Opinion of the Scientific Panel on Plant Protection Products and their Residues on a request from the Commission related to the revision of Annexes II and III to Council Directive 91/414/EEC concerning the placing of plant protection products on the market - Toxicological and metabolism studies

(Question N° EFSA-Q-2006-118)

adopted on 31 January 2007

SUMMARY OF OPINION

The PPR Panel has reviewed the proposed revisions to toxicological data requirements in Annexes II and III of Directive 91/414/EEC, as set out in the Commission Working Document, SANCO/10482/2006. The Panel concludes that the proposed revisions are generally appropriate in the context of current scientific understanding, but that there are a number of ways in which they could be improved or usefully augmented.

The PPR Panel makes the following main recommendations:

- It should always be open to the notifier to submit a reasoned scientific argument as to why a particular study is not needed. Guidance on modifications to the standard data requirements may be helpful for some categories of plant protection product (*e.g.* pheromone mating disruptors).
- As a general rule, the PPR Panel believes that the data requirements should specify the use of validated test protocols (accepted either by the EU or the OECD) where they are available and fit for purpose. Where no validated method is currently available, notifiers should justify their choice of non-standard tests, and provide details of their performance. Furthermore, should a suitable validated test be developed in the future, this should then be used in preference to non-standard tests.
- To allow for the possibility that, in the future, benchmark doses may come to be viewed as a preferable reference point to No Observed Adverse Effect Levels (NOAELs), it may be better to refer in the data requirements to "reference points" rather than specifically to NOAELs.
- Data requirements that are out of line with those of other regulatory agencies could in some circumstances lead to undesirable duplication of effort, with unnecessary use of animals. OECD protocols should therefore be referenced in the data requirements where they would be acceptable as alternatives to EU protocols. Furthermore, data requirements should be drafted in a way that facilitates harmonisation with those of other regulatory authorities as scientific understanding evolves, for example, by allowing, where appropriate, several alternative methods to address a particular aspect of the risk assessment.
- The use of a tiered approach to toxicity testing is already embraced in some sections of the draft data requirements, and the PPR Panel believes that this strategy could be developed further in the future. In Section 1.3.2, the Panel identifies several other circumstances in which it believes that a tiered approach to data generation would be appropriate.

- The PPR Panel recommends better integration of data on disposition and kinetics when interpreting the relevance of animal tests to toxicity in humans. In Section 1.3.2, the Panel discusses how this might be approached.
- In general, the PPR Panel believes that there should be a drive towards use of fewer but more informative studies in toxicological risk assessment for plant protection products.
- The value of several individual tests that currently form part of the standard data package for plant protection products – in particular, the 1-year dog study and the mouse carcinogenicity study – is questionable. The PPR Panel suggests that the need for these studies should be reviewed.
- The draft data requirements incorporate separate exposure assessments for residents and bystanders. However, any exposure experienced by a bystander could also occur in a resident (since residents can be bystanders on the day of spraying). Thus, separate exposure assessments should only be necessary if the estimated exposures will be assessed against different reference values.
- The PPR Panel believes there is a need for further scientific development in the assessment of potential exposures to operators, workers and bystanders/residents. To this end, the Panel proposes that an expert group be established to assess critically new predictive modelling approaches, and develop further guidance on the assessment of operator, worker and bystander/resident exposures.
- The PPR Panel notes with concern the proposal in the draft data requirements that toxicological studies in humans should never be used to derive regulatory reference values. The Panel believes that this approach is both scientifically unsound and ethically dubious.
- The PPR Panel recommends that where they are available, historical control data should be provided routinely in relation to studies of carcinogenicity, developmental toxicity and *in vivo* genotoxicity, and also for measurements of blood chemistry, haematological parameters and urinalysis in other studies. The data submitted should only be for endpoints that could represent critical adverse effects, and should be strain-specific and from the same laboratory as that which carried out the index study. They should come from a five-year period, centred as closely as possible on the date of the index study.
- The PPR Panel considers that the assessment of findings from toxicological studies will be facilitated if, where possible, results are presented in a standardised format. To this end, the Panel suggests that guidelines be produced setting out templates for the reporting of results.
- One area of development in regulatory toxicology, which the PPR Panel believes is not adequately reflected in the draft proposals is the consideration of combined toxicity, in particular where two or more active substances are co-formulated in the same product. The Panel suggests that in these circumstances, there should be a routine requirement for the notifier to provide adequate reassurance that adverse health effects will not occur as a consequence of combined action or interaction. In most cases, this should be possible through reasoned argument, but occasionally it might be necessary to generate additional data.
- The PPR Panel notes that the draft data requirements in several places use terms such as “relevant” and “appropriate” without making clear exactly what is implied. Some guidance on this, with examples, might be helpful.

- The PPR Panel anticipates that before the revised Annexes are finalised, their wording will be amended in some places to improve the clarity of the English. When such revisions are made, it will be important to check that the intended scientific meaning has not been altered or obscured.
- It is important to ensure that the data requirements in each individual area of risk assessment for plant protection products are consistent with those for the other areas. Therefore, there needs to be an holistic check of all six proposed revisions to the data requirements before they are finalised.

In addition to these main recommendations, the PPR Panel makes specific comments and recommendations on various sections of the draft data requirements.

Key words: toxicology, metabolism studies, plant protection product, data requirements, active substance, Annex II and III, Council Directive 91/414/EEC.

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BACKGROUND[1]

The Commission is revising the data requirements for authorisation of active substances and plant protection products in the framework of Council Directive 91/414/EEC.

The revision process involves also Part A of Annexes II and III of Council Directive 91/414/EEC. The revision process has been organised in order to amend current Directives laying down the data requirements for active substances and plant protection products, particularly Directive 94/79/EC (toxicological and metabolism studies). With regard to that Directive, the Commission has prepared a Working Document (SANCO/10482/2006) containing the now proposed data requirements to revise Annexes II and III to Directive 91/414/EEC.

The text has been drafted in collaboration with national experts under the coordination of the Competent Authorities of France and has been subject to extensive consultation with Member States and industry, whose comments have been taken into account.

TERMS OF REFERENCE

The PPR Panel is requested to provide observations and/or possible recommendations on the draft data requirements, in particular to verify that the methodology and the approaches presented in the draft data requirements are in line with the state of the art in the relevant field and the extent of its applicability with respect to the risk assessment of plant protection products.

ASSESSMENT

1.1. Introduction

In the framework of the revision of Council Directive 91/414/EEC, the data requirements for authorisation of active substances and plant protection products as laid down in six particular directives[2] are being revised. The PPR Panel has already provided opinions on the first three of the proposed revisions: physical and chemical properties, analytical methods and residues (EFSA 2006 a, b, c). The Panel has now reviewed the data requirements in the draft Commission working document SANCO/10482/2006 on toxicology, primarily for their relevance to the risk assessment process but also for their completeness, clarity and compatibility with the state of the art in the relevant scientific fields.

This SANCO working document SANCO/10482/2006 describes the requirements for key data from studies of toxicity and metabolism for the authorisation of active substances and plant protection products in the framework of Council Directive 91/414/EEC. The PPR Panel acknowledges the extensive consultation by the Commission with Member States, and the efforts towards harmonisation with other international requirements and documents. The Panel also notes that the draft requirements do not set out detailed test guidelines, but indicate that appropriate methods should be sought in guidance documents elsewhere. The principles underpinning the provision of all required data are the use, wherever possible, of well-standardised and validated methods, clear reporting, and rigorous and appropriate quality assurance. At the same time, data requirements must recognise advances in science and technology as they evolve.

[1] Submitted by the European Commission

[2] 94/37/EC physical and chemical properties, 96/46/EC analytical methods, 94/79/EC toxicological and metabolism studies, 96/68/EC residues, 95/36/EC fate and behaviour in the environment, 96/12/EC ecotoxicological studies

All opinions of the PPR Panel reviewing SANCO working documents on the revision of Annexes II and III have a common structure. General comments and principles are covered first before more-detailed comments and suggested changes to the existing draft texts. The Panel has already proposed that a glossary be constructed, setting out terminology and definitions applicable to all documents of Annexes II and III. This would reduce the chance of inconsistent or erroneous interpretations of documents from different sections of the Annexes. These terms can be marked in the texts to indicate their entry in the glossary. Appended to each PPR Panel opinion is a suggested basis for such a common and comprehensive glossary. The glossary for this opinion is at Appendix 1. It lists a number of terms, and for most of them, proposes a definition.

It is important to ensure that the data requirements in each individual area of risk assessment for plant protection products are consistent with those for the other areas. Therefore, there needs to be an holistic check of all six proposed revisions to the data requirements before they are finalised.

1.2. Comments on the composition and structure of the document

It is the purpose of the SANCO document to give a detailed description of the data requirements from studies of toxicity and metabolism that are needed for authorisation of active substances and plant protection products in the framework of Council Directive 91/414/EEC. The PPR Panel considers that the working document (Sanco/10482/2006 rev. 10) is a valuable contribution, reflecting continuous efforts to improve the data requirements given in Directive 94/79/EC (Annex I and II), and incorporating revisions and adaptations in response to the comments of the Member States and European Crop Protection Association (ECPA).

The draft proposal is divided into two parts: Annex II relating to the data requirements for active substances and Annex III to those for plant protection products.

1.3. Remarks and recommendations

1.3.1. General remarks and recommendations

1.3.1.1 The need for flexibility

The PPR Panel recognises that in the drafting of data requirements, a balance must be drawn between detailed prescription and flexibility. Detailed prescription helps to promote equity (one notifier is not required to generate more data than another in similar circumstances), transparency (notifiers know exactly what data they have to produce), and consistency of regulatory decisions. On the other hand, flexibility may be needed to address compound- or product-specific issues that cannot always be anticipated in advance, and may prevent unnecessary use of animals or resources. It can also enable a timely response to future developments in scientific understanding and methodology without the need for amendments to legislation.

Some plant protection products, because of their chemical characteristics and/or mode of use, may not warrant all elements of the standard data requirements. Thus, it should always be open to the notifier to submit a reasoned scientific argument as to why a particular study is not needed. Handling of such cases will generally be assisted by dialogue between the notifier and regulatory authority at an early stage in the approval process. Guidelines on modifications to the

standard data requirements may be helpful for some categories of plant protection products (*e.g.* pheromone mating disruptors).

Flexibility is also appropriate in relation to those aspects of the risk assessment for which no validated study method is currently available. This applies, for example, to the *in vitro* assessment of eye irritation, and to the assessment of developmental neurotoxicity and some aspects of genotoxicity. As a general rule, the PPR Panel believes that the data requirements should specify the use of validated test protocols (accepted either by the EU or the OECD) where they are available and fit for purpose. Where no validated method is currently available, notifiers should justify their choice of non-standard tests, and provide details of their performance criteria so that the assessor can form a view on interpretation. Furthermore, should a suitable validated test be developed in the future, this should then be used in preference to non-standard tests.

Another area in which future scientific developments may support changes to current practice is in the toxicological starting point for derivation of reference values such as the ARfD, ADI and AOEL. It may be, for example, that benchmark doses will come to be viewed as an alternative and often preferable reference point to No Observed Adverse Effect Levels (NOAELs). To allow for this possibility, it may be better to refer in the data requirements to "reference points" rather than specifically to NOAELs.

1.3.1.2 THE INTERNATIONAL CONTEXT

In specifying data requirements, it is important to consider the international context. Requirements that are out of line with those of other regulatory agencies could in some circumstances lead to undesirable duplication of activities, with unnecessary use of animals or resources. Thus, OECD protocols should be referenced in the data requirements where they would be acceptable as alternatives to EU protocols. Furthermore, data requirements should be drafted in a way that facilitates harmonisation with those of other regulatory authorities as scientific understanding continues to evolve in the future. For example, in some circumstances, several alternative methods might be allowed to address a particular aspect of the risk assessment.

1.3.1.3 EMERGING DEVELOPMENTS IN SCIENTIFIC UNDERSTANDING

An example of the way in which scientific understanding on risk assessment for plant protection products is currently evolving can be found in the proposals for radical changes to data requirements that recently have been published by the ILSI Health and Environmental Sciences Institute (HESI) (Carmichael *et al.*, 2006, Barton *et al.*, 2006, Doe *et al.*, 2006, Cooper *et al.*, 2006).

A major feature of the ILSI HESI proposals is the application of a tiered approach to toxicity testing, in which progression to further studies is triggered by findings in a standard, first level of investigation. This strategy is already embraced in the draft data requirements, for example, in relation to neurotoxicity, immunotoxicity and endocrine disruption, and the PPR Panel believes that it could be developed further in the future, particularly if other regulatory authorities such as the US Environmental Protection Agency move along a similar route. In making specific comments on the data requirements (Section 1.3.2), The PPR Panel highlights a number of other circumstances in which it believes that a tiered approach to the generation of data would be appropriate.

The PPR Panel also supports the ILSI HESI proposal for better integration of data on disposition and kinetics (ADME) when interpreting the relevance of animal tests to toxicity in humans. In its detailed comments, the Panel makes several specific recommendations for collection of additional ADME data where this could be useful for risk assessment, and the data could be obtained without substantially increasing the use of animals.

Another important question raised in the ILSI HESI report is the value of several individual tests that currently form part of the standard data package for plant protection products – in particular, the 1-year dog study, the current multi-generation study, and the mouse carcinogenicity study. The PPR Panel gives its views on the place for these tests when commenting on specific details of the data requirements later in this opinion. In general, however, the Panel believes that the drive should be towards fewer but more informative studies.

1.3.1.4 EXPOSURE ASSESSMENT FOR HUMANS

A further general principle is that all required data should have the potential to impact in some way on regulatory decisions. This principle appears to have been overlooked in the proposed incorporation of separate exposure assessments for residents and bystanders. Any exposure experienced by a bystander could also occur in a resident (since residents can be bystanders on the day of spraying). Thus, separate exposure assessments should only be necessary if the estimated exposures will be assessed against different reference values. If the same reference value is to be used, then it should be sufficient to estimate the exposure that is potentially the highest – *i.e.* that of the resident. The reference value against which the exposures of bystanders are currently assessed is the AOEL, which is considered appropriate for repeated exposures over the course of a spraying season, year upon year. It follows that separate exposure assessments for residents and bystanders would only be justifiable if the Commission intended either to adopt a more stringent reference value than the AOEL for residents, or alternatively to change the reference value that it currently uses for bystander exposures to one that can be higher than the AOEL. This is discussed further in the Panel's detailed comments on Section 7.2 of the draft data requirements.

More generally, the PPR Panel believes there is a need for further scientific development in the assessment of potential exposures to operators, workers and bystanders/residents. In commenting on Section 7.2 of the draft data requirements, the Panel proposes that an expert group should be established to assess critically new predictive modelling approaches, and develop further guidance on the assessment of operator, worker and bystander/resident exposures.

1.3.1.5 USE OF HUMAN DATA

It is also important that regulatory decisions make the best possible use of all available scientific data. The PPR Panel notes with concern the proposal in the draft data requirements that toxicological studies in humans should not be used to derive regulatory reference values. The Panel believes that this approach would be both scientifically unsound and ethically dubious. The Panel sets out its reasoning in its detailed comments on Section 5.9.2 of the draft data requirements.

