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Review

Guidance on setting of acute reference dose (ARfD) for pesticides ☆

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Abstract

This paper summarises and extends the work developed over the last decade by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) for acute health risk assessment of agricultural pesticides. The general considerations in setting of acute reference doses (ARfDs) in a step-wise process, as well as specific considerations and guidance regarding selected toxicological endpoints are described in detail. The endpoints selected are based on the practical experience with agricultural pesticides by the JMPR and are not a comprehensive listing of all possible relevant endpoints. Haematotoxicity, immunotoxicity, neurotoxicity, liver and kidney toxicity, endocrine effects as well as developmental effects are taken into account as acute toxic alerts, relevant for the consideration of ARfDs for pesticides. The general biological background and the data available through standard toxicological testing for regulatory purposes, interpretation of the data, conclusions and recommendations for future improvements are described for each relevant endpoint. The paper also considers a single dose study protocol. This type of study is not intended to be included in routine toxicological testing for regulatory purposes, but rather to guide further testing when the current database indicates the necessity for an ARfD but does not allow a reliable derivation of the value.

Keywords: Acute reference dose; Acute toxicity; Agricultural pesticides; Food contaminants; Food safety; Risk assessment

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Abbreviations: ADI, Acceptable daily intake; ARfD, Acute reference dose; AUC, Area-under-the-curve; bw, Body weight; CSAF, Chemical-specific adjustment factor; EHC, Environmental Health Criteria; FAO, Food and Agriculture Organisation of the United Nations; IPCS, International Programme on Chemical Safety; JMPR, Joint Meeting on Pesticide Residues; LOAEL, Low-Observed-Adverse-Effect-Level; MRL, Maximum residue level; MetHb, Methaemoglobin; NOAEL, No-Observed-Adverse-Effect-Level; OECD, Organisation for Economic Co-operation and Development; C_{max}, Peak concentrations; RBC, Red blood cell; WHO, World Health Organisation.

☆ The opinions in this paper represent the opinion of the authors and not of their respective agencies or employers.

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1. Introduction

1.1. The acute reference dose (ARfD) concept for pesticides

In terms of World Health Organisation/Food and Agriculture Organisation of the United Nations (WHO/FAO) programs, one of the first references to the need to consider the acute effects of pesticide residue intake appears in Environmental Health Criteria document (EHC) 104, "consideration should be given to the potentially acute toxic effects that are not normally considered in the assessment of the ADI" (acceptable daily intake) (IPCS, 1990). Relatively shortly thereafter, the Codex Alimentarius Commission requested the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) to address this issue. Although the JMPR had discussions in 1993 regarding establishing ADIs based on short-term exposures to acutely toxic pesticides through food, the term ARfD was not coined until 1994. Since that time there has been a progressive increase in the establishment of ARfDs for particular pesticides.

A first definition of the ARfD was published in the report of the 1998 JMPR meeting (JMPR, 1999a). In 2002, the JMPR recognised that consumption databases are available for daily intakes but generally cannot be further divided into individual meals. Thus the original definition of the ARfD was re-worded from "over a short period of time, usually during one meal or one day" to the following definition of the ARfD:

"The ARfD of a chemical is an estimate of the amount a substance in food and/or drinking water, normally expressed on a body weight basis, that can be ingested in a period of 24 h or less without appreciable health risk to the consumer on the basis of all known facts at the time of the evaluation" (JMPR, 2002).

Because the consumption data are only available for a 24-hour period this will provide a conservative approach for rapidly reversible effects (e.g., carbamate cholinesterase inhibitors) where the ARfD is applicable to a shorter time period.

1.2. Development of JMPR guidance on setting ARfDs

In 1998 the JMPR Toxicology Panel published some brief guidance on procedures for setting ARfDs (JMPR, 1999a). At the 2001 meeting the JMPR established an international Working Group to compile a table of all available ARfDs and to collate information from different national agencies about their approaches to setting ARfDs (JMPR, 2001b). Based on an analysis of this inventory and a comparison of the technical policy approaches of different countries to setting ARfDs, further guidance was drafted by a Working Group and published by the 2002 meeting of the JMPR (JMPR, 2002).

At the direction of the 2003 meeting of the JMPR, the Working Group elaborated further guidance, including detailed advice on interpretation of some specific toxicology endpoints which may be particularly relevant to acute exposure to pesticides; and recommendations about a protocol for an appropriate single dose toxicity study, with guidance on (1) data collection for acute effects on target organs/systems; (2) how to design and perform appropriate single dose studies; and (3) sample collection (e.g., blood, urine) at early time points in repeat dose studies. This work built on the guidance prepared and finalised at the 2002 JMPR. As far as was appropriate, it also took into account other work and previous reports on ARfD setting published by various national or regional agencies.

1.3. Derivation of short-term threshold values for other chemicals

The need to set an ARfD should be considered for chemicals other than agricultural pesticides to which the population may be exposed, such as non-agricultural pesticides, veterinary drugs as well as food and drinking water contaminants such as certain mycotoxins. Based on the general considerations in this paper, further guidance on short-term reference values should be developed. The guidance provided in this document for agricultural pesticides should be of value in the general considerations of the necessity of establishing an ARfD, as well as in the specific endpoint considerations in the derivation of an ARfD. It is hoped that when establishing acute health guidance values for other compounds in food and drinking water, a harmonised approach is followed.

2. Inventory of ARfDs established by different agencies

An analysis of the ARfDs set by several regulatory bodies (1995–2002) was performed in 2002. The purpose of this inventory was to compare the ARfDs established

for selected pesticides by different regulatory bodies, i.e., in Australia, in Germany, in the European Union, in the Netherlands, in the United Kingdom, in the United States and by the JMPR in order to identify any inconsistencies in the practice of derivations of the threshold and to identify areas where more detailed guidance is required for further harmonisation of acute risk assessments. The inventory, which included entries up to September 2002, included 1050 entries for 494 active substances. For 387 pesticides, an ARfD-value was established, while for the other 107 pesticides it was concluded that an ARfD was unnecessary.

There were large differences (up to 2500-fold) in the ARfD values set for some individual pesticides. Inconsistencies were mostly due to the fact that ARfDs were based on different No-Observed-Adverse-Effect-Levels (NOAELs)/Low-Observed-Adverse-Effect-Levels (LOAELs) from different studies with different species and selection of different endpoints in the same study. With a lower priority, different safety factors based on animal/human studies or on severity of lesions were responsible