and other forms of pharmacokinetic/toxicokinetic models are considered part of *in silico* (i.e., computer simulation) models. Also within the term "*in silico*" are contained QSAR/QSPR/SAR models. This is very different from *in vitro* and *in vivo* models, where the term "model" is used to describe a test system that give us some idea of what happens in humans (i.e., the "rat" is an "*in vivo*" model and the "Caco-2 cells" is an "*in vitro*" model).

50. Primary cell cultures from liver cells and fresh tissue slices can be used to address similar questions as with liver microsomes. In certain cases, it may be possible to answer specific questions using cell lines with defined expression of the relevant enzyme.

Bridging with Toxicokinetic Data from Toxicity Studies

51. Additional approaches beyond the *in vivo* experiments described above may provide useful information on the absorption, distribution, metabolism or elimination of a chemical in certain species. Analysis of blood, tissue and/or excreta samples obtained during the conduct of any other toxicity studies can provide data on bioavailability, changes in plasma concentration in time (AUC, Cmax), bioaccumulation potential, clearance rates, and gender or life-stage changes in metabolism and kinetics.

52. Study design consideration could include questions such as:

Have saturation of absorption or detoxification pathways been reached at the higher doses tested? Has excretion been saturated at the higher doses tested? Are new pathways becoming operational at the higher doses? Are toxic metabolites limited to higher dose?

53. Other hazard assessment considerations could include issues such as:

- Age-related sensitivity due to differences in the status of the blood-brain barrier, the kidney and/or detoxification capacities;
- Sub-population sensitivity due to differences in detoxification capacities or other TK differences;
- Extent of exposure of the fetus by transplacental transfer of chemicals or of the newborn through lactation.

Use of Toxicokinetic Modeling

54. Toxicokinetic models may have utility for various aspects of hazard and risk assessment as for example in the prediction of systemic exposure and internal tissue dose. Furthermore specific questions on mode of action may be addressed, and these models can provide a basis for extrapolation across species, routes of exposure or dosing patterns. Data useful for developing PBTK models for a chemical in any given species are 1) partition coefficients, 2) biochemical constants and physiological parameters, 3) route-specific absorption parameters and 4) *in vivo* kinetic data for model evaluation (e.g. clearance parameters for relevant (> 10 percent) excretion pathways, Km and Vmax for metabolism). The experimental data used in model development should be generated with scientifically sound methods and the model results validated. Chemical- and species-specific parameters such as absorption rates, blood:tissue partitioning and metabolic rate constants are often determined to facilitate development of non-compartmental or physiologically-based models.

DATA AND REPORTING

55. The completed study should be presented in the following format:

Title/Cover Page

56. Title page and any appropriate certifications (e.g., requirements for data submission, good laboratory practice, statements of data confidentiality claims and quality assurance) should precede the content of the study formatted below.

Table of Contents

57. A concise listing is to precede the body of the report, containing all essential sections of the study and the page and table number where the section is located in the final report of the study. Essential sections of the Table of Contents should include a summary, an introduction, the materials and methods section, results, discussion/conclusions, references, tables, figures, appendices, and key subsections as appropriate. The Table of Contents should include the page number of each of these sections.

Body of the Report

58. The body of the report should include information covered by this Test Guideline organized into sections and paragraphs as follows:

Summary

59. This section of the study report is to contain a summary and analysis of the test results and a statement of the conclusions drawn from the analysis. This section should highlight the key findings regarding the nature and magnitude of metabolites, tissue residue, rate of clearance, bioaccumulation potential, sex differences, etc. The summary should be presented in sufficient detail to permit evaluation of the findings.

Introduction

60. This section of the report should include the study objectives, rationale and design, as well as an overview of the study, appropriate references and background history, if any.

Materials and Methods

61. This section of the report is to include detailed descriptions of all pertinent information including:

(a) Test Substance

62. This subsection should include identification of the test substance – chemical name, molecular structure, qualitative and quantitative determination of its chemical composition, chemical purity and type and quantities of any impurities, whenever possible.

63. This subsection should also include information on physical/chemical properties including physical state, colour, gross solubility and/or partition coefficient, stability, and if appropriate, corrosivity.

64. The type or description of any vehicle, diluents, suspending agents, and emulsifiers or other materials used in administering the test substance should be stated.

65. If the test substance is radiolabelled, information on the following should be included in this subsection: the type of radionuclide, position of label, specific activity, and radiochemical purity.