1.3.1.6 HISTORICAL CONTROL DATA

The draft data requirements refer in several places to the use of data on historical controls. The PPR Panel recommends that where they are available, provision of such data should be routine in relation to studies of carcinogenicity, developmental toxicity and *in vivo* genotoxicity, and also for measurements of blood chemistry, haematological parameters and urinalysis in other studies. The data submitted should only be for endpoints that could represent critical adverse effects, and should be strain-specific and from the same laboratory as that which carried out the index study. They should come from a five-year period, centred as closely as possible on the date of the index study. Guidance on the use of historical control data might come better in the introduction to Section 5 rather than in the subsections on individual components of the toxicological data requirements.

1.3.1.7 FORMAT FOR SUBMISSION OF DATA

More generally, the PPR Panel considers that the assessment of findings from toxicological studies will be facilitated if, where possible, results are presented in a standardised format. To this end, the Panel suggests that guidelines be produced setting out templates for the reporting of results. The PPR Panel notes that OECD has developed such templates for toxicological studies.

1.3.1.8 COMBINED TOXICITY OF CO-FORMULATED ACTIVE SUBSTANCES

One area of development in regulatory toxicology, which the PPR Panel believes is not adequately reflected in the draft proposals, is the consideration of combined toxicity, in particular where two or more active substances are co-formulated in the same product. The Panel suggests that in these circumstances, there should be a routine requirement for the notifier to provide adequate reassurance that adverse health effects will not occur as a consequence of combined action or interaction. In most cases, this should be possible through reasoned argument based on data that have already been collected, but occasionally it might be necessary to generate additional data to satisfy the requirement, for example on mechanisms of action. If in the future, consideration of combined toxicity is extended to cumulative and aggregate risk assessment for dietary exposures, the need for such data may increase.

1.3.1.9 CLARIFICATION OF TEXT

The PPR Panel notes that the draft data requirements in several places use terms such as "relevant" and "appropriate" without making clear exactly what is implied. Some guidance on this, with examples, might be helpful. The Panel also recommends that a consistent approach be adopted to use of the terms "significant" and "significance", which have a technical meaning in statistics that is more specific than their meaning in everyday language.

The PPR Panel anticipates that before the revised Annexes are finalised, their wording will be amended in some places to improve the clarity of the English. When such revisions are made, it will be important to check that the intended scientific meaning has not been altered or obscured.

1.3.2. SPECIFIC COMMENTS

In this section, the Panel comments on specific elements of the draft data requirements. For ease of reference, the original text of the draft data requirements (in full and with its original numbering) is presented indented and in a reduced font size. Those parts of the original text to which comments refer are highlighted.

ANNEX II

5. TOXICOLOGICAL AND METABOLISM STUDIES

The PPR Panel suggests that this section should also include general guidance on the use of historical controls (see General Remarks and Recommendations).

Introduction

(i) The information provided, taken together with that provided for one or more preparations containing the active substance, must be sufficient to permit an evaluation to be made as to the risks for man, associated with handling and use of plant protection products containing the active substance, and the risk for man arising from residual traces remaining in food and water. In addition, the information provided must be sufficient to:

- permit a decision to be made as to whether, or not, the active substance can be included in Annex I,

- specify appropriate conditions or restrictions to be associated with any inclusion in Annex II,

- classify the active substance as to hazard,

-establish , where relevant an acceptable daily intake (ADI) level for man,

-establish acceptable operator exposure level(s) (AOEL),

-establish where relevant an acute reference dose, (ARfD),

- specify the hazard symbols, the indications of danger, and the risk and safety phrases for the protection of man, animals and the environment to be included in packaging (containers),

- identify relevant first aid measures as well as appropriate diagnostic and therapeutic measures to be followed in the event of poisoning in man,

and

- permit an evaluation to be made as to the nature and extent of the risks for man, animals (species normally fed and kept or consumed by man) and of the risks for other non-target vertebrate species.

The PPR Panel notes that information on mammalian toxicity is also used in assessing risks to vertebrate wildlife. This may have implications for the testing strategy.

The PPR Panel believes that there is a need for updated guidance on study design and data interpretation for the purpose of establishing ARfDs. A publication by Solecki *et al.* (2005) would serve as a useful starting point for this exercise.

(ii) The relevance of generating toxicity data in animal models with dissimilar metabolic profiles to those found in humans must be addressed in order to reduce animal usage. Discussion should be based on reliable information (comparative in vitro metabolism studies and in vivo toxicokinetic data).

In general, it is not possible to determine the metabolic relevance of animal models used for toxicity testing without undertaking specific studies. Hence, this section implies a need for routine performance of comparative *in vitro* studies of metabolism and *in vivo* studies of toxicokinetics in humans. The PPR Panel proposes that this section should be more flexible, requiring such metabolic justification of the species and study design used in toxicological tests on a case by case basis. For example, for compounds with low toxicity and little metabolism, there would be no need to justify the relevance of toxicity data on the basis of metabolic profiles.

(iii) There is a need to investigate and report all potentially adverse effects found during routine toxicological investigations (including effects on organs and special systems such as immunotoxicity and neurotoxicity) and to undertake and report such additional studies which may be necessary to investigate the probable mechanism involved, to establish NOAEL (No Observed Adverse Effect Levels) and to assess the significance of these effects.

All available biological data and information which is relevant to the assessment of the toxicological profile of the substance tested, must be reported.

The PPR Panel suggests rewording as "…. to report all potentially adverse effects found during toxicological investigations (including effects on organs and special systems such as immunotoxicity and neurotoxicity), and to undertake and report such additional studies as may be necessary to investigate the mechanisms underlying effects that could be critical to hazard identification or risk assessment."

Reason: There is no need to investigate the mechanisms of effects that will not be critical to classification and labelling or to the establishment of reference values.

(iv) In the context of the influence that impurities (by-products and/or isomers) can have on toxicological behaviour, it is essential that for each study submitted, a detailed quantitative description (specification) of the material used, as mentioned under section 1 point 11 [To check in revision section 1] be provided. Studies should be conducted using active substance of that specification to be used in the manufacture of preparations to be authorized, except where radio labelled material is required or permitted.

The PPR Panel suggests rewording this sentence as "Where possible, studies should be ..... preparations to be authorized."

Reason: For technical reasons, the impurity profile of an active substance may sometimes differ between batches produced during the development of a new plant protection product. This possibility is correctly recognised in the next paragraph.

Where studies are conducted using an active substance of different purity or which contains different impurities to the technical specification, the significance of the differences must be addressed either by data or scientific case. In cases of uncertainty, appropriate studies using the active substance as manufactured for commercial sale (to check with phys chem section) must be submitted to serve as a basis for a decision.

The use of the term "significance" is ambiguous. It is unclear whether it is intended to imply statistical significance.

In the case of studies in which dosing extends over a period, dosing should preferably be done using a single batch of active substance if stability permits.

(v) For all studies actual achieved dose in mg/kg body weight, as well as in other convenient units, must be reported.

The PPR Panel is unclear what is intended by the phrase "as well as in other convenient units". Is this a requirement or an optional extra in addition to using mg/kg body weight? If the latter, is there a need to make this clearer?

(vi) Absorption, distribution, metabolism and excretion of the active substance by oral route is normally reported for the rat. Information on blood and tissues concentration of the active substance and/or relevant metabolite(s) (around Cmax) should be generated in short and long term studies in all relevant species to enhance the value of the toxicological data generated in terms of understanding the toxicity studies. The main objective of the blood concentration data is to describe the systemic exposure achieved in animals and its relationship to the dose levels and the time course of the toxicity studies.

The PPR Panel recommends that the need for data on concentrations of the parent compound, determined by specific methods, should be emphasised here. Guidance should be developed, explaining what is meant by a "relevant metabolite". The extent to which such testing is required (all relevant species, short and long term studies) needs to be considered. Thought should be given to obtaining a core data set in the rat, supplemented by studies in other species during toxicity testing, either in the test animals or in satellite groups as convenient. Data are required not only on Cmax values but also on AUC and trough values during repetitive administration. Toxicokinetic data obtained by routes other than oral can be invaluable for route-to-route extrapolation, based on systemic exposure.

The other objectives of toxicokinetic data are :

-to relate the achieved exposure in toxicity studies to toxicological findings and contribute to the assessment of the relevance of these findings to operator, bystander, worker, resident and consumer safety,

-to confirm the design of a toxicity study (choice of species and treatment regimen) with respect to kinetic and metabolism, -to provide information which in relation with the findings of toxicity studies, contributes to the design of complementary toxicity studies.

Toxicokinetic data may also be of value in the derivation of chemical-specific adjustment factors (IPCS, 2005), and more generally in risk characterisation, for example in identification of potentially susceptible sub-groups.

The PPR Panel suggests that toxicokinetic data should be used to help in study design, for example in choice of the dose groups, and not just to confirm that a design is appropriate. In this way, it should be possible to improve the efficiency of toxicity testing and avoid excessive use of animals.

It might be helpful to include a paragraph on the purpose and value of metabolic profile studies in the rat. There are several benefits of such studies, but currently they are often regarded simply as a routine data requirement, only rarely being used to support data interpretation. Amongst the reasons for conducting such studies are: distinguishing, where possible, between parent compound- and metabolite-related toxicity, interspecies extrapolation (this often requires data on human metabolism, obtained *in vitro* or *in vivo*), confirmation that plant and soil metabolites have been evaluated for their toxicity, confirmation where possible that the toxicological test species is not irrelevant to humans, on the basis of unique metabolic characteristics of one or the other, understanding modes of action, derivation of chemical-specific adjustment factors, and identification of potentially susceptible sub-groups (*e.g.* genetic- or life stage-specific generation of a toxicologically important metabolite (*i.e.* of toxicity similar to or greater than the parent compound) or failure to detoxify such a metabolite).

(vii) The analytical method(s) to be used in toxicity studies should be specific for the entity to be measured and must be adequately validated. The limit of quantification should be adequate for the measurement of the range of concentration anticipated to occur in the generation of the toxicokinetic data.

(viii) Where, as a result of metabolism or other processes in or on treated plants, in soil, or in ground water, or open air, or as a result of processing of treated products, the terminal residue (to which consumers or workers as defined in Annex III, point 7.2.3 will be exposed) ***[To check in revision section7]*** contains a substance which is not the active substance itself and is not identified as a significant metabolite in mammals, it will be necessary to carry out toxicity studies on these components of the terminal residue unless it can be demonstrated that consumer or worker exposure to these substances does not constitute a relevant risk to health.

The PPR Panel suggests rewording “consumer or worker” as “human”.

Reason: The intention should be to cover all human exposures, including operators, bystanders and residents as well as consumers and workers.

The term “significant metabolite” requires definition and a reference to relevant guidance notes.

Toxicokinetic and metabolism studies relating to metabolites and degradation products should only be conducted if toxicity findings of the metabolite cannot be evaluated by the available results relating to the active substance.

(ix) The route of administration of the test substance depends on the main exposure routes. In cases where exposure of the operator is mainly by the gas phase, it can be more appropriate to perform some of the studies via inhalation instead of oral route.

The PPR Panel suggests that oral administration should always be used if it is practical. Exposure by inhalation should be used where oral administration is not practical, or (in addition to oral administration) where there is a particular reason, based on a compound’s toxicology or

kinetics. The Panel also suggests replacing the words "the operator" with "humans", since, for example, bystanders might also be exposed mainly by the gas phase.

(x) For ethical consideration, study designs should be carefully considered taking reduction, refinement and replacement of animal tests into consideration, for example by introducing an additional dose group(s) and/or time points for blood sampling into a given study, in order to avoid repeating the studies.

The PPR Panel suggests rewording as "For ethical reasons, study designs should be carefully considered, taking into account the scope for reduction, refinement and replacement of animal tests. For example, by including one or more additional dose groups or time points for blood sampling in one study, it may be possible to avoid the need for another study." The Panel also recommends that reference be made to OECD guidance on the recognition, assessment and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (OECD, 2000).

(xi) Studies conducted in the past although not fully compliant with today's standards and GLP may be integrated into the assessment, if scientifically valid. thereby removing the need for repeating animal tests, especially for carcinogenicity and reprotoxicity studies.

The PPR Panel suggests adding commas before and after this phrase.

Reason: Clarification of meaning.

(xii) Relevant data from the published literature on the active substance and/or preparations containing the active substance must be submitted and evaluated.

The PPR Panel suggests specifying "All relevant data ….", and omitting "… and evaluated"

Reason: It is important that all relevant data be submitted and not just a selection. Evaluation is a task for the regulatory authority.

(xiii) Current *adopted* EU guidance document on acceptable operator exposure, acute reference dose, dermal absorption, relevant metabolite should be taken into consideration in the evaluation. (COM to check wording and place of the sentence)

The PPR Panel notes that not all of this EU guidance is yet adopted. Possibly the sentence should be reworded as "Evaluation should take into account any EU guidance documents that have been adopted on acceptable operator exposure, acute reference dose, dermal absorption, and relevant metabolites."

5.1. Studies on absorption, distribution, metabolism and excretion in mammals

5.1.1 Absorption, distribution, metabolism and excretion by oral route

Limited data, as described below and restricted to one in vivo test species (normally the rat) may be all that is required in this area. These data can provide information useful in the design and interpretation of subsequent toxicity tests. However, it must be remembered that information on interspecies differences is crucial in extrapolation of animal data to man and information on metabolism following administration via other routes may be useful in operator risk assessments.

Information more generally on toxicokinetics (ADME) via other routes, not just on metabolism or indeed even mainly on metabolism, can be useful for risk assessment for exposures other than by the oral route. This applies not only to operators, but also, for example, to workers, residents and bystanders. In general, as indicated above, some toxicokinetic data would be desirable in other test species, such as the dog, to help in study design and data interpretation. These data should be obtained during the conduct of the toxicity test, not in separate toxicokinetic studies.

It is not possible to specify detailed data requirements in all areas, since the exact requirements will be dependant upon the results obtained for each particular test substance.

Aim of the study:

The studies should provide sufficient information about the kinetics of the active substance and/or its metabolite(s) in relevant species after

- a single oral dose (low and high dose levels),
- an intravenous dose (low dose level),
- and a repeated dose (low dose level).

Some consideration needs to be given to the objective of the repeat dose study as currently performed according to EU B.36 and OECD 417 (OECD 1984). From a toxicological perspective, the main concerns are accumulation of the parent compound or its metabolites after repeated dosing and time-dependent changes in kinetics due, for example, to enzyme induction or inhibition. Both enzyme induction and inhibition (but particularly inhibition) are more likely to occur at high than at low doses. Therefore, the dose chosen should be dictated by the high doses that are likely to be used in repeat dose toxicity studies. The information derived from this kinetic study should be supplemented by complementary toxicokinetic measurements made during the toxicity studies themselves, provided that the procedures used do not intrude on the expression of toxicity due to the test compound alone. Kinetic studies conducted early in pesticide development are likely to involve the use of radio-labelled parent compound. In later studies, in particular those designed to investigate the effects of repeat dosing on pesticide disposition, the use of analytical methods specific to the parent and metabolites will be necessary. The simple use of radioactivity measurements is inadequate for this purpose, particularly within the limitations of this test as currently conducted, where a single radio-labelled dose is administered after repeated administration of the unlabelled compound and data are recorded as parent compound-equivalents. An alternative, the use of radio-labelled material throughout the dosing period, would become excessively expensive and greatly increase the risk of environmental contamination.

