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EC 7th Amendment: Role of Mammalian Toxicokinetic and Metabolic Studies in the Toxicological Assessment of Industrial Chemicals

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SUMMARY

Experience obtained with pharmaceuticals and crop protection chemicals suggests that information on the absorption, distribution, metabolism and elimination of chemicals in mammals can assist in the interpretation of toxicity studies. The fate of a chemical as described by toxicokinetics and metabolism is closely related to the toxic effects. This awareness has led the EC to include toxicokinetic and metabolic information in its legislation governing the toxicity testing of industrial chemicals. Information requested for new chemicals is dependent on the amount of substance to be placed on the market. Progressing through notification Levels 1 and 2, an increasing amount of toxicokinetic and metabolic data is required. The concept of compound-tailored approaches is allowed and rigid procedures have not been defined in the regulatory requirements. The resulting requirements at Level 1 ("basic toxicokinetic information") and at Level 2 ("additional toxicokinetic studies which cover biotransformation, pharmacokinetics") have to be interpreted.

The principles and methods used in the conduct of toxicokinetic and metabolic studies are reviewed to provide background and some key reference material is given. Much of this science has been developed from research on pharmaceuticals, veterinary medicines and crop protection chemicals. Each of these classes is treated differently in legislation and there are good reasons to conclude that industrial chemicals have to be considered as a separate class. Toxicokinetic and metabolic studies are lengthy and sophisticated and because of the varied nature and use of industrial chemicals, non-routine approaches will be necessary.

Various factors affecting toxicokinetics and metabolism are reviewed. One of the most significant of these, species differences, leads on to a

consideration of the extrapolation of the experimental data to man. The use of toxicokinetic and metabolic studies in toxicology, including the interpretation of results and their impact on hazard assessment is discussed.

The basic toxicokinetic and metabolic information required at notification Level 1 is interpreted by ECETOC to mean an assessment of absorption and excretion of the test substance. Physico-chemical properties and analogies based on structure-activity relationships can be of predictive value. Results of toxicity testing may also offer evidence for absorption and elimination. If such approaches provide no information, absorption-excretion studies based on elements of the EC testing guideline are proposed. If there is evidence of non-absorption then no further testing is needed.

"Additional toxicokinetic studies which cover biotransformation, pharmacokinetics", as required at notification Level 2, is interpreted by ECETOC to mean that quantitative data should be obtained on absorption, excretion, and possibly information on distribution and metabolism. The acquisition of information required at this Level is best approached in two stages. Stage 1 is the conduct of a single oral dose mass-balance study using quantitative analytical methods. If non-absorption can be demonstrated and no toxicity has been observed at this stage in the general testing and if no structure-activity considerations give cause for concern, it may be that no further work need be carried out. If these conditions are not fulfilled, or if there is a specific toxicological problem to address, Stage 2 testing will be necessary. This may involve tissue distribution, blood kinetics or biotransformation.

Testing may be impossible under certain circumstances due to the physical and chemical properties of the test substance. Many industrial chemicals are either insoluble, polymeric, highly reactive, or ill-defined. It is recommended that, particularly at Level 2, an extensive evaluation be conducted between the Manufacturer and the Authorities, with a view to generating an agreed approach.

1. INTRODUCTION

Protection of man against potential risks from chemicals requires that appropriate precautionary measures for their safe handling, use and disposal are observed. The nature and extent of the measures to be taken for a given chemical depend on its inherent toxicological properties and the potential exposure.

National Authorities and International Organisations have provided guidance on how to determine and evaluate these two factors of risk assessment for different categories of chemicals. Registration schemes have been enforced for pharmaceuticals and agrochemicals in many countries; these specify in detail the design, performance, interpretation and reporting for the required safety investigations.

Legislation exists for industrial chemicals. Within Europe production volume is the main criterion for the initiation of a process with steps of increasing toxicity information. This approach is governed by EC Council Directive 67/548/EEC, the 7th Amendment of which is presently in preparation (EEC, 1990).

This Council Directive requires information on the toxicokinetics of industrial chemicals at Levels 1 and 2 of notification. As the wording of the 7th amendment is somewhat vague, guidance regarding a rational approach for this category of chemicals is required. To meet this aim ECETOC established a Task Force with the following Terms of Reference:

- review the regulatory requirements for mammalian toxicokinetic and metabolism studies with respect to industrial chemicals,

- review the contribution of toxicokinetic and metabolic information to the design and interpretation of toxicity studies,

- discuss the relevance of toxicokinetics and metabolism in the assessment of hazard from industrial chemicals for man,

- propose a suitable range and appropriate sequence of studies which provide the toxicokinetic and metabolic information required for industrial chemicals.

This report describes the regulatory requirements (Section 2), gives the scientific background pertaining to toxicokinetics and metabolism (Sections 3 - 5) and makes recommendations on a stepwise approach for the evaluation of new chemicals (Section 6).