(b) Test Animals

66. This subsection should include information on the test animals, including selection and justification for species, strain, age at study initiation, sex as well as body weight, health status, and animal husbandry.

(c) Methods

67. This subsection should include details of the study design and methodology used. It should include a description of:

(1) How the dosing solution was prepared and the type of solvent or vehicle, if any, used;

(2) Number of treatment groups and number of animals per group;

(3) Dosage levels and volume;

(4) Route(s) and methods of administration;

(5) Frequency of dosing;

(6) Fasting period (if used);

(7) Total radioactivity per animal;

(8) Animal handling;

(9) Sample collection and handling;

(10) Analytical methods used for separation, quantitation and identification of metabolites;

(11) Other experimental measurements and procedures employed (including validation of test methods for metabolite analysis).

(d) Statistical Analysis

68. If statistical analysis is used to analyze the study findings, then sufficient information on the method of analysis and the computer program employed should be included so that an independent reviewer/statistician can re-evaluate and reconstruct the analysis.

69. In the case of systems modelling studies, presentation of models should include a full description of the model to allow independent reconstruction and validation of the model.

Results

70. All data should be summarized and tabulated with appropriate statistical evaluation and placed in the text of this section. Radioactivity counting data should be summarized and presented as appropriate for the study, typically as disintegrations per minute and microgram or milligram equivalents, although other units may be used. This section should include graphic illustrations of the findings, reproduction of representative chromatographic and spectrometric data, and proposed metabolic pathways including molecular structure of metabolites. In addition the following information is to be included in this section, if applicable:

(1) Justification for modification of exposure conditions, if applicable;

(2) Justification for selection of dose levels;

(3) Description of pilot studies used in the experimental design of the follow-up studies, if applicable. Pilot study supporting data should be submitted;

(4) Quantity and percent recovery of radioactivity in urine, faeces, and expired air, as appropriate. For dermal studies, include recovery data for treated skin, skin washes, and residual radioactivity in the covering apparatus and metabolic unit as well as results of the dermal washing study;

(5) Tissue distribution reported as percent of administered dose and concentration (microgram equivalents per gram of tissue), and tissue-to-blood or tissue-to-plasma ratios;

(6) Material balance developed from each study involving the assay of body tissues and excreta;

(7) Plasma concentrations and toxicokinetic parameters (bioavailability, AUC, Cmax, plasma concentration time-course, clearance, half-life) after administration by the relevant routes of exposure;

(8) Rate and extent of absorption of the test substance after administration by the relevant routes of exposure;

(9) Quantities of the test substance and metabolites (reported as percent of the administered dose) collected in excreta;

(10) Individual animal data;

(11) A figure with the proposed metabolic pathways and the molecular structures of the metabolites.

Discussion and Conclusions

71. In this section the author(s) should:

(1) Provide an explanation of the metabolic pathway for the test substance;

(2) Emphasize species and sex differences whenever possible;

(3) Tabulate and discuss the nature and magnitude of metabolites, rates of clearance, bioaccumulation potential, and level of tissue residues of parent, and/or metabolite(s), as well as possible dose-dependent changes in TK parameters, as appropriate;

(4) Integrate into this section any relevant TK data obtained in the course of conducting toxicity studies;

(5) Be able to derive a concise conclusion that can be supported by the findings of the study.
(6) Add Sections (as needed or appropriate)

The authors may need to include additional sections such as appendices, bibliography, tables, etc.

ALTERNATIVE ROUTES OF EXPOSURE

Dermal

Dermal Treatment

72. Alternative routes only need to be considered if human exposure occurs in this way. Dermal doses could be selected based on expected human exposure and/or doses at which toxicity occurred in dermal toxicity study. One (or more if needed) dose levels for the test substance should be used in the dermal portion of the study. The test substance (e.g. neat, diluted or formulated material containing the test chemical which is applied to the skin) should be the same (or a realistic surrogate) as that to which humans or other potential target species might be exposed (4). The low dose level should be selected in accordance with paragraph 20 of this Test Guideline. The dermal doses should be dissolved, if necessary, in a suitable vehicle and applied in a volume adequate to deliver the doses. Shortly before testing, fur should be clipped from the dorsal area of the trunk of the test animals. Shaving may be employed, but it should be carried out approximately 24 h before the test. When clipping or shaving the fur, care should be taken to avoid abrading the skin, which could alter its permeability. Approximately 10 percent of the body surface should be cleared for application of the test substance. With highly toxic substances, the surface area covered may be less than approximately 10 percent, but as much of the area as possible is to be covered with a thin and uniform film. The same treatment surface area should be used for all dermal test groups. The dosed areas are to be protected with a suitable covering which is secured in place. The animals should be housed separately.