When intravenous dosing is not feasible a justification is required. The design of the kinetic studies required should include :

i) - an evaluation of the rate and extent of oral absorption (including C max, AUC, Tmax, plasmatic half life(s)) and other appropriate parameters

A key parameter here is systemic bioavailability (F), obtained by comparison of the AUC after oral and intra venous dosing. Note that plasma half-life does not normally reflect rate or extent of absorption. Perhaps half-life determination should be moved to (ii) below.

ii) - the potential for bioaccumulation
- the distribution in major organs and tissues
- information on the distribution in blood cells.

iii) - the chemical structure, and quantities of metabolites in biological fluids and tissues- the different metabolic pathways

On a case by case basis, there may be value in identifying the main enzymes involved in elimination. This would be of particular value, for example, when toxicity is due to a metabolite, or when toxicokinetics play a key role in inter-species differences in toxic potency.

iv) - the route and time course of excretion of active substance and metabolites.
- the investigation of enterohepatic recycling where relevant

Consideration should be given to providing an option also to investigate differences in toxicokinetics following dietary and gavage dosing, on a case by case basis, for example when there is a very marked difference in toxicity by the two modes of administration.

> Information on plasma protein binding, comparison between rats, mice, other species and human proteins may be useful to enhance the value of the toxicological data generated in terms of understanding the relevance to human.

Studies on total protein binding in plasma will have limited value, although information on species differences in the free fraction of the compound may be useful in data interpretation. Information on binding to specific proteins, on a case by case basis, could be more valuable in interspecies extrapolation and determination of the relevance of findings in experimental animals.

> Comparative in vitro metabolism studies must be performed on animal species used in pivotal studies and on human material (microsomes or hepatocytes) in order to determine the relevance of the toxicological animal data base.

It is questionable whether this should be mandatory ("must be performed"). It should certainly be an option, but perhaps some flexibility is needed, this approach being adopted on a case by case basis, as part of a "second tier" of toxicokinetics.

Some additional options should be provided here. For example, depending on the nature of the metabolites, tissues (including plasma) other than liver and subcellular fractions other than microsomes might be more appropriate, as might recombinant expressed enzymes.

The PPR Panel suggests rewording "hepatocytes" as "intact cell systems, *e.g.* hepatocytes".

Reason: There are other possible systems such as precision-cut liver slices.

The role of such studies in investigating local tissue metabolism in bioactivation might be mentioned here.

> Further investigation and/or argumentation is needed when a metabolite is detected in vitro in human material and not in the tested animal species. In rare cases, if another species is considered relevant, additional studies on another species may be considered.

See comments above on the need for information on ADME in species used for toxicity testing, obtained as part of the toxicity testing strategy, rather than in a specific toxicokinetic/ADME study.

If exposure to the metabolite of concern does not occur in the species used for toxicity testing, the need for specific toxicity testing of the metabolite should be considered.

> Test guidelines
>
> EU testing Method B.36 (Annex V Directive 67/548/EEC) – Toxicokinetics, in addition intravenous route of administration must be conducted and the following parameters must be reported : Tmax, Cmax, AUC, plasmatic half life.

The explanatory text on the parameters to be reported is not necessary, as this level of detail is not provided elsewhere when test guidelines are identified in the document. Also, there may be other relevant parameters that should be reported, *e.g.* F (bioavailability).

5.1.2 Absorption, distribution, metabolism and excretion by other routes

> Distribution, metabolism and excretion after exposure by dermal route may be essential in operator and worker risk assessments and should be considered unless the active substance is a severe irritant or the dermal absorption is demonstrated to be low.

It is not clear why absorption is not included here (*i.e.* absorption, distribution, …). The original wording "may be useful in operator and worker risk assessments …" is perhaps preferable to

that now proposed. Dermal absorption can be relevant to risk assessments for residents and bystanders as well as operators and workers.

> Dermal absorption estimation from data generated in these studies should be critically assessed for relevance to operator and worker risk evaluation with the preparation. Dermal absorption estimation of the preparation is specifically considered under Annex III 7.3.

Again, this should also apply to risk assessment for residents and bystanders. It is not clear how the relevance of data obtained with the active substance to the preparation should be determined.

> For volatile substances (vapour pressure >10^{-2} Pascal), distribution, metabolism and excretion after exposure by inhalation is essential in operator and worker risk assessments and should be considered. Expert judgement is required to decide whether the study has to be performed by oral or inhalation exposure.

It is not clear why absorption is not included along with distribution, metabolism and excretion. This information could be very useful in route to route extrapolation of toxicity data.

The information is also relevant to risk assessment for residents and bystanders.

The words "and should be considered" could be omitted. Consideration of essential data is an onus on the regulator, but not a data requirement.

Some clarification is needed on what is meant by "oral or inhalation" exposure. Is this nose-only versus whole body? If not, some comment on the desirability of nose-only exposure should be included.

Again, "… exposure by inhalation may be useful in operator, worker, resident and bystander risk assessments …" is perhaps preferable to the wording currently proposed.

> Test guidelines
>
> EU testing Method B.36 (Annex V Directive 67/548/EEC)
>
> – Toxicokinetics, in addition intravenous route of administration must be conducted and the following parameters must be reported : Tmax, Cmax, AUC, plasmatic half life.

The meaning of "Toxicokinetics, in addition intravenous route of administration" is unclear.

The text following "… must be conducted" could be deleted as suggested above.

The possibility of including provision for higher tier studies on trans-placental and/or lactational transfer, on a case by case basis, should be considered.

Other issues that should be included to reflect state of the science are physiologically-based toxicokinetic models (PBTK) and kinetic/ADME studies in human volunteers.

PBTK models require generic and chemical-specific information, some of which can be obtained *in vitro*. They can be of value in extrapolating from high to low dose, from daily to intermittent exposure, from one route of exposure to another, and from one age, sex or species to another. They can be of considerable help in understanding modes of toxic action and non-linearities in response.

Comments on studies in human volunteers are provided elsewhere. Such studies can be invaluable for obtaining data on comparative absorption by different routes of exposure, metabolite profiles, routes and rates of elimination and the relationship between external and internal dose. These data could be used in extrapolation of toxicity data obtained in experimental animals, in the development of PBTK models, and in the derivation of chemical-specific adjustment factors.

5.2. Acute toxicity

The studies, data and information to be provided and evaluated must be sufficient to permit the identification of effects following a single exposure to the active substance, and in particular to establish, or indicate:

- the toxicity of the active substance,

- the time course and characteristics of the effects with full details of behavioural changes and possible gross pathological findings at post-mortem,

- where possible mode of toxic action, and

- the relative hazard associated with the different routes of exposure.

While the emphasis must be on estimating the toxicity ranges involved, the information generated must also permit the active substance to be classified in accordance with Council Directive 67/548/EEC. The information generated through acute toxicity testing is of particular value in assessing hazards likely to arise in accident situations.

The PPR Panel suggests that opportunities for obtaining information that might be of value in establishing an acute reference dose need to be highlighted. An absence of substance-related mortalities at doses up to 1000 mg/kg bw in acute oral studies is one of the main criteria for not setting an ARfD (FAO/WHO, 2004). Moreover, where an ARfD needs to be set, this could be assisted by interim measurements (after 1 or a few days) in short and long term studies in rats, dogs or other species. The possibility of conducting a single dose toxicity test could also be considered, on a case by case basis, in which non-lethal endpoints were investigated. The guidance on endpoints that might be suitable for establishing an acute reference dose published by Solecki *et al.* (2005) might usefully be referenced here.

5.2.1. Oral

Circumstances in which required

The acute oral toxicity of the active substance must always be reported.

Test guidelines

EU testing Method B.1bis (Annex V Directive 67/548/EEC) - Acute oral toxicity - fixed dose method,

EU testing Method B.1tris (Annex V Directive 67/548/EEC) - Acute oral toxicity - Acute toxic class method,

OECD guideline 425 - Acute oral toxicity : up-and-down procedure.

For the assessment of risks to human health, any of these test methods would be acceptable, and the aim should be to minimise the use of laboratory animals. However, where there is any lethality at 2000 mg/kg body weight, a point estimate of LD_{50} will be needed as part of the environmental risk assessment for mammalian wildlife. In these circumstances, the up-and-down procedure should be used. Thus, if it is anticipated in advance that the LD_{50} is likely to be less than 2000 mg/kg body weight, it would be more efficient (and use fewer animals) to proceed directly to an up-and-down study without doing a limit test.

5.2.2. Dermal

Circumstances in which required

The acute dermal toxicity of the active substance must always be reported. Both local and systemic effects must be investigated. When relevant, skin irritation data provided in the study should be considered to using this instead of a specific irritation study.

Test guideline

EU testing Method B.3 (Annex V Directive 67/548/EEC) - Acute toxicity (dermal).

The PPR Panel suggests that acute dermal toxicity should only be required when a) the oral LD_{50} is <200 mg/kg or b) the oral LD_{50} is in the range 200-2000 mg/kg and the dermal absorption is > 10%.

5.2.3. Inhalation

Circumstances in which required

The acute inhalation toxicity of the active substance must be reported except when the active substance is :

- a substance with a vapour pressure <1 × 10-2 Pa and not containing particles of diameter

< 50 µm (< 1% on weight basis).

A fixed concentration procedure is recommended.

The head/nose only exposure should be used, whole body exposure must be justified.

Test guideline

– EU testing Method B.2 (Annex V Directive 67/548/EEC) - Acute toxicity (inhalation).

The extent to which an active substance is made up of particles < 50 µm may depend on how it is formulated. Moreover, it is unclear whether 50 µm is the most appropriate cut-point.

The PPR Panel suggests deleting the recommendation for a fixed concentration procedure.

Reason: There is only a draft OECD guideline for the fixed concentration procedure. The cited guideline Method B.2 is not consistent with a fixed concentration procedure.

5.2.4. Skin irritation

Aim of the study

The results of the study will provide the potential of skin irritancy of the active substance including the potential reversibility of the effects observed.

This method includes the recommendation that before undertaking in vivo studies for corrosion/irritation of the substance, a weight-of-evidence analysis be performed on the existing relevant data. Where insufficient data are available, they can be developed through application of sequential testing.

The testing strategy should include,

1) the use of an in vitro dermal irritation/corrosion study to predict dermal corrosion prior to consideration of an in vivo dermal study,

2) the performance of validated and accepted in vitro dermal irritation studies and

3) in addition, where appropriate, the successive, instead of simultaneous application of the three test patches to the animals in the in vivo study.

Circumstances in which required

The skin irritancy of the active substance must always be documented. Consideration should be given to the use the dermal toxicity study to provide irritancy information.

Test guidelines

–EU testing Method B.4 (Annex V Directive 67/548/EEC) - Acute toxicity: dermal irritation/corrosion,

–EU testing Method B.40 (Annex V Directive 67/548/EEC) - Skin corrosion.

The PPR Panel suggests that the wording should make clearer that the testing strategy should be hierarchical and tiered, starting with *in vitro* methods and proceeding to *in vivo* as necessary.

Currently, a validated *in vitro* method is available only for corrosion and not for irritation.

5.2.5. Eye irritation

Aim of the study

The results of the study will provide the potential of eye irritancy of the active substance including the potential reversibility of the effects observed.

This method includes the recommendation that before undertaking in vivo studies for eye corrosion/irritation of the substance, a weight-of-evidence analysis be performed on the existing relevant data. Where insufficient data are available, they can be developed through application of sequential testing.

The testing strategy should include,

1) the use of an in vitro dermal irritation/corrosion test to predict eye corrosion prior to consideration of an in vivo eye study,

2) the performance of validated and accepted in vitro eye irritation studies and

3) in addition, where appropriate, the successive, instead of simultaneous application to the animal in the in vivo study.

Circumstances in which required

The eye irritancy of the active substance must always be documented except where it is likely that severe effects on the eyes may be produced.

Test guidelines

EU testing Method B.5 (Annex V Directive 67/548/EEC) - Acute toxicity: eye irritation/corrosion,

EU testing Method B.40 (Annex V Directive 67/548/EEC) - Skin corrosion.

It is unclear to what method “This method” refers.

The currently validated *in vitro* method is for dermal corrosion and not for dermal irritation.

The PPR Panel notes that there is currently no fully validated *in vitro* method for evaluating eye irritation or corrosion. The PPR Panel suggests adding “… studies if indicated, and”

For reasons of clarity, the PPR Panel suggests substituting “tested” for “documented”.

5.2.6. Skin sensitisation

Aim of study

The study will provide sufficient information to assess the potential of the active substance to provoke skin sensitisation reactions.

Circumstances in which required

The study must always be carried out except where the substance is a known sensitiser.

The local lymph node assay should be used preferably. In case the local lymph node assay can not be conducted, the Guinea Pig Maximisation Test should be performed.

The PPR Panel suggests rewording as "Where the local lymph node assay cannot be conducted, this must be justified. In these circumstances, a Guinea Pig Maximisation test or Buehler test should be performed. References to these tests can be found in Annex V of Council Directive 67/548/EEC Method B6 and in OECD 406 (OECD, 1992)."

Reason: The PPR Panel believes that a Buehler test provides an adequate test of potential to cause skin sensitisation. The Guinea Pig Maximisation test is considered to be a more sensitive assay than the Buehler test. However, the Buehler test is preferable to the Guinea Pig Maximisation test with regard to animal welfare.

Since an active substance identified as a skin sensitiser, can potentially induce hypersensivity reaction, potential respiratory sensitisation must be taken into consideration unless there is no anticipated respiratory exposure for the operator or worker during normal handling and use.

All known respiratory sensitisers to date have been identified from observations in humans. Moreover, there are few examples of typical skin sensitisers producing respiratory hypersensitivity. It is unclear whether this is because of lack of exposure or differences in immune mechanisms (typically skin sensitisers produce preferentially Th1-type cell-mediated delayed responses, whereas respiratory sensitisers usually induce preferentially Th2-type immune responses). All low molecular weight chemical respiratory sensitisers tested so far have been positive in the local lymph node assay. However, the PPR Panel notes that currently there are no validated models of respiratory sensitisation. Thus, the above paragraph does not carry any implications for data requirements.

Test guidelines

EU testing Method B.42 (Annex V Directive 67/548/EEC) - Skin sensitisation : Local lymph node assay,

EU testing Method B.6 (Annex V Directive 67/548/EEC) - Skin sensitisation (Guinea Pig Maximisation Test).

The PPR Panel suggests deleting the words "(Guinea Pig Maximisation Test)" for the reasons set out above.

5.2.7. Phototoxicity

Aim of the study

The study will provide the potential of certain active substances to induce photoxicity in combination with light, e.g. active substances that are phototoxic in vivo after systemic exposure and distribution to the skin, as well as active substances that acts as photoirritants after dermal application to the skin.

Circumstances in which required

For active substance suspected to have photoreactive potential.

Before biological testing is considered, a UV/vis absorption spectrum of the active substance and its major metabolites must be determined according to OECD Test Guideline 101. If the molar extinction / absorption coefficient is less than 10 litre x mol^{-1} x cm^{-1} the chemical has no photoreactive potential and may not need to be tested.

Test guideline

Directive 2000/33/EEC Method B.41 - Phototoxicity – In vitro 3T3 NRU phototoxicity test.