2. REGULATIONS FOR INDUSTRIAL CHEMICALS REGARDING TOXICOKINETICS AND METABOLISM

2.1. Legislative Position: Europe (EC)

2.1.1. Regulatory Requirements

Legislation is in a transitional phase, currently in annex VII of Council Directive 79/831/EEC relating to the classification, packaging and labelling of dangerous substances (6th amendment) applies (EEC, 1979). At notification Level 2 toxicokinetic studies are required for new substances which are placed on the EC market in quantities reaching 1,000 tonnes/year or exceeding a total of 5,000 tonnes irrespective of time (Level 2). The type of studies to be carried out are selected by the notifier in consultation with the competent authority.

In the proposal for the 7th Amendment (EEC, 1990) "basic toxicokinetic information" is required at Level 1 of notification (100 tonnes/year or when the total market exceeds 500 tonnes irrespective of time). In the legislation "basic toxicokinetic information" is not explained. At Level 2 (5,000 tonnes) additional "toxicokinetic studies which cover biotransformation, pharmacokinetics" are required. The interpretation of these terms is given in Section 3.1. and Section 6.

2.1.2. Test Guidelines

Examples of toxicokinetic tests are outlined in Commission Directive 88/302/EEC (EEC, 1988) [included as Appendix 1] which is an addition to the annex V of the 6th amendment and can be summarised as follows (the terms used are those quoted in the legislation):

Absorption studies,

- the determination of test substance and/or its metabolites in excreta, exhaled air and carcass (i.e. radioactivity balance);
- the comparison of biological response between test and reference groups (eg. oral vs iv);

- the determination of plasma levels of test substance and/or its metabolites.

Distribution studies,

- whole body autoradiography;
- the determination of test substance and/or its metabolites in organs/tissues at various times after administration.

Excretion studies,

- the determination of test substance and/or its metabolites in excreta and exhaled air;
- the determination of test substance and/or its metabolites in milk.

Biotransformation studies,

- the determination of metabolites in excreta in vivo;
- the determination of metabolites in vitro;
- biochemical studies.

This guideline is very similar to OECD Guideline No 417 (OECD, 1984).

2.2. Legislative Position: United States (US)

The EPA is open to toxicokinetic and metabolic studies in the testing of industrial chemicals. Normally no such studies are required by the EPA for Pre-Manufacturing Notification (PMN) of new chemicals.

The EPA is currently proposing study guidelines (EPA, 1991) for testing required under TSCA and FIFRA. This proposed rule is a joint guideline to harmonise the "pharmacokinetics" testing guidelines of the Office of Toxic Substances (OTS) and the Office of Pesticide Programmes (OPP). The main features of the US guidelines are presented below:

- absorption by relevant routes of exposure;
- biological half-life of parent compound and its metabolites and of their accumulation;
- tissue distribution of parent compound and its metabolites;

- metabolism pathways;
- routes and rates of elimination of parent compound and its metabolites.

The preferred species is the rat. The number of animals required is 5/sex/dose for a total of at least 10 animals. At least 2 dose levels should be used. The low dose should correspond with a no-effect-level whereas the higher dose should produce toxicity. The routes of administration are the oral, the dermal, the inhalation and the i.v. route.

The following dosing schedules may be envisaged:

- single i.v.-dose at an appropriate dose level;
- single oral dose at a low dose and a high dose level;
- single 6 hour inhalation exposure at a low and at a high concentration level;
- repeated dosing studies by the oral, the dermal and the inhalation route.

In all studies, except the repeated dose studies, the animals should be kept in individual metabolism cages for seven days or until 90% of the administered dose is excreted.

2.3. Comment

Test guidelines for toxicokinetic and metabolic studies exist only in EC and US legislation. The EC-guideline is almost an identical copy of OECD Guideline No 417 (OECD, 1984). The general concept of this legislation is that data should be obtained on absorption, distribution, excretion and metabolism in order to assist in the evaluation of test results from toxicology studies and in the extrapolation of data from animals to man. The requirements of the EC and the US are kept as broad as possible to allow for a compound-tailored study design.

3. PRINCIPLES AND METHODS USED IN TOXICOKINETIC AND METABOLIC STUDIES

3.1. General

3.1.1. Definitions

The terms used to address kinetic studies have been the subject of extensive discussion and considerable confusion over their meaning. Therefore some explanation and definition is essential. Kinetics, in the context of this report, describes the extent and the time course of adsorption, distribution and excretion of a foreign compound in the body following intentional or incidental exposure. Within this context the objective of "pharmacokinetic studies" is the optimisation of the dosage of medicines, whereas the objective of "toxicokinetics" is the assessment of systemic exposure to chemicals. Thus "pharamacokinetic studies" are an integral part of the pre-clinical evaluation of drugs whilst exposure to industrial chemicals is incidental to occupation (ie. multiple low exposures) or accidental (ie. single high exposure).