Dermal Washing

73. A washing experiment should be conducted to assess the removal of the applied dose of the test substance by washing the treated skin area with a mild soap and water. A single dose should be applied to two animals in accordance with paragraph 19 of this Test Guideline. After application (e.g. 2 to 5 min) the treated areas of the animals should be washed with a mild solution of soap and water. The amounts of test substance recovered in the washes should be determined to assess the effectiveness of removal by washing.

74. Unless precluded by corrosiveness, the test substance should be applied and kept on the skin for a minimum of 6 h. At the time of removal of the covering, the treated area should be washed following the procedure as outlined in the dermal washing study (Paragraph 73). Both covering and the washes should be analyzed for residual test substance. At the termination of the studies, each animal should be humanely killed in accordance with (1), and the treated skin removed. An appropriate section of treated skin should be analyzed to determine residual substance (radioactivity).

Inhalation

75. A single concentration (or more if needed) of test substance should be used in this portion of the study. The concentration should be selected in accordance with paragraphs 19-23 of this Test Guideline. Inhalation treatments are to be conducted using a "nose-cone" or "head-only" apparatus to prevent absorption by alternate routes of exposure. If other inhalation exposure conditions are used, justification for the modification must be documented. A single exposure over a defined period should be used for each group; a typical exposure is 4 – 6 h.

REFERENCES

(1) *OECD (2000) Guidance Document on Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation; Environmental Health and Safety Monograph Series on Testing and Assessment N°19.*

(2) *OECD Guideline for Testing of Chemicals: 428 "Skin Absorption: In Vitro Method" (Adopted 13 April 2004)*

(3) Loizou G, Spendiff M, Barton HA, Bessems J, Bois FY, d'Yvoire MB, Buist H, Clewell HJ 3rd, Meek B, Gundert-Remy U, Goerlitz G, Schmitt W. (2008): Development of good modelling practice for physiologically based pharmacokinetic models for use in risk assessment: The first steps. Regulatory toxicology and pharmacology 50, 400 – 411

(4) *OECD Guideline for Testing of Chemicals: 427 "Skin Absorption: In Vivo Method" (Adopted 13 April 2004)*

(5) *Barton, H.A., et al. (2006), The Acquisition and Application of Absorption, Distribution, Metabolism, and Excretion (ADME) Data in Agricultural Chemical Safety Assessments, Critical Reviews in Toxicology, 36: 9-35.*

ANNEX: DEFINITIONS

Absorption: Process(es) of uptake of substances into or across tissues. Absorption refers to parent compound and all its metabolites. Not to be confused with "bioavailability";

Accumulation (Bioaccumulation): Increase of the amount of a substance over time within tissues (usually fatty tissues); if the input of a substance into the body is greater than the rate at which it is eliminated, the organism accumulates the substance and toxic concentrations of a substance might be achieved;

ADME: Acronym for "Absorption, Distribution, Metabolism, and Excretion";

AUC: (Area under the plasma concentration-time curve): Area under the curve in a plot of concentration of substance in plasma over time. It represents the total amount of substance absorbed by the body within a predetermined period of time. Under linear conditions, the AUC (from time zero to infinity) is proportional to the total amount of a substance absorbed by the body, irrespective of the rate of absorption;

Autoradiography: (Whole-body autoradiography): Used to determine the tissue localization of a radioactive substance, this technique uses X-ray film to visualize radioactively labelled molecules or fragments of molecules by recording on a photographic plate the radiation emitted within the object under study;

Biliary excretion: Excretion from the bile ducts;

Bioaccumulation: See "Accumulation";

Bioavailability: Fraction of an administered dose that reaches the systemic circulation or is made available at the site of physiological activity. Usually, bioavailability of a substance refers to the parent compound, but it could refer to its metabolite. It considers only one chemical form. Nota Bene: bioavailability and absorption are not the same. The difference between e.g. oral absorption (i.e. presence in gut wall and portal circulation) and systemic bioavailability (i.e. presence in systemic blood and in 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 (5). Systemic bioavailability of the toxic component (parent compound or a metabolite) is a critical parameter in human risk assessment (high-to-low dose extrapolation, route-to-route extrapolation) for derivation of an internal value from the external NOAEL or BMD (applied dose). For liver effects upon oral administration, it is the oral absorption that suffices. However, for every effect other than at the portal of entry, it is the bioavailability that is in general a more reliable parameter for further use in risk assessment, not the absorption;