The PPR Panel suggests that it may be preferable to refer to induction of cytotoxicity in combination with UV radiation, and to substances that are "photoirritating" rather than phototoxic.

The study should be required where the active substance absorbs electromagnetic radiation in the range 290-700 nm, and is liable to reach the eyes or light exposed areas of skin, either by direct contact or through systemic distribution.

Guidance should be provided on what is meant by "major metabolites".

The PPR Panel suggests that it would be better to say, "is probably not photoreactive …".

The PPR Panel suggests including also "OECD 432 *In vitro*3T3 NRU phototoxicity test 2004" (OECD, 2004).

5.3. Short-term toxicity

Short-term toxicity studies must be designed to provide information as to the amount of the active substance that can be tolerated without adverse effects under the conditions of the study. Such studies provide useful data on the risks for those handling and using preparations containing the active substance. In particular, short-term studies provide an essential insight into possible cumulative actions of the active substance and the risks to workers who may be exposed. In addition short-term studies provide information useful in the design of chronic toxicity studies.

The PPR Panel suggests adding at the end of this sentence, "…chronic toxicity studies, and they may sometimes be used in the derivation of an ARfD."

Reason: An ARfD is often based on a relevant NOAEL from a repeat-dose study, where the critical end-point is considered relevant also for a single exposure (a single dose study investigating the most relevant acute endpoint is not yet currently required in the data package according to Annex II). Short-term studies may represent one of the most useful sources of data for setting ARfDs. In particular, if changes in toxicological parameters are observed early in a repeated-dose study and do not appear to progress during the course of the study, then such effects can be considered as relating to acute exposure to the substance.

The studies, data and information to be provided and evaluated, must be sufficient to permit the identification of effects following repeated exposure to the active substance, and in particular to further establish, or indicate:

- the relationship between dose and adverse effects,
- toxicity of the active substance including where possible the NOAEL
- target organs, where relevant (especially immune, nervous and endocrine systems),
- the time course and characteristics of adverse effects with full details of behavioural changes and possible pathological findings at post-mortem,

- specific adverse effects and pathological changes produced,

- where relevant the persistence and reversibility of certain adverse effects observed, following discontinuation of dosing,

- where possible, the mode of toxic action,

-the relative hazard associated with the different routes of exposure

-relevant critical endpoints at appropriate time points for setting Acceptable Operator Exposure Level, and Acute Reference Dose when necessary.

Toxicokinetic data (i.e.:blood concentration) should be included in short term studies. In order to avoid increased animal use the data can be derived in range finding studies.

If nervous system or endocrine system are *specific* targets in short term studies at dose levels not producing marked toxicity additional second tier tests including functional testing should be considered.

The PPR Panel welcomes the inclusion of a tiered testing strategy, and suggests adding ",immune system", after "nervous system"

Reason: Assessment of potential adverse effects on the immune system is an important component of the overall toxicity evaluation of chemicals. When appropriate endpoints are included in the study design, evidence of immunotoxicity can be observed in standard toxicology studies such as the proposed core 28-day and 90-day studies. Typically, the outcome of short-term toxicity testing is sufficient to detect any potential effects on the immune system. If one or more of the predictive markers is altered by treatment and this cannot be ascribed as secondary to general systemic toxicity, then consideration should be given to conducting additional functional tests. The PPR panel notes that for drugs, EMEA only requires a functional test to assess immune responses where there is prior cause for concern about immunotoxicity – *e.g.* because of observed pathology of immune tissues or because the compound belongs to a group of chemicals with known immunotoxicity.

5.3.1. Oral 28-day study

Circumstances in which required

It is not mandatory to perform 28-day short term studies. When conducted as range finding tests they must be reported.

If 28-day studies are used as basis for AOEL and/or ARfD the animal number and investigated parameters have to meet the requirements of 90-day or one-year studies.

The PPR Panel proposes rewording this paragraph as: "If a 28-day study is used as the basis for setting an AOEL and/or ARfD, the animal number and parameters investigated must meet the requirements of EU testing Method B.7 (Annex V Council Directive 67/548/EEC) and must be sufficient to permit the identification of all relevant effects following repeated exposure to the active substance."

Reason: The reference to 90-day and one-year studies is potentially misleading, since for non-rodent species, only eight animals are used at each dose level.

Test guidelines

–EU testing Method B.7 (Annex V Directive 67/548/EEC) - Repeated dose (28 days) toxicity (oral).

5.3.2 Oral 90-day and one-year study

Circumstances in which required

The short-term oral toxicity of the active substance to rodents (90-day), usually the rat, a different rodent species must be justified, and non rodents (90-day or 12-month toxicity study in dogs), must always be reported.

The PPR Panel proposes that a 90-day study in rodents may not be needed if a full 28-day study (OECD, 1995) in rodents is available. The short-term toxicity of the active substance to non-rodents should always be reported from a 90-day study (usually in dogs). However, there is no need to require a 12-month dog study in addition to a 90-day study in dogs. Any use of non-rodents other than dogs should be justified. If not all studies are used, there may be advantages in increasing the power of those studies that are conducted by using larger numbers of animals per study (while overall numbers are still reduced).

Reason: The 90-day study in rodents rarely impacts importantly on reference values when an adequate 28-day study is available. Based on extensive evidence (to which the PPR Panel can provide references if required), the PPR Panel has concluded that extension of a dog toxicity study beyond a 13-week duration provides little additional information, and that a 12-month dog study therefore constitutes an unnecessary use of animals.

In the 90-day rodent study, a functional test to assess immune responses (e.g. T cell dependent antibody assay) after an adequate period of exposure with the active substance should be included in the study plan.

Test guidelines

EU testing Method B.26 (Annex V Directive 67/548/EEC) - Sub-chronic oral toxicity test. Repeated dose 90-day oral toxicity study in rodents,

EU testing Method B.27 (Annex V Directive 67/548/EEC) - Sub-chronic oral toxicity test. Repeated dose 90-day oral toxicity study in non-rodents,

EU testing Method B.30 (Annex V Directive 67/548/EEC) - Chronic toxicity test.

The PPR Panel supports the inclusion of a functional test to assess immune responses only where there is prior cause for concern about immunotoxicity – *e.g.* because of observed pathology of immune tissues, because the plant protection product belongs to a group of chemicals with known immunotoxicity, or based on the mechanisms underlying other observed toxic effects.

The PPR Panel suggests that the reference to Method B.30 be deleted (see above).

5.3.3. Other routes

Circumstances in which required

For operator and worker risk assessment additional dermal studies should be considered unless the active substance is a severe irritant or the dermal absorption is demonstrated to be low.

For volatile substances (vapour pressure >10-2 Pascal) expert judgement is required to decide whether the short term studies have to be performed by oral or inhalation exposure.

The PPR Panel suggests that the need for separate dermal or inhalational studies should be guided in part by route-specific kinetic data.

Test guidelines

EU testing Method B.9 (Annex V Directive 67/548/EEC) - Repeated dose (28 days) toxicity (dermal)

EU testing Method B.28 (Annex V Directive 67/548/EEC) - Sub-chronic dermal toxicity test : 90-day repeated dermal dose study using rodent species,

EU testing Method B.8 (Annex V Directive 67/548/EEC) - Repeated dose (28 days) toxicity (inhalation),

EU testing Method B.29 (Annex V Directive 67/548/EEC) - Sub-chronic inhalation toxicity test : 90-day repeated inhalation dose study using rodent species.

5.4. Genotoxicity testing

Aim of the studies

These studies are of value in :

- the prediction of genotoxic potential
- the early identification of genotoxic carcinogens
- the elucidation of the mechanism of action of some carcinogens

Appropriate toxic dose levels, depending on the test requirements, must be used in either in vitro or in vivo assays.

The physico-chemical properties of the test chemical and its purity can affect the results of the studies, they must be taken into account when elaborating the study plan and in the interpretation of the results.

The PPR Panel suggests that this could be reworded as "When elaborating a study plan, available data on the test substance, such as its physico-chemical properties (*e.g.* volatility), purity, reactivity (*e.g.* rate of hydrolysis, electrophilicity) and structure-activity relationships of chemical analogues, should to be taken into account." This sentence might better be moved to the introduction of the toxicology section since it is not specific to genotoxicity.

This approach should be regarded as general guidance. It is important that a flexible approach is adopted, with selection of further tests being dependant upon interpretation of results at each stage.

The PPR Panel suggests adding "Special testing requirements in relation to photomutagenicity may be indicated by the structure of a molecule, its light absorbing potential or its potential to be photoactivated (TGD). In order that negative results can be accepted with confidence, protocols for such tests should be designed and applied so as to optimise the potential to detect a mutagenic response."

5.4.1. In vitro studies

Circumstances in which required

In vitro mutagenicity tests (bacterial assay for gene mutation, combined tests for structural and numerical chromosome aberrations in mammalian cells and test for gene mutation in mammalian cells) must always be performed.

If there are indications of numerical chromosome aberrations in mammalian cells, this should be confirmed using appropriate staining procedures such as FISH (fluorescence in-situ hybridization) to highlight alterations in the number of copies of chromosomes. If chromosome non disjunction is observed, the no observed effect concentration must be reported.

The PPR Panel suggests rewording as "If there are indications of numerical chromosome aberrations in mammalian cells, appropriate staining procedures, such as FISH (fluorescence in-situ hybridization) or chromosome painting, could be applied as supplementary tests to highlight alterations in the number of copies of chromosomes. If chromosomal non-disjunction is observed, an appropriate reference concentration (*i.e.* the NOEL or BMDL10) should be reported."

Active substances which display highly bacteriostatic properties as demonstrated in a range finding test must be tested in two different in vitro mammalian cell tests for gene mutation . Non performance of the Ames test should be justified.

Substances bearing structural alerts that have given negative results in the standard test battery may necessitate limited additional testing, if the standard tests have not been optimised for these alerts. The choice of additional study or study plan modification(s) depend on the chemical nature, the known reactivity and the metabolism data on the structurally alerting substance.

Test guidelines

Acceptable test guidelines are:

EU testing Method B.13/14 (Annex V Directive 67/548/EEC) - Mutagenicity- reverse mutation test using bacteria,

EU testing Method B.10 (Annex V Directive 67/548/EEC) - Mutagenicity-in vitro mammalian chromosome aberration test,

EU testing Method B.17 (Annex V Directive 67/548/EEC) - Mutagenicity-in vitro mammalian cell gene mutation test (mouse lymphoma assay is recommended).

EU testing Method B.18 (Annex V Directive 67/548/EEC) – DNA damage and repair- Unscheduled DNA synthesis - mammalian cells in vitro.

OECD 487 in vitro micronucleus test

In vitro Comet assay could be used when justified .

5.4.2. In vivo studies in somatic cells

Circumstances in which required

If all the results of the in vitro studies are negative, at least one in vivo study must be done with demonstration of exposure (e.g. cell toxicity and/or toxicokinetic data).

The PPR Panel proposes that an *in vivo* study is not needed in this circumstance.

Reason: The PPR Panel knows of no compounds that are consistently negative in *in vitro* studies but positive when tested *in vivo*. The Panel's proposal is in line with the approach applied to biocides.

A negative result in the first in vivo test in somatic cells will provide sufficient reassurance for substances that are negative in the three in vitro tests.

For substances for which an equivocal or a positive test result is obtained in any in vitro test, the nature of additional testing needed should be considered on a case-by-case basis taking into account all relevant information using the same endpoint as in the in vitro test. If the in vitro mammalian chromosome aberration test is positive, an in vivo test for clastogenicity using somatic cells (metaphase analysis in rodent bone marrow or micronucleus test in rodents or in vivo Comet assay) must be conducted.

If the in vitro test for numerical chromosome aberrations on mammalian cells is positive, an in vivo micronucleus test should be conducted. In case of positive result, appropriate staining procedure such as FISH (fluorescence in-situ hybridization) to highlight alterations in the number of copies of chromosomes should be used.

If either of the in vitro gene mutation tests are positive, an in vivo test to investigate unscheduled DNA synthesis test must be conducted.

A study using transgenic animal models can also provide valuable information as supplementary tests, in particular in investigating mutagenic activity in specific tissues which are often the site of initial contact with the active substance. Taking animal welfare into account, this test should only be considered for high-risk substances.

The PPR Panel suggests adding "Normally, when conducting *in vivo* genotoxicity studies, only relevant exposure routes and methods (*e.g.* admixture to diet, drinking water, skin application, inhalation) should be used,. Other exposure techniques (*e.g.*, intraperitoneal or subcutaneous injection) that are likely to result in abnormal kinetics, distribution and metabolism are not recommended. The comet assay could be applied, as a supplementary test, to evaluate the genotoxic activity of the compound in specific target tissues. The importance of excluding false positives when using relatively non-specific endpoints, (*e.g.* DNA strand breakage (comet assay) at cytotoxic doses) should be noted."

The PPR Panel notes that no standard protocols are currently available for gene mutation tests with transgenic mice, that such tests are not commonly performed, and that interpretation of the results can be difficult.

Test guidelines

Acceptable test guidelines are:

EU testing Method B.12 (Annex V Directive 67/548/EEC) - Mutagenicity - in vivo mammalian erythrocyte micronucleus test,

EU testing Method B (Annex V Directive 67/548/EEC) 11 - Mutagenicity - in vivo mammalian bone-marrow chromosome aberration test,

OECD guideline 486 - Unscheduled DNA synthesis (UDS) test with mammalian liver cells in vivo.

The in vivo comet assay could be used when justified.

5.4.3. In vivo studies in germ cells

Circumstances in which required

The necessity for conducting these tests will have to be considered on a case by case basis, taking into account information regarding toxicokinetics, use and anticipated exposure.

For most of the substances recognised as in vivo somatic cell mutagens no further genotoxicity testing is necessary since they will be considered to be potential genotoxic carcinogens and potential germ cell mutagens.

However, in some specific cases germ cells studies may be undertaken to demonstrate whether a somatic cell mutagen is or is not a germ cell mutagen.

Consideration of the type of mutation produced in earlier studies i.e. gene, numerical chromosome or structural chromosome changes is important when selecting the appropriate assay.

Suitable tests to provide information on genotoxicity to germ cells are the mammalian spermatogonial chromosome aberration test, the rodent sperm-FISH assay, the in vivo Comet assay in germ cells and the rodent dominant lethal assay.

A study for the presence of DNA adducts in gonad cells, or a study using transgenic animal models can also be considered.

Test guidelines

Acceptable test guidelines are:

EU testing Method B.23 (Annex V Directive 67/548/EEC)– Mammalian spermatogonial chromosome aberration test.

The in vivo comet assay in germ cells, the rodent sperm-FISH assay, the alkaline elution of testicular DNA and the Method B.22 (rodent dominant lethal assay) could be used when justified.

The PPR Panel recommends that these references to the rodent dominant lethal assay be deleted.

Reason: The assay requires large numbers of animals, produces relatively little information, and it is not always clear whether observed effects are attributable to mutation.

The PPR Panel notes that currently there are no agreed protocols for studies of mutation in transgenic animals.

5.5. Long term toxicity and carcinogenicity

Aim of the study

This section should be harmonised with section 5.3 of the data requirements on short-term toxicity. It might help to include a sentence highlighting the added value from long-term studies relative to short-term studies.