While kinetics describes the extent and time course of phenomena, the term metabolism or synonymously biotransformation describes the chemical modification of a foreign compound following exposure. The parent compound is usually converted into another compound often with completely different chemical, physico-chemical or biological properties. Although such reactions have kinetic aspects they are not normally included within toxicokinetics.

It should be noted that in the 7th Amendment (EEC, 1990) toxicokinetics is defined as the combination of pharmacokinetics (sic) and metabolism.

3.1.2. Prerequisites

Experimental approaches to the study of metabolism are different from those used for kinetic studies and require non-routine research effort in contrast to other studies in experimental toxicology.

The most relevant routes of exposure for industrial chemicals are the dermal and the inhalation routes. This is in contrast to the routine toxicity testing, for which initial information on toxicokinetics and metabolism is obtained generally from oral tests. It is only after having established the toxicokinetic parameters and metabolism of the chemical in oral tests that additional tests can be designed to measure absorption via the dermal and the inhalation route.

3.1.3. Methods

Toxicokinetic and metabolism studies can be carried out using non-labelled compounds, stable-isotope-labelled compounds, radioactively labelled compounds or using dual (stable and radio-) labelling. The labels should be placed in metabolically stable positions, the placing labels such as ^{14}C in positions from which they can enter the carbon pool of the test animal should be avoided. The radiolabelled compound must be of high radiochemical purity and of adequate specific activity to ensure sufficient sensitivity in radioassay methods. Separation techniques are used in metabolism studies to purify and to separate the several radioactive fractions in biota such as urine, plasma and bile. These techniques range from relatively simple approaches such as liquid-liquid extraction and column chromatography to more sophisticated techniques such as high pressure liquid chromatography. These methods also allow for the establishment of a metabolite profile when radiolabelled material is used.

Quantitative analytical methods are required to follow concentrations of parent compound and metabolites in the body as a function of time. The most common techniques used are gas chromatography coupled to sensitive and specific detectors such as electron capture, Fourier transform infra-red and mass detectors and high performance liquid chromatography with UV detection. The cost of radiosynthesis is often seen as an impediment to the use of radiochemicals. Often it will be cheaper and faster than approaches which require the development of other analytical methods for parent compound and major metabolite(s). It is important to note that kinetic parameters cannot be calculated from measurement of

total radioactivity. An analytical step is required to define the radioactivity as chemical species. This is also usually faster than cold analytical techniques. "Dual" labelling (eg. ^{13}C and $^{14}C/^{12}C$) is the method of choice for structural elucidation of metabolites (by MS and NMR spectroscopy). A cold analytical techniques which incorporates stable isotope labelling (for GC/MS or LC/MS analysis) is a useful combination. Unless this latter method has already been developed for the test compound in various matrices (urine, faeces, blood, fat, liver, kidney, etc), the use of radiolabelled compound will be less costly than other methods. The decision must be made on a case-by-case basis but it will usually be the case that where minimal toxicokinetic information is sought, the method development costs (radiosynthesis or cold GC/LC methods) will outweigh the cost of the dosing/analysis phase of the study.

It is important therefore, to attempt a preliminary assessment of absorption, elimination and metabolism without specific toxicokinetic and metabolic testing. This can be done by using information which is already available:

Molecular weight: small molecules are more likely to be absorbed than large molecules (e.g. >1000 daltons).

Structure: a consideration of the fate of structural analogues is often useful. Possible routes of metabolism can be readily predicted from structure but it is not possible to predict which of these many options will occur for a specific compound.

Stability: if low, then exposure to decomposition products may be important and absorption of the given compound may be limited.

Ionisation: passive diffusion across membranes occurs only for unionised forms, therefore pH may well affect absorption (and elimination).

Water solubility: water soluble compounds are often rapidly

absorbed and eliminated (via the kidneys in urine). Compounds of low solubility may be deposited at the dosage site and be poorly absorbed.

Fat solubility (or octanol/water partition coefficient): if high, ready passage across membranes will occur; accumulation in fat may occur (if biotransformation is slow); binding to plasma proteins and lipoproteins is likely.

Vapour pressure; if high, inhalation exposure is likely, as is elimination via the lungs in expired air.

Toxicity: When a clear dose-effect relationship can be established in toxicity studies this presents a clear indication that the substance is absorbed. Difference in response in a mutagenicity test with and without S9 indicates that the substance is metabolised and that the metabolite(s) formed are biologically active.

3.2. Absorption

3.2.1. Principles

The process by which foreign compounds cross body membranes and enter the bloodstream is referred to as absorption. In most cases, this consists of passive transport down a concentration gradient. Small hydrophilic molecules may pass through aqueous channels or pores in the membranes. For some chemicals, absorption via facilitated diffusion, active transport or pinocytosis has been reported.

The most important sites of absorption are gastrointestinal tract, skin and lungs [fig.1]. In the case of absorption from the gastrointestinal tract, the compound reaches the systemic circulation usually after passage through the liver via the hepatic portal vascular system. As the liver possesses considerable metabolic capacity, the chemical may not reach the circulation intact. Some metabolic activity also exists in the