Biopersistence: See "Persistence";

Biotransformation: (Usually enzymatic) chemical conversion of a substance of interest into a different chemical within the body. Synonymous with "metabolism";

C_{max}: Either maximal (peak) concentration in blood (plasma/serum) after administration or maximal (peak) excretion (in urine or feces) after administration;

Clearance: Quantitative measure of the rate at which a substance is removed from the blood, plasma or a certain tissue within a specified period of time;

Compartment: Structural or biochemical portion (or unit) of a body, tissue or cell, that is separate from the rest;

Detoxification pathways: Series of steps leading to the elimination of toxic substances from the body, either by metabolic change or excretion;

Distribution: Position, arrangement, or frequency of occurrence (as of the members of a group) over an area or throughout a space or unit of time;

Enzymes/Isoenzymes: Proteins that catalyse chemical reactions. Isoenzymes are enzymes that catalyse the same chemical reaction but differ in their amino acid sequence;

Enzymatic Parameters: K_m : Michaelis constant and V_{max}: maximum velocity;

Excretion: Process(es) by which an administered substance and/or its metabolites are removed from the body;

Exogenously: Introduced from or produced outside the organism or system;

Extrapolation: Inference of one or more unknown values on the basis of that which is known or has been observed;

Half-life ($t_{1/2}$): Time required for the decay of half of a sample;

Induction/Enzyme induction: Enzyme synthesis in response to an environmental stimulus or inducer molecule;

Linearity/linear kinetics: A process is linear in terms of kinetics when all transfer rates between compartments are proportional to the amounts or concentrations present, i.e. first order. Consequently, clearance and distribution volumes are constant, as well as half-lives. The concentrations achieved are proportional to the dosing rate (exposure), and accumulation is more easily predictable;

Mass balance: Accounting of test material entering and leaving the system;

Material balance: See mass balance;

Mechanism (Mode) of toxicity/Mechanism (Mode) of action: Mechanism of action refers to specific biochemical interactions through which a substance produces its effect. Mode of action refers to more general pathways leading to the toxicity of a substance;

Metabolism: Synonymous with “biotransformation”;

Metabolites: Products of metabolism or metabolic processes;

Partition coefficient: Also known as the distribution coefficient, it is a measure of the differential solubility of a substance in two solvents;

Peak blood (plasma / serum) levels: Maximal (peak) blood (plasma/serum) concentration after administration (see also "C_{max}");

Persistence (biopersistence): Long-term presence of a substance (in a biological system) due to resistance to degradation/elimination;

Route of administration (oral, oral gavage, intravenous, dermal, inhalation, etc.): Refers to the means by which substances are administered to the body (e.g., orally by gavage, orally by diet, dermal, by inhalation, intravenously, etc);

Saturation: State of being saturated, having reached maximum levels of absorption;

Sensitivity: Capability of a method or instrument to discriminate between measurement responses representing different levels of a variable of interest;

Steady-state blood (plasma) levels: Non-equilibrium state of an open system in which all forces acting on the system are exactly counter-balanced by opposing forces, in such a manner that all its components are stationary in concentration although matter is flowing through the system;

Systems Modeling/Modeling (Pharmacokinetic-based, Physiologically-based Pharmacokinetic, Biologically-based, etc.): Abstract model that uses mathematical language to describe the behaviour of a system (eg. Pharmacokinetic-based, physiologically-based pharmacokinetic, biologically-based, etc.);

Target tissue: Tissue in which the principal adverse effect of a toxicant is manifested;

Tissue distribution: Reversible movement of a substance from one location in the body to another;

T_{max}: Time to reach C_{max};

Toxicokinetics (Pharmacokinetics): Study of the absorption, distribution, excretion, and metabolism of substances;

Validation of models: Statistical and visual comparison of model predictions with experimental values against a common independent variable (e.g., time, space, concentration). Specific to physiologically-based pharmacokinetic models, validation includes assessment of each the following: (i) model purpose, (ii) model structure, (iii) mathematical representation, (iv) parameter values, (v) computer implementation, and (vi) predictive capacity. The extent of the validation should be justified in relation to the intended use of the model. In addition, validated models should be sufficiently documented to allow independent reconstruction and evaluation. Suggestions for documentation include: (i) text description of the model including species, route, exposure duration, and final parameter values; (ii) schematic diagram of the model; (iii) software and method used for optimizing parameter values; (iv) model code used for simulations should be included in an appendix or made available upon request.