The long-term studies conducted and reported, taken together with other relevant data and information on the active substance, must be sufficient to permit the identification of effects, following repeated exposure to the active substance, and in particular must be sufficient to:

- identify adverse effects resulting from exposure to the active substance,
- identify target organs, where relevant,
- establish the dose-response relationship,

- establish the NOAEL

Similarly, the carcinogenicity studies taken together with other relevant data and information on the active substance, must be sufficient to permit the evaluation of hazards for humans, following repeated exposure to the active substance, to be assessed, and in particular must be sufficient:

- to identify carcinogenic effects resulting from exposure to the active substance,
- to establish the species, sex, and organ specificity of tumours induced,
- to establish the dose-response relationship, and
- for non-genotoxic carcinogens, to identify the maximum dose eliciting no carcinogenic effect.

NOAELs also exist for some genotoxic carcinogens, particularly those acting by a mechanism other than direct reaction with DNA. Hence, where appropriate, supported by relevant mechanistic data, it may be possible to establish no effect levels for some carcinogens in addition to those acting by non-genotoxic mechanisms.

The PPR Panel recommends adding an additional bullet point: " – where possible, to determine the mode of action and human relevance of any carcinogenic response."

Circumstances in which required

The long-term toxicity and carcinogenicity of all active substances must be determined. If in exceptional circumstances, it is claimed that such testing is unnecessary, that claim must be fully justified.

Test conditions

A long-term oral toxicity and carcinogenicity study (two years) of the active substance must be conducted using the rat as test species, these studies can be combined.

The PPR Panel recommends rewording as,

"... where possible, these studies should be combined."

Reason: Reduced use of animals.

A second carcinogenicity study of the active substance must be conducted using the mouse as test species, unless it can be justified scientifically that this is not necessary.

Several reviews of the contribution of long-term studies in the mouse to the establishment of health-based guidance values have indicated that use of this species does not provide any additional contribution to risk assessment (*e.g.* Doe *et al.*, 2006). Hence, there is a strong case for deleting this requirement, and for focusing more on understanding modes of action when assessing risks of carcinogenicity.

In such cases scientifically validated alternative carcinogenicity in vivo models such as transgenic mice could be used instead of a second carcinogenicity study. If comparative metabolism data indicate that either rat or mouse is an inappropriate model for human cancer risk assessment, an alternative species could be considered if justified.

There is little evidence that a transgenic mouse model provides any additional benefit in risk assessment, other than to address specific mechanistic questions (MacDonald *et al.*, 2004).

Where a non-genotoxic mechanism for carcinogenicity is suggested, a well argued case, supported with relevant experimental data, including that necessary to elucidate the possible mechanism involved, must be provided.

Recent emphasis has been more on the mode of action rather than the mechanism, recognising that a detailed mechanistic understanding is rarely if ever available. The application of the International Programme on Chemical Safety (IPCS) human relevancy framework for carcinogenesis (Boobis *et al.*, 2006) should be encouraged, as a means of increasing transparency and harmonisation in cancer risk assessment. The important thing is that evidence be submitted sufficient to allow an assessment of the relevance of the observed carcinogenicity to human exposures.

For active substances which have shown effects in carcinogenicity studies with no clear evidence for a non-genotoxic mechanism and which were negative in standard genotoxicity testing (see 5.4), additional genotoxicity testing may be conducted including modified conditions for metabolic activation in in vitro studies or in vivo studies measuring genetic damage in target organs of tumour induction.

The nature and interpretation of such studies need to be considered carefully. There are only a few tests suitable for this purpose, and there are no agreed or accepted guidelines for *in vivo* studies. Presumably, the *in vitro* concern is that there may be site-specific formation of an active metabolite that could be detectable using sub-cellular fractions from the target tissue. Perhaps some clarification of the concerns and the available options could be provided here.

While the standard reference points for treatment responses are concurrent control data, historical control data may be helpful in the interpretation of particular carcinogenicity studies. Where submitted, historical control data should be from the same species and strain, maintained under similar conditions in the same laboratory and should be from contemporaneous studies. Additional historical control data from other laboratories can be reported separately as supplementary information.

See general comment on the use of historical control data. The PPR Panel recommends that historical control data should be submitted routinely for carcinogenicity studies.

The information on historical control data provided must include :

- identification of species and strain, name of the supplier, and specific colony identification, if the supplier has more than one geographical location,
- name of the laboratory and the dates when the study was performed,
- description of the general conditions under which animals were maintained, including the type or brand of diet and, where possible, the amount consumed,
- approximate age, in days, and weight of the control animals at the beginning of the study and at the time of killing or death,
- description of the control group mortality pattern observed during or at the end of the study, and other pertinent observations (e.g. diseases, infections),
- name of the laboratory and the examining scientists responsible for gathering and interpreting the pathological data from the study, and
- a statement of the nature of the tumours that may have been combined to produce any of the incidence data.

The historic control data should be presented on a study by study basis giving absolute values plus percentage, relative or transformed values where these would assist in the evaluation. If combined or summary data are submitted, these must contain information on the range of values, the mean, median and, if applicable, standard deviation.

The PPR Panel suggests that it would be better to transfer consideration of historical controls to the introduction to Section 5 (see General Remarks and Recommendations).

> The doses tested, including the highest dose tested, must be selected on the basis of the results of short-term testing and where available at the time of planning the studies concerned, on the basis of metabolism and toxicokinetic data. The highest dose level in the carcinogenicity study and in the long term toxicity study should elicit signs of toxicity without substantially altering normal lifespan due to effects other than tumours. Dose selection should consider toxicokinetic data such as saturation of absorption measured by systemic availability of substance and/or metabolite(s).

Toxicokinetic data should also be obtained during the course of the study, either from the test animals or from a satellite group maintained for this purpose. Treatment-related changes in kinetics that might occur include induction and inhibition of metabolism and pathology-related changes in the function of organs of elimination, *e.g.* renal dysfunction.

An impact on normal lifespan from effects other than tumours cannot always be avoided because effects of long-term exposure (*e.g.* nephropathy) and some effects of age may not be predictable at the beginning of the study. Furthermore, this point is addressed in test guideline B.32, and does not need to be mentioned here.

> Doses, causing excessive toxicity are not considered relevant to evaluations to be made. Information on blood concentration of the active substance (around Cmax) should be generated in long term studies in all relevant species.

The PPR Panel recommends that information be obtained on the average, maximum and minimum concentrations during the inter-dosing interval.

Reason: It is the concentration at around the time at which the peak occurs that is of interest (*i.e.* Tmax). Such information, when combined with data on the trough (minimum) concentrations can provide an indication of the total systemic exposure over a 24 hour period, and the extent to which this differs after a single exposure.

The PPR Panel suggest that the phrase "in all relevant species" can be deleted here because it is redundant.

> In the collection of data and compilation of reports, incidence of benign and malignant tumours must not be combined, unless there is clear evidence of benign tumours becoming malignant with time. Similarly, dissimilar, un-associated tumours, whether benign or malignant, occurring in the same organ, must not be combined, for reporting purposes.

Many malignant tumours arise from benign tumours, *e.g.* carcinomas from adenomas. Even where there is a precursor relationship, data should be provided on the incidence of benign and malignant tumours separately for each tissue. For example, findings might be interpreted differently if an increase were due entirely to benign lesions.

> In the interests of avoiding confusion, conventional histopathological terminology commonly used when the study is conducted such as that published by the International Agency for Research on Cancer should be used in the nomenclature and reporting of tumours. The system used must be identified.

> It is essential that biological material selected for histopathological examination includes material selected to provide further information on lesions identified during gross pathological examination. Where relevant to the elucidation of mechanism of action and available, special histological (staining) techniques, histochemical techniques and electron microscopic examinations, must be conducted and reported.

Perhaps more flexibility should be permitted here, and rather than "...must be conducted and reported", this might be better as "might be of value, and when conducted, must be reported."

Test guidelines

– EU testing Method B.30 (Annex V Directive 67/548/EEC) – Chronic toxicity test,

– EU testing Method B.32 (Annex V Directive 67/548/EEC) – Carcinogenicity test,

– EU testing Method B.33 (Annex V Directive 67/548/EEC) – Combined chronic toxicity/carcinogenicity test.

5.6. Reproductive toxicity

Adverse reproductive effects are of two main types:

- impairment of male or female fertility, and

- impacts on the normal development of progeny (developmental toxicity).

The PPR Panel recommends rewording as:

- Impairment of male and female reproductive functions or capacity, *e.g.* from effects on oestrus cycle, sexual behaviour, any aspect of spermatogenesis or oogenesis, or hormonal activity or physiological response which would interfere with the capacity to fertilise, fertilisation itself or development of the fertilised ovum up to and including implantation.
- Induction of harmful effects on the progeny, *e.g.* any effect interfering with normal development, both before and after birth. This includes morphological malformations and functional disturbances (*e.g.* reproductive and neurological effects).

Possible effects on all aspects of reproductive physiology in both males and females, as well as possible effects on pre-natal and post-natal development, must be investigated and reported.

It is recommended to measure the active substance and/or its relevant metabolite(s) in milk if severe adverse effects are observed in offspring.

The PPR Panel recommends that this measurement should be performed as a second tier investigation where relevant effects are observed in the offspring or are expected (*e.g.* from a range-finding study). Not all severe adverse effects in the offspring should trigger measurements in the milk (*e.g.* they would not be necessary in relation to effects apparent at the time of birth such as decreased pup weight or litter size).

If in exceptional circumstances, it is claimed that such testing is unnecessary, that claim must be fully justified. While the standard reference point for treatment responses are concurrent control data, historical control data may be helpful in the interpretation of particular reproductive studies. Where submitted, historical control data should be from the same species and strain, maintained under similar conditions in the same laboratory and should be from contemporaneous studies.

The information on historical control data provided must include :

- identification of species and strain, name of the supplier, and specific colony identification, if the supplier has more than one geographical location,

- name of the laboratory and the dates when the study was performed,

- description of the general conditions under which animals were maintained, including the type or brand of diet and, where possible, the amount consumed,

- approximate age, in days, and weight of the control animals at the beginning of the study and at the time of killing or death,

- description of the control group mortality pattern observed during or at the end of the study, and other pertinent observations (e.g. diseases, infections),

- name of the laboratory and the examining scientists responsible for gathering and interpreting the pathological data from the study.

The historic control data should be presented on a study by study basis giving absolute values plus percentage, relative or transformed values where these would assist in the evaluation. If combined or summary data are submitted, these must contain information on the range of values, the mean, median and, if applicable, standard deviation.

The PPR Panel suggests that it would be better to transfer consideration of historical controls to the introduction to Section 5 (see General Remarks and Recommendations).

The PPR Panel suggests an additional paragraph here along the lines of the following: "During recent years many *in vitro* test systems have been proposed as alternatives to animal testing for developmental toxicity (Genschow *et al*., 2002). These tests usually address single events of the reproductive cycle and are therefore insufficient for the assessment of adverse *in vivo* effects and do not replace animal testing in the risk assessment of chemicals. They may, however, be useful for screening of closely related chemicals and for elucidating the modes of action underlying observed effects.

In order to provide useful information in the design and interpretation of subsequent toxicity studies, information on blood concentration of the active substance in parents and fetus/offspring should be included in range finding studies and reported.

The PPR Panel notes that measurement of blood concentrations in the fetus is resource-intensive and will not be necessary for all compounds. The Panel proposes that this should be a second tier requirement on a case by case basis.

5.6.1. Multi-generation studies

Aim of the study

The studies reported, taken together with other relevant data and information on the active substance, must be sufficient to permit the identification of effects for reproduction, following repeated exposure to the active substance, and in particular must be sufficient :

- to identify direct and indirect effects on reproduction resulting from exposure to the active substance,

- to identify any enhancement of general adverse effects (noted during short-term and chronic toxicity testing),

- to establish the NOAELs for parental toxicity, reproductive outcome and pup development.

The PPR Panel suggests rewording as "non-reproductive adverse effects occurring at lower doses than in short-term and chronic toxicity testing".

Reason: Clarification of meaning.

Circumstances in which required

A reproduction toxicity study in rats over at least two generations must always be reported.

Test guideline

– EU testing Method B.35 (Annex V Directive 67/548/EEC) - Two-generation reproduction toxicity study.

The ILSI HESI report has proposed an 'F1-extended one generation study', which might be used as a substitute for the two-generation study based on the available database and as part of a weight of evidence approach (Cooper *et al.*, 2006). If and when it has been validated and adopted in the EU/OECD, this test could be considered as an alternative to the present two-generation toxicity test. The study should cover a dosing period from pre-mating, during mating and through the lactation period and development of offspring up to at least 70 days of age. The flexible study design should include modules for the assessment of specific effects depending on the toxicological profile of the test substance – for example, clinical pathology, a functional observational battery, immunotoxicity endpoints, oestrous cyclicity and semen analysis, developmental neurotoxicity, and investigation of the second generation according to a modified developmental study based on the OECD 414 protocol (OECD, 2001).

Supplementary studies

Where necessary for a better interpretation of the effects on reproduction and as far as this information is not yet available it could be necessary to perform supplementary studies in order to provide the following information :

- the affected gender

- the possible mechanism(s)

5.6.2. Developmental toxicity studies

Aim of the study

The studies reported, taken together with other relevant data and information on the active substance, must be sufficient to permit effects on embryonic and foetal development, following repeated exposure to the active substance, to be assessed, and in particular must be sufficient:

- to identify direct and indirect effects on embryonic and foetal development resulting from exposure to the active substance,

- to identify any maternal toxicity,

- to establish the relationship between observed responses and dose in both dam and offspring,

- to establish NOAELs for maternal toxicity and pup development.

Furthermore, the tests will give additional information on enhancement of adverse effects in pregnant as opposed to non-pregnant females.

The PPR Panel suggests rewording as "... on adverse effects in pregnant as compared with ...".

Reason: Qualitative as well as quantitative differences are relevant.

Circumstances in which required

The studies must always be carried out.

Test conditions

Developmental toxicity must be determined both to rat and rabbit by the oral route.

Based upon the available data (including exposure data), it may be reasonable to omit the prenatal developmental toxicity study in the rat if: a) no reproductive or endocrine effects or toxicity to offspring are observed either in a two-generation toxicity test or in repeated dose studies in rats performed according to accepted guidelines, and b) no developmental effects are

observed in rabbits. Nor would a separate developmental test in the rat according to EU testing Method B.31 be necessary if such an investigation formed part of an extended 1-generation test.

Additional routes may be useful in operator or worker risk assessment. Malformations and variations should be reported separately and combined in such a way that all relevant changes which are observed to occur in characteristic patterns in individual fetuses or those that can be considered to represent different grades of severity of the same type of change are reported in a concise manner.

This may also apply to risk assessment for residents and bystanders.

Diagnostic criteria for malformations and variations should be given in the report. The glossary of terminology under development by the International Federation of Teratology Societies should be considered where possible.

Test guideline

EU testing Method B.31 (Annex V Directive 67/548/EEC) - Prenatal developmental toxicity study.

Where necessary for a better understanding of developmental effects or when indicated by the mode of action of the test substance and/or observations in other studies, supplementary studies or information may become necessary to provide information on the postnatal manifestation of effects such as developmental neurotoxicity or immunotoxicity.

The PPR Panel suggests adding "For example, where a substance has been shown to cause structural abnormalities of the central nervous system, or where clear signs of adverse behavioural or functional effects on the nervous system have been observed in standard adult studies, at dose levels that are relevant for risk assessment, testing for developmental neurotoxicity effects should be considered."

At the same time, the PPR Panel notes that a comparison of the NOAELs and LOAELs by toxicity endpoint in each of four related studies (developmental toxicity, multigeneration reproductive toxicity, and acute and short-term neurotoxicity studies) on cholinesterase inhibitors showed that, in general, most developmental neurotoxicity studies did not identify importantly lower NOAELs or LOAELs (FAO/WHO 2002; JMPR, 2003).

The PPR Panel notes also that developmental neurotoxicity studies, such as draft OECD 426, are designed to provide information on potential functional and morphological hazards to the nervous system in the offspring from exposure of the mother during pregnancy and lactation. They investigate changes in behaviour due to effects on the central and peripheral nervous system. However, as behaviour may be affected also by the function of other organs such as liver, kidneys and the endocrine system, toxic effects on these organs in offspring may also lead to changes in behaviour.

The PPR Panel is not aware of any adopted guidelines for studies of developmental immunotoxicity.

Finally, the PPR Panel notes that should the toxicological profile of a chemical indicate concerns about developmental neurotoxicity or immunotoxicity, appropriate testing parameters could be incorporated into an extended 1-generation test or a multigeneration reproductive toxicity study.

5.7. Neurotoxicity studies

5.7.1. Neurotoxicity studies in rodents

Aim of the study

The study shall provide sufficient data to evaluate the potential neurotoxicity of the active substance (neurobehavioural and neuropathological effects) after single and repeated exposure. It may be combined with standard repeated dose toxicity studies.

Circumstances in which required

This study must be performed for substances with structures that are similar or related to those capable of inducing neurotoxicity, and for substances which induce specific indications of potential neurotoxicity, neurological signs or neuropathological lesions in toxicity studies at dose levels not associated with marked general toxicity.

Consideration should be given to including neurotoxicity investigations in routine toxicology studies.

The PPR Panel notes the proposal of the ILSI HESI committee that where appropriate neurotoxicity endpoints are included in the standard 28-day or 90-day study in rats, this may be sufficient for risk assessment. If there are indications of neurotoxicity in the 28-day or 90-day rat study, a specific neurotoxicity study may be required to better characterise the effects.

Test guideline

EU testing Method B.43 (Annex V Directive 67/548/EEC) - Neurotoxicity study in rodents.

5.7.2. Delayed neurotoxicity studies

The PPR Panel suggests that here and elsewhere in this section, "delayed polyneuropathy" might be substituted for "delayed neurotoxicity".

Reason: The study required is aimed specifically at detecting potential to cause delayed polyneuropathy, and does not address other types of neurotoxicity.

Aim of the study

The study shall provide sufficient data to evaluate if the active substance could provoke delayed neurotoxicity after acute and repeated exposure.

The repeated exposure study can be waived unless: a) there are indications that the compound accumulates, and b) significant inhibition of neuropathy target esterase or clinical/histopathological signs of delayed polyneuropathy occur at around the hen LD_{50} as determined in the single dose test. This is similar to the approach adopted by the US Environmental Protection Agency (OPPTS 870.6100 method) (EPA, 1998). To date, for organophosphorus pesticides, the repeated dose study has never provided additional information that was critical to risk assessment (see Moretto (1999) for more details).

Circumstances in which required

These studies have to be performed for substances of similar or related structures to those capable of inducing delayed neurotoxicity such as organophosphates

The PPR Panel suggests changing "organophosphates" to "organophosphorus compounds"

Reason: Organophosphates are not the only organophoshorus compounds with potential to cause delayed polyneuropathy.

Test guidelines

EU testing Method B.37 (Annex V Directive 67/548/EEC) - Delayed neurotoxicity of organophosphorus substances after acute exposure,

EU testing Method B.38 (Annex V Directive 67/548/EEC) - Delayed neurotoxicity of organophosphorus substances: 28-day repeated dose.

5.8. Other toxicological studies

5.8.1. Toxicity studies of metabolites as referred to in the introduction point, (x)

For reasons of clarity, the PPR Panel suggests omission of the word "point".

Supplementary studies, where they relate to substances other than the active substance, are not a routine requirement. Decisions as to the need for supplementary studies must be made on a case by case basis.

Where as a result of metabolism or other processes, metabolites from plants, soil, groundwater, open air differ from those in animals or are detected in low proportions in animals, further testing may apply on a case by case basis, taking into account the amount of metabolite and the chemical structure of the metabolite compared to the parent.

5.8.2. Supplementary studies on the active substance

In certain cases it can be necessary to carry out supplementary studies to further clarify observed effects. These studies could include :

- studies on absorption, distribution, excretion and metabolism, in a second species
- studies on the immunotoxicological potential,
- studies on potential effects on the endocrine system,
- studies on other routes of administration,- studies on the carcinogenic potential.

The PPR Panel suggests that follow-up analysis of stored blood samples from repeat dose toxicity studies could be of value in clarifying possible effects on the endocrine system, and might avoid the need for additional animal studies.

Decisions as to the need for supplementary studies must be made on a case by case basis, taking into account the results of the available toxicological and metabolism studies and the most important exposure routes.

Studies required must be designed on an individual basis, in the light of the particular parameters to be investigated and the objectives to be achieved.

5.9. Medical data

Where available, and without prejudice to the provisions of Article 10 of Council Directive 98/24/EC of 7 April 1998 on the protection of workers from the risks related to chemical, physical and biological agents at work (1), practical data and information relevant to the recognition of the symptoms of poisoning, and on the effectiveness of first aid and therapeutic measures have to be submitted. More specific references to the investigation for antidote pharmacology or safety pharmacology using animals should be provided. Where relevant, the effectiveness of potential antagonists to poisoning, should be investigated and reported.

The PPR Panel suggests replacing this sentence with "This should include reports of any studies investigating antidote pharmacology or safety pharmacology".

Reason: The meaning of the sentence as currently worded is unclear. Relevant studies may not only be in animals. They might, for example, be carried out *in vitro*.

> Data and information relevant to the effects of human exposure, where available and of the necessary quality, are of particular value, in confirming the validity of extrapolations made and conclusions reached with respect to target organs, dose-response relationships, and the reversibility of adverse effects. Such data can be generated following accidental or occupational exposure.

The PPR Panel suggests expanding this sentence to read "...occupational exposure, or incidents of intentional self-poisoning, and must be reported if available."

Reason: Data on intentional poisoning may be available for older plant protection products that are under review. Any data that are available on the health effects of exposure in humans should be submitted, regardless of the notifier's opinion about their quality.

5.9.1. Medicinal surveillance on manufacturing plant personnel, monitoring studies

> Reports of occupational health surveillance programs and of monitoring studies, supported with detailed information on the design of the program, on exposure to the active substance and exposure to other chemicals, must be submitted. Such reports should, where feasible, include data relevant to the mechanism of action of the active substance. These reports shall, where available, include data from persons exposed in manufacturing plants or after application of the active substance (e.g . from monitoring studies in operators, workers, residents, bystanders or victims of accidents). Available information on adverse health effects including allergenic response of workers and others exposed to the active substance, must be provided, and include where relevant details of any incident. The information provided should include details of frequency, level and duration of exposure, symptoms observed and other relevant clinical information.

The PPR Panel suggests changing "Medicinal" to "Medical".

Reason: "Medicinal" means "related to medicines".

The PPR Panel suggests additional wording to read "... studies be submitted, supported with detailed information on the design of the programme, the number of exposed persons included in the programme, the nature of their exposure to the active substance, and their exposure to other potentially hazardous agents."

Reason: Information about the number of exposed persons included in surveillance programmes is crucial for interpretation, as is the nature of their exposure. Information is also needed on other potentially hazardous agents (physical and biological agents may be relevant as well as chemicals), but not on innocuous exposures.

The PPR Panel suggests changing "after" to "during or after".

Reason: Important exposures may occur during as well as after application. In particular, the highest exposures of operators often occur during mixing and loading.

For clarity, the PPR Panel suggests changing "response of" to "responses in".

5.9.2. Information from studies with human volunteers

> Where available, reports from studies with human volunteers, such as tests on toxicokinetics and metabolism, or tests on skin irritation or skin sensitisation, must be submitted.

> Toxicological studies conducted in humans with the purpose of determining a human No Observed Effect Level of an active substance have not been and will not be used *per se* to derive

regulatory limit values (such as an Acceptable Daily Intake, an Acceptable Operator Exposure Level or an Acute Reference Dose) for the substance. Rather, such studies if they are scientifically and ethically valid, will be evaluated and used as supplementary information to confirm the validity of regulatory limit values but will not be used to change any uncertainty factor in the evaluation of regulatory limit values. Regulatory limit values will continue to be derived from extrapolations from appropriate studies in laboratory model species.

The PPR Panel believes that this proposal is scientifically unsound and ethically dubious. The purpose of reference values such as the Acceptable Daily Intake and Acceptable Operator Exposure Level is to ensure adequate protection of human health. Normally, these reference values are set on the basis of toxicological data from animals, but this entails scientific uncertainty because of possible inter-species differences in sensitivity. To account for the uncertainty, an assessment factor (usually tenfold) is applied. However, where adequate human data are available, this source of scientific uncertainty is eliminated and a more reliable assessment of acceptable exposure can be made.

It could be argued that where setting human data aside leads to a lower reference value, any error is on the side of safety and therefore is tolerable. However, this argument ignores the potential benefits from approval of a plant protection product. Setting an unnecessarily low reference value might, for example, lead to approval being withheld, and substitution by another product with a lower margin of safety.

The disadvantages of excluding human data are even more stark where the effect is to produce an inappropriately high reference value. This will only occur if humans are exceptionally sensitive relative to the animal species tested, but is a theoretical possibility. The revision as currently drafted states that human studies, if scientifically and ethically valid, will be used as supplementary information to confirm the validity of regulatory "limit values", but it does not indicate how a reference value would be set if the reference value from animal studies were shown to be potentially unsafe.

One alternative approach, might be to allow the use of data from scientifically valid human studies in setting reference values:

a) where the study was observational rather than experimental in design, or

b) where the study investigated ADME at low levels of exposure in humans, and the results enabled the derivation of a chemical-specific adjustment factor, or

c) where the study was initiated before the proposed revisions to Annexes II and III came into force, and was ethically acceptable by the standards that applied at the time when it was conducted.

5.9.3. Direct observation, e.g.: clinical cases and poisoning incidents

Available reports from the open literature, relating to clinical cases and poisoning incidents, where they are from refereed journals or official reports, must be submitted together with reports of any follow-up studies undertaken. Such reports should contain complete descriptions of the nature, level and duration of exposure, as well as the clinical symptoms observed, first aid and therapeutic measures applied and measurements and observations made. Summary and abstract information is not of value. *If the poisoning cases involved a plant protection product, it can be useful if the signs / symptoms can be identified as related to the active substance or a co-formulant in the specific product.*

Where supported with the necessary level of detail, such documentation can be of particular value in confirming the validity of extrapolations from animal data to man and in identifying unexpected adverse effects which are specific to humans.

The PPR Panel recommends rewording as "Insofar as they are available, such reports should contain ….".

Reason: These data may not always be available in full.

5.9.4. Observations on exposure of the general population and epidemiological studies if appropriate

Where available, and supported with data on levels and duration of exposure, and conducted in accordance with recognized standards (2), epidemiological studies are of particular value and must be submitted.

The PPR Panel suggests amending this sentence to read "Relevant epidemiological studies are of particular value, and must be submitted where available."

Reason: Epidemiological studies may be of value even if they do not include data on levels and duration of exposure or fully conform to currently prescribed standards. It would be inappropriate to ignore such research, particularly if it pointed to a problem with the safety of a plant protection product.

5.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

A detailed description of the clinical signs and symptoms of poisoning, including the early signs and symptoms and full details of clinical tests useful for diagnostic purposes, where available, must be provided and include full details of the time courses involved relevant to the ingestion, dermal exposure or inhalation of varying amounts of the active substance.

The PPR Panel proposes that the words "where available" be moved to the beginning of the sentence.

Reason: At present the wording implies that this qualifier only applies to the details of clinical tests, but often a detailed description of clinical signs and symptoms will not be available.

5.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

The first aid measures to be used in the event of poisoning (actual and suspected) and in the event of contamination of eyes must be provided. Therapeutic regimes for use in the event of poisoning or contamination of eyes, including where available the use of antidotes, must be described in full. Information based on practical experience, where it exists and is available, in other cases on theoretical grounds, as to the effectiveness of alternative treatment regimes, where relevant, must be provided. Contraindications associated with particular regimes, particularly those relating to 'general medical problems' and conditions, must be described.

5.9.7. Expected effects of poisoning

Where known, the expected effects and the duration of these effects following poisoning must be described and include the impact of :

- the type, level and duration of exposure, or ingestion, and
- varying time periods between exposure, or ingestion, and commencement of treatment.

5.10. Summary of mammalian toxicity and overall evaluation

A summary of all data and information provided under paragraphs 5.1 through 5.10, must be submitted, and include a detailed and critical assessment of those data in the context of relevant evaluative and decision making criteria and guidelines, with particular reference to the risks for man and animals that may or do arise, and the extent, quality and reliability of the data base.

Where relevant, in the light of findings with respect to the analytical profile of batches of the active substance (paragraph 1.11) and any bridging studies conducted (paragraphs 5 (v)), the relevance of the data as submitted to the assessment of the toxicological profile of the active substance as manufactured, must be argued.

On the basis of an assessment of the data base, and the relevant decision making criteria and guidelines, justifications must be submitted for the NO(A)ELs proposed for each relevant study.

On the basis of these data scientifically reasoned proposals for the establishment of ADI, AOEL(s) and ARfD for the active substance must be submitted. If an ARfD is not required, an explanation must be provided, based on the toxicological profile of the compound.

ANNEX III

7. TOXICOLOGICAL STUDIES

For proper evaluation of the toxicity of preparations sufficient information should be available on acute toxicity, irritation and sensitisation of the active substance. If possible, additional information on mode of toxic action, toxicological profile and all other known toxicological aspects of the active substance should be submitted.

For reasons of clarity, the PPR Panel suggests substituting “Where available”.

In the context of the influence that impurities and other components can have on toxicological behaviour, it is essential that for each study submitted, a detailed description (specification) of the material used, be provided. Studies must be conducted using the plant protection product to be authorised.

The PPR Panel recommends that a Guidance Note setting out the format for this description might be helpful.

7.1. Acute toxicity

The studies, data and information to be provided and evaluated, must be sufficient to permit the identification of effects following a single exposure to the plant protection product, to be assessed, and in particular to establish, or indicate:

- the toxicity of the plant protection products,

- toxicity of the plant protection product relative to the active substance,

- the time course and characteristics of the effect with full details of behavioural changes and possible gross pathological findings at post-mortem,- where possible the mode of toxic action, and

- the relative hazard associated with the different routes of exposure.

While the emphasis must be on estimating the toxicity ranges involved, the information generated must also permit the plant protection product to be classified in accordance with

Council Directive 99/45/EC. Council Directive 99/45/EC can be invoked for waiving studies when the outcome of the study is highly predictable i.e. high concentration of active substance in the preparation. Before generating studies, the possibility to extrapolate results from related preparations should be taken into account. The information generated through acute toxicity testing is of particular value in assessing hazards likely to arise in accident situations.

7.1.1. Oral

Circumstances in which required

An acute oral test should always be carried out unless the applicant can justify to the satisfaction of the competent authority that Council Directive 99/45/EC can be invoked.

Test guidelines

EU testing Method B.1bis (Annex V Directive 67/548/EEC) - Acute oral toxicity - fixed dose method,

EU testing Method B.1tris (Annex V Directive 67/548/EEC) - Acute oral toxicity - Acute toxic class method,

OECD guideline 425 - Acute oral toxicity : up-and-down procedure.

In selecting a test method, the aim should be to minimise the use of laboratory animals.

7.1.2. Dermal

Circumstances in which required

An acute dermal test should always be carried out unless the applicant can justify to the satisfaction of the competent authority that Council Directive 99/45/EC can be invoked.

When relevant, skin irritation data provided in the study should be considered to using this instead of a specific irritation study.

The wording of this sentence could be amended to make its meaning clearer.

Test guideline

EU testing Method B.3 (Annex V Directive 67/548/EEC) - Acute toxicity (dermal).

7.1.3. Inhalation

Aim of the study

The study will provide the inhalation toxicity to rats of the plant protection product or of the smoke it generates.

Circumstances in which required

The study must be carried out where the plant protection product:

- is a gas or liquified gas,
- is a smoke generating formulation or fumigant,
- is used with fogging equipment,

- is a vapour releasing preparation,

- is an aerosol,

- is a powder containing a significant proportion of particles of diameter <50 µm (> 1 % on a weight basis),

- is to be applied from aircraft in cases where inhalation exposure is relevant,

- contains an active substance with a vapour pressure > 1 × 10^{-2} Pa and is to be used in enclosed spaces such as warehouses or glasshouses,

- is to be applied in a manner which generates a significant proportion of particles or droplets of diameter <50 µm (> 1 % on a weight basis) unless the applicant can justify to the satisfaction of the competent authority that Council Directive 99/45/EC can be invoked.

A fixed concentration procedure is recommended.

The head/nose only exposure should be used, whole body exposure must be justified.

Test guideline

EU testing Method B.2 (Annex V Directive 67/548/EEC) - Acute toxicity (inhalation).

The meaning of "significant proportion" needs to be clarified.

The PPR believes that a strong case could be made for setting this limit at 100 µm rather than 50 µm (CEN, 1993).

The PPR Panel suggests deleting the recommendation for a fixed concentration procedure.

Reason: There is only a draft OECD guideline for the fixed concentration procedure. The cited guideline Method B.2 is not consistent with a fixed concentration procedure.

7.1.4. Skin Irritation

Aim of the study

The results of the study will provide the potential of skin irritancy of the plant protection product including the potential reversibility of the effects observed.

This method includes the recommendation that before undertaking in vivo studies for corrosion/irritation of the substance, a weight-of-evidence analysis be performed on the existing relevant data. Where insufficient data are available, they can be developed through application of sequential testing. The testing strategy should include,

1) the use of an in vitro dermal irritation/corrosion study to predict dermal corrosion prior to consideration of an in vivo dermal study,

2) the performance of validated and accepted in vitro dermal irritation studies and

3) in addition, where appropriate, the successive, instead of simultaneous application of the three test patches to the animals in the in vivo study.

Consideration should be given to the use the dermal toxicity study to provide irritancy information..

Circumstances in which required

The skin irritancy of the plant protection product must always be reported, unless the applicant can justify to the satisfaction of the competent authority that Council Directive 99/45/EC can be invoked.

Test guidelines

EU testing Method B.4 (Annex V Directive 67/548/EEC) - Acute toxicity : dermal irritation/corrosion,

EU testing Method B.40 (Annex V Directive 67/548/EEC) - Skin corrosion.

The PPR Panel suggests that the wording should make clearer that the testing strategy should be hierarchical and tiered, starting with *in vitro* methods and proceeding to *in vivo* as necessary.

Currently a valid *in vitro* method is available only for corrosion and not for irritation.

7.1.5. Eye irritation

Aim of the study

The results of the study test will provide the potential for eye irritation of the plant protection product, including the potential reversibility of the effects observed.

This method includes the recommendation that before undertaking in vivo studies for eye corrosion/irritation of the substance, a weight-of-evidence analysis be performed on the existing relevant data. Where insufficient data are available, they can be developed through application of sequential testing. The testing strategy should include,

1) the use of an in vitro dermal irritation/corrosion study to predict eye corrosion prior to consideration of an in vivo eye study,

2) the performance of validated and accepted in vitro eye irritation studies and

3) in addition, where appropriate, the successive, instead of simultaneous application to the animals in the in vivo study.

Circumstances in which required

Eye irritation tests must always be reported, unless the applicant can justify to the satisfaction of the competent authority that Council Directive 99/45/EC can be invoked.

Test guidelines

EU testing Method B.5 (Annex V Directive 67/548/EEC) Acute toxicity: eye irritation/corrosion,

EU testing Method B.40 (Annex V Directive 67/548/EEC) - Skin corrosion.

It is unclear to what method “This method” refers.

The currently validated *in vitro* method is for dermal corrosion and not for dermal irritation.

The PPR Panel notes that there is currently no fully validated *in vitro* method for evaluating eye irritation or corrosion.

7.1.6. Skin sensitisation

Aim of the study

The study will provide sufficient information to assess the potential of the plant protection product to provoke skin sensitisation reactions.

Circumstances in which required

The studies must always be carried out unless the active substance(s) or co-formulants are known to have sensitising properties and/or the applicant can justify to the satisfaction of the competent authority that Council Directive 99/45/EC can be invoked.

The local lymph node assay should be used preferably. In case the local lymph node assay can not be conducted, the Guinea Pig Maximisation Test should be performed.

Test guidelines

EU testing Method B.42 (Annex V Directive 67/548/EEC) - Skin sensitisation : Local lymph node assay,

EU testing Method B.6 (Annex V Directive 67/548/EEC) - Skin sensitisation (Guinea Pig Maximisation Test).

The PPR Panel recommends that this be reworded as "Where the local lymph node assay cannot be conducted, the Guinea Pig Maximisation test or Buehler test should be performed." Reference can be made to both of these tests in the test guideline, B.6 (Council Directive Directive 67/548/EEC) (and also OECD 406) (OECD, 1992).

Reason: A Buehler test is an acceptable alternative.

7.1.7. Supplementary studies on the plant protection product

Aim of the study

In certain cases it may be necessary to carry out short term studies on the plant protection product to be authorised.

Decisions as to the need for supplementary additional studies on the plant protection product must be made case by case based on expert judgement. The study must be clearly justified (e.g.: for plant protection product containing active substances suspected to have synergistic or additive toxicological effects).

The type of the study should be adapted to the endpoint of concern.

The PPR Panel suggests deleting "short term" since other types of study might also be relevant. Additional studies on ADME may be appropriate, for example, to determine effects of co-formulants on absorption. These should be on a case by case basis, depending on the properties of the co-formulants and any toxicological differences from the active ingredient.

The PPR Panel recommends that where a product contains more than one active substance, the notifier should justify why additional studies on the product to assess combined toxicity are not necessary.

7.1.8. Supplementary studies for combinations of plant protection products

Aim of the study

In certain cases it may be necessary to carry out studies for a combination of plant protection products where the product label includes requirements for use of the plant protection product with other plant protection products and/or with adjuvants as a tank mix. Decisions as to the need for supplementary studies must be made on a case by case basis, taking into account the results of the acute toxicity studies of the individual plant protection products and the toxicological properties of the active substance(s), the possibility for exposure to the combination of the products concerned and available information or practical experience with the products concerned or similar products.

7.2 Data on exposure

The PPR Panel suggests including a short introduction along the following lines:

"Non-dietary exposure to plant protection products can occur in several groups of people:

- operators who apply plant protection products (who may be professionals or amateurs),
- workers who occupationally handle treated commodities (*e.g.* picking or packing crops) and/or work in a treated area at the time of or after application,
- bystanders who casually are in the vicinity at the time of or after application of plant protection products, but not for the purpose of working on the treated area or with the treated commodity, and
- residents who live or work long-term near to areas that are treated with plant protection products."

Reason: The different groups of people involved are not self-evident.

The PPR Panel also suggests that this section should be re-ordered, focusing on operators in 7.2.1, workers in 7.2.2 and bystanders and residents in 7.2.3.

7.2.1. Operator[3] exposure

The risks for those using plant protection products depend on the physical, chemical and toxicological properties of the plant protection product as well as the type of the product (undiluted/diluted), on the use conditions, and on the route, the degree and duration of exposure.

The PPR Panel suggests rewording as: "...and on the route, degree, duration and frequency of exposure."

Reason: The frequency of exposure is also an important determinant of risk.

Sufficient information and data must be generated and reported to permit an assessment of the extent of exposure to the active substance(s) and/or toxicologically relevant compounds in the plant protection product likely to occur under the proposed conditions of use. It must also provide a basis for the selection of the appropriate protective measures including personal protective equipment to be used by operators and to be specified on the label.

The PPR panel suggests adding the following sentence on amateurs:

[3] The operator is the person who is actively involved in the handling and/or application of a plant protection product.

"....on the label. For amateur operators no personal protective equipment should be assumed in the risk assessment."

Reason: Amateur operators are generally untrained, and in practice cannot be relied upon to make appropriate use of personal protective equipment.

7.2.1.1. Estimation of operator exposure

Aim of the estimation

An estimation shall be made, using where available a suitable calculation model, in order to permit an evaluation of the operator exposure likely to arise under the proposed conditions of use.

The PPR Panel notes that the predictive exposure models that are used currently in risk assessment for Annex I inclusion (UK-POEM and BBA model) were never critically assessed at EU level. Nor was EUROPOEM. More recently, however, a further approach, the Agricultural Handlers Exposure Database (AHED), has been developed. The Panel strongly recommends that suitable methodology and guidance be developed to assess worker exposure for use in EU and national risk assessments.

The PPR Panel also notes that assessment of exposure in amateur operators generally requires different models from those used for professional operators.

Circumstances in which required

An estimation of operator exposure must always be completed.

Estimation conditions

An estimation shall be made for each type of application method and application equipment proposed for use of the plant protection product taking account of the requirements resulting from the implementation of the classification and labelling provisions of Directive 99/45/EC for handling the undiluted or diluted product.

The estimation must address mixing/loading and application and should include clean-up activities and routine maintenance of the application equipment. Specific information on local use conditions (types and sizes of containers to be used, typical work rates, field sizes, crop growing climatic conditions) should be included.

The PPR Panel suggests this sentence be adapted to read:

"...(types and sizes of containers to be used, application equipment, typical work rates and application rates, field sizes, crop growing climatic conditions)...".

Reason: Application rate can also be an important determinant of exposure.

At first an estimation shall be made with the assumption that the operator is not using any personal protective equipment.

The PPR Panel suggests deleting this sentence.

Reason: In certain use scenarios, some plant protection products cannot or should not be used without personal protective equipment. It is of no value to estimate exposure for an irrelevant scenario. Furthermore, for some scenarios, experimental data may only be available for application with the use of personal protective equipment.

Where appropriate, a second estimation shall be made with the assumption that the operator is using effective and readily obtainable protective equipment which is feasible to be used by the

operator. Where protective measures are specified on the label, the estimation will take these into account.

The PPR Panel suggests rewording as: "Where appropriate, an estimation shall ..... which could reasonably be used in practice."

Reason: Improved clarity.

The PPR Panel suggests deleting the last sentence, which will be redundant if its earlier proposal is accepted.

7.2.1.2. Measurement of operator exposure

Aim of the study

The study shall provide sufficient data to permit an evaluation of the operator exposure likely to arise under the specific proposed conditions of use. The study should be performed in a way that guarantees the complete safety of the operator.

The PPR Panel suggests changing the last sentence to read:

"The study should be ethically sound. In particular, it will need to be approved by an appropriate medical-ethical review board."

Reason: Complete safety can never be guaranteed.

Circumstances in which required

Actual exposure data for the relevant exposure route(s) must be reported where the model- based risk assessment indicates that AOEL is exceeded or where there are no representative data in available calculation models.

The PPR Panel suggests replacing the word "actual" with "experimental (field)".

Reason: The word "actual" in this context may be misunderstood since it could be interpreted as referring to exposure of the operator, taking account of personal protective equipment (as opposed to "potential exposure" meaning in the absence of personal protective equipment).

This will, for example, be the case when the results of the estimation of operator exposure provided for under point 7.2.1.1 indicate that:

- the Acceptable Operator Exposure Level(s) (AOEL) established in the context of inclusion of the active substance(s) in Annex I, and/or

- the Limit Values established for the active substance and/or toxicologically relevant compound(s) of the plant protection product in accordance with Council Directive, 91/322/EEC, 96/94/EC, 98/24/EC, 2000/39/EC and Council Directive 90/394/EEC, 97/42/EC on the protection of workers from the risks related to exposure to carcinogens at work, may be exceeded.

Actual exposure data must also be reported when no appropriate calculation model or no appropriate data are available to do the estimation provided for under point 7.2.1.1.

Again, the PPR Panel recommends replacing the word "actual" with "experimental (field)".

Study conditions

The study must be done under realistic exposure conditions taking into account the proposed conditions of use and in accordance with the current OECD guidance document for the conduct of studies of occupational exposure to pesticides during agricultural application.

The PPR Panel suggests including the full title of the OECD Guidance Document:

Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides During Agricultural Application, OECD Series on Testing and Assessment No. 9, OCDE/GD(97) 148. (OECD, 1997)

Reason: Clarity.

Overall, the PPR Panel believes that there is a pressing need for an expert group to assess critically new predictive modelling approaches and develop further guidance on operator exposure assessment.

7.2.2. Bystander[4] and resident exposure

The PPR Panel suggests that a short introduction is needed:

"Bystanders and residents are people who are not involved in the application or handling of plant protection products and/or professionally handling treated commodities. Bystanders are persons who casually are located within or directly adjacent to an area where application of a plant protection product is in process or has taken place. Residents are people who live or work long-term near to areas that are treated with plant protection products."

Reason: The term, resident, is relatively new and the methods for assessing exposure to bystanders and residents are not yet well developed.

7.2.2.1 Bystander exposure

Bystanders can be exposed during the application of plant protection products. Sufficient information and data must be reported to provide a basis for the selection of appropriate conditions of use, including the exclusion of bystanders from treatment areas and separation distances.

The PPR Panel suggests including the words 'or after' in the above sentence "Bystanders can be exposed during or after the application….".

Reason: Bystander exposure may also occur after application, *e.g.* for soil fumigants.

Aim of the estimation

An estimation shall be made, using where available a suitable calculation model in order to permit an evaluation of the bystander exposure likely to arise under the proposed conditions of use.

Circumstances in which required

An estimation of bystander exposure must always be completed.

The PPR Panel suggests changing the above sentence to read:

"An estimation of bystander exposure must be completed when there is reason to expect that the exposure could be higher, or carry a higher risk, than that of residents."

Reason: Residential exposure will generally be higher and more frequent than bystander exposure, in which case there is no reason to estimate bystander exposure, unless it will be compared with a different reference value from that used for residents.

[4] The bystander is a person who is not actively involved in the application of a plant protection product but might be incidentally exposed during the application.

Estimation conditions

An estimation of bystander exposure must be made for each type of application method. The estimation shall be made with the assumption that bystanders do not use any personal protective equipment. Measurement of bystander exposure may be required when estimates indicate a cause for concern or there are no representative data available in calculation models.

The PPR Panel suggests rewording the first sentence as:

"When an estimation of bystander exposure is needed, it must be made for each relevant type of application method."

Reason: Separate estimation of bystander exposure may not always be needed.

Overall, the PPR Panel believes that there is a pressing need for an expert group to assess critically new predictive modelling approaches and develop further guidance on the assessment of bystander and resident exposure.

7.2.2.2 Resident exposure

Residents living in agricultural areas may be indirectly exposed to active(s) substance(s) mainly by inhalation and dermal route.

The PPR Panel suggests changing this sentence as follows:

"Residents living and/or working near to areas that are treated with plant protection products may be directly and indirectly exposed…"

Reason: Residents may not only live, but also work in the vicinity of areas treated with plant protection products, and these are not always agricultural areas (*e.g.* plant protection products may be applied to sports fields in urban locations). Residents may also be exposed directly, in the same way as bystanders.

The PPR Panel notes that for infants and toddlers appreciable exposure might also occur by the oral route (*e.g.* through hand-mouth transfer).

Sufficient information and data must be generated and reported to permit an assessment of the extent of exposure to the active substance(s) and/or toxicologically relevant compounds likely to occur under the proposed conditions of use and to provide a basis for the selection of appropriate conditions of use, including the exclusion of treatment areas and separation distances.

Aim of the estimation

An estimation shall be made, using where available a suitable calculation model in order to permit an evaluation of the residents exposure likely to arise under the proposed conditions of use.

Circumstances in which required

An estimation of resident exposure must always be completed.

Estimation conditions

An estimation of resident exposure must be made for each type of application method. The estimation shall be made with the assumption that residents do not use any personal protective equipment. Measurement of resident exposure may be required when estimates indicate a cause for concern.

Where appropriate, this evaluation should address separate subgroups of the residential populations such as infants and toddlers.

Overall, the PPR Panel believes that there is a pressing need for an expert group to assess critically new predictive modelling approaches and develop further guidance on the assessment of bystander and resident exposure.

7.2.3. Worker[5] exposure

Workers can be exposed following application of plant protection products, when entering treated fields or premises or handling treated plants or plant products on which residues remain. Sufficient information and data (e.g.: description of post-applications activities, exposure duration) must be reported to provide a basis for the selection of appropriate protective measures, including waiting and re-entry periods.

The first sentence will become redundant if the earlier suggestions of the PPR Panel are taken on board.

7.2.3.1. Estimation of worker exposure

Aim of the estimation

An estimation shall be made using where available a suitable calculation model, in order to permit an evaluation of the worker exposure likely to arise under the proposed conditions of use, including systemic and local effects (cf. 7.1.4 to 7.1.6).

The PPR Panel notes that the last part of the above sentence, "including systemic and local effects (cf. 7.1.4 to 7.1.6)" does not apply exclusively to workers. It should either be included for all the groups of people considered in Section 7.2 or deleted. Moreover, if it is retained, the wording "that would be relevant to systemic …" should be substituted for "including systemic …".

Circumstances in which required

The estimation of worker exposure must always be completed when it is likely to arise under the proposed conditions of use.

The PPR Panel suggests rewording as: "….. when such exposure could arise ….."

Reason: There is a danger that the original wording could be misinterpreted as implying that no estimation of worker exposure would be required if such exposure were rare.

Estimation conditions

An estimation of worker exposure must be made for crops and tasks to be carried out.

At first the estimation shall be made using available data on the exposure to be expected with the assumption that the worker is not using any personal protective equipment. Where appropriate, a second estimation shall be made with the assumption that the worker is using effective and readily obtainable protective equipment which is feasible to be used. Where appropriate, a further estimation shall be made using data generated on the amount of dislodgeable residues under the proposed conditions of use.

[5] The worker is a person who is actively working in crops or with commodities that have been previously treated with a plant protection product.

The PPR Panel believes that a risk assessment assuming that workers use protective equipment would only be appropriate where the use of such equipment was already standard for the occupational tasks that might give rise to exposure, or in very specific circumstances to be judged on a case by case basis.

The PPR Panel considers that there is a pressing need to develop an improved calculation model for worker exposure, based on developments in EUROPOEM II and parallel work in the USA. Currently, there is no consensus approach and/or guidance available.

7.2.3.2. Measurement of worker exposure

Aim of the study

The study shall provide sufficient data to permit an evaluation of the worker exposure likely to arise under the proposed conditions of use. The study should be performed in a way that guarantees the complete safety of the worker.

The PPR Panel suggests changing the last sentence in the following way:

"The study should be ethically sound. In particular, it should be approved by an appropriate medical-ethical review board."

Reason: Complete safety can never be guaranteed.

Circumstances in which required

Actual exposure data for the relevant exposure route(s) must be reported where the model- based risk assessment indicates that AOEL value is exceeded or where there are not representative data in available calculation models. This will, for example, be the case where the results of the estimation of worker exposure provided for under point 7.2.3.1 indicate that:

- the AOEL(s) established in the context of inclusion of the active substance(s) in Annex I,

and/or

- the Limit Values established for the active substance and/or toxicologically relevant compound(s) of the plant protection product in accordance with Council Directives 91/322/EEC, 96/94/EC, 98/24/EC, 2000/39/EC and 90/394/EEC, 97/42/EC may be exceeded.

Actual exposure data must also be reported when no appropriate calculation model or no appropriate data are available to do the estimation provided for under point 7.2.3.1.

The PPR Panel suggests replacing the word "actual" by "experimental (field)".

Reason: The word "actual" in this context may be misunderstood since it could be interpreted as referring to exposure of the worker, taking account of personal protective equipment (as opposed to "potential exposure" meaning in the absence of personal protective equipment).

Study conditions

The study must be done under realistic exposure conditions taking into account the proposed conditions of use and in accordance with the OECD current guidance document for the conduct of studies of occupational exposure to pesticides during agricultural application.

The PPR Panel recommends including full title of the OECD Guidance Document:

Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides During Agricultural Application, OECD Series on Testing and Assessment No. 9, OCDE/GD(97)148. (OECD, 1997).

Reason: Clarity - although the Guidance Document focuses on operators and not on workers, many elements are relevant to workers.

Overall, the PPR Panel believes that there is a pressing need for an expert group to assess critically new predictive modelling approaches and develop further guidance on the assessment of worker exposure.

7.3. Dermal absorption

Aim of the study

The studies shall provide a measurement of the absorption through the skin of the active substance(s) and/or toxicologically relevant substances in the plant protection product to be authorised.

The PPR Panel recommends changing the wording of this sentence as follows: "The studies shall provide a measurement of absorption through the skin of the active substance(s) and/or toxicologically relevant substances in the plant protection product to be authorised, both from the plant protection product in its concentrated form, and from in-use dilutions.

Reason: Absorption from in-use dilutions may differ from that from the concentrated product, and this difference needs to be taken into account in risk assessment.

Some guidance should be provided as to what is meant by "toxicologically relevant" substances.

Study conditions

In principle data of in vitro human /rat dermal absorption study must be reported.

When the results of the systemic operator exposure estimation using these in vitro dermal absorption data are incorporated into the risk assessment, and indicate that AOEL value is exceeded, it may be necessary to perform an in vivo dermal absorption study on rats.

The PPR Panel suggests modifying the text as follows:

"Data from absorption studies, preferably using human skin *in vitro*, must be reported. Results should include quantification of the material in the skin as well as receptor fluid. Results should be expressed as % absorbed and maximum flux.

If estimates of human exposure using the human *in vitro* dermal absorption data indicate exceedance of a relevant reference value, it may be necessary to perform an *in vitro* dermal absorption study with rat skin and an *in vivo* dermal absorption study on rats. A refined estimate of human absorption *in vivo* would then be calculated as: "*in vivo* rat dermal absorption" X "*in vitro* human dermal absorption"/"*in vitro* rat dermal absorption."

In case studies do not correspond with the anticipated exposure situation, e.g. with regard to the type of formulant or the concentration, scientific argumentation should be provided before such data can be used with confidence.

For reasons of clarity, the PPR Panel recommends using "argument" instead of "argumentation".

Test guideline

OECD guideline 428 - Skin absorption: in vitro method,

OECD guideline 427 - Skin absorption: in vivo method.

The PPR Panel proposes adding the following Guidance Document:

SANCO/222/2000 rev.7 Guidance Document on Dermal Absorption (EC, 2000).

Consideration could also be given to mention of EHC 235 Dermal Absorption (IPCS, 2006).

Furthermore, the Panel notes that work is currently in progress to develop harmonised OECD guidance on dermal absorption.

7.4. Available toxicological data relating to non-active substances

A copy of the notification summary dossier, when relevant, and the safety data sheet submitted in the context of Directive 67/548/EEC and Commission Directive 91/155/EEC of 5 March 1991 defining and laying down the detailed arrangements for the system of specific information relating to dangerous preparations in implementation of Article 16 and 18 of Council Directive 99/45/EC (1) must be submitted for each formulant. All other available information should be submitted.

For formulants used in plant protection product which display carcinogenic or mutagenic properties or effects on the reproduction parameters and classified for such effects according to the Directive 67/548/EEC and/or Directive 99/45/EC, operator and worker risk assessment must be performed.

(1) OJ No L 200, 30. 7. 1999, p. 2-3.

The PPR Panel suggests that in these circumstances, a risk assessment for bystanders/residents should also be performed.

CONCLUSIONS AND RECOMMENDATIONS

The Scientific Panel on Plant Protection Products and their Residues concludes that the proposed revisions to Annexes II and III of Council Directive 91/414/EEC relating to toxicology and metabolism studies are generally appropriate in the context of current scientific understanding. However, the Panel believes that there are a number of ways in which they could be improved or usefully augmented.

The Panel makes the following main recommendations:

- It should always be open to the notifier to submit a reasoned scientific argument as to why a particular study is not needed. Guidance on modifications to the standard data requirements may be helpful for some categories of plant protection product (*e.g.* pheromone mating disruptors).
- As a general rule, the PPR Panel believes that the data requirements should specify the use of validated test protocols (accepted either by the EU or the OECD) where they are available and fit for purpose. Where no validated method is currently available, notifiers should justify their choice of non-standard tests, and provide details of their performance. Furthermore, should a suitable validated test be developed in the future, this should then be used in preference to non-standard tests.
- To allow for the possibility that, in the future, benchmark doses may come to be viewed as a preferable reference point to No Observed Adverse Effect Levels (NOAELs), it may be better to refer in the data requirements to "reference points" rather than specifically to NOAELs.
- Data requirements that are out of line with those of other regulatory agencies could in some circumstances lead to undesirable duplication of effort, with unnecessary use of animals. OECD protocols should therefore be referenced in the data requirements where they would be acceptable as alternatives to EU protocols. Furthermore, data requirements should be drafted in a way that facilitates harmonisation with those of

other regulatory authorities as scientific understanding evolves, for example, by allowing, where appropriate, several alternative methods to address a particular aspect of the risk assessment.

- The use of a tiered approach to toxicity testing is already embraced in some sections of the draft data requirements, and the PPR Panel believes that this strategy could be developed further in the future. In Section 1.3.2, the Panel identifies several other circumstances in which it believes that a tiered approach to data generation would be appropriate.
- The PPR Panel recommends better integration of data on disposition and kinetics when interpreting the relevance of animal tests to toxicity in humans. In Section 1.3.2, the Panel discusses how this might be approached.
- In general, the PPR Panel believes that there should be a drive towards use of fewer but more informative studies in toxicological risk assessment for plant protection products.
- The value of several individual tests that currently form part of the standard data package for plant protection products – in particular, the 1-year dog study and the mouse carcinogenicity study – is questionable. The PPR Panel suggests that the need for these studies should be reviewed.
- The draft data requirements incorporate separate exposure assessments for residents and bystanders. However, any exposure experienced by a bystander could also occur in a resident (since residents can be bystanders on the day of spraying). Thus, separate exposure assessments should only be necessary if the estimated exposures will be assessed against different reference values.
- The PPR Panel believes there is a need for further scientific development in the assessment of potential exposures to operators, workers and bystanders/residents. To this end, the Panel proposes that an expert group be established to assess critically new predictive modelling approaches, and develop further guidance on the assessment of operator, worker and bystander/resident exposures.
- The PPR Panel notes with concern the proposal in the draft data requirements that toxicological studies in humans should never be used to derive regulatory reference values. The Panel believes that this approach is both scientifically unsound and ethically dubious.
- The PPR Panel recommends that where they are available, historical control data should be provided routinely in relation to studies of carcinogenicity, developmental toxicity and *in vivo* genotoxicity, and also for measurements of blood chemistry, haematological parameters and urinalysis in other studies. The data submitted should only be for endpoints that could represent critical adverse effects, and should be strain-specific and from the same laboratory as that which carried out the index study. They should come from a five-year period, centred as closely as possible on the date of the index study.
- The PPR Panel considers that the assessment of findings from toxicological studies will be facilitated if, where possible, results are presented in a standardised format. To this end, the Panel suggests that guidelines be produced setting out templates for the reporting of results.
- One area of development in regulatory toxicology, which the PPR Panel believes is not adequately reflected in the draft proposals is the consideration of combined toxicity, in particular where two or more active substances are co-formulated in the same product. The Panel suggests that in these circumstances, there should be a routine requirement

for the notifier to provide adequate reassurance that adverse health effects will not occur as a consequence of combined action or interaction. In most cases, this should be possible through reasoned argument, but occasionally it might be necessary to generate additional data.

- The PPR Panel notes that the draft data requirements in several places use terms such as "relevant" and "appropriate" without making clear exactly what is implied. Some guidance on this, with examples, might be helpful.
- The PPR Panel anticipates that before the revised Annexes are finalised, their wording will be amended in some places to improve the clarity of the English. When such revisions are made, it will be important to check that the intended scientific meaning has not been altered or obscured.
- It is important to ensure that the data requirements in each individual area of risk assessment for plant protection products are consistent with those for the other areas. Therefore, there needs to be an holistic check of all six proposed revisions to the data requirements before they are finalised.

In addition to these main recommendations, the PPR Panel makes specific comments and recommendations on various sections of the draft data requirements (see Section 1.3.2).

DOCUMENTATION PROVIDED TO EFSA

1. Letter, dated 03 August 2006, with ref. SANCO/E3/FA/bp (2006) D/530804] from P. Testiri Coggi from the Health & Consumer Protection Directorate-General to EFSA.
2. EC (1994). Commission Directive 94/79/EC of 21 December 1994 amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market, E.C.O.J. L n° 354, 31.12.1994, p. 16–31.
3. Working Document of the Services of the European Commission 2006. Draft data requirements. Revision of Annex II and III to Directive 91/414/EEC – Toxicology SANCO/10482/2006 rev.10, 2 March 2006, 42p.
4. Comments from Member States and PETA to the draft Working Document rev. 10, and comments from ECPA to the draft Working Document rev 8, including remarks from the Commission's services to those comments from ECPA.

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APPENDIX

Glossary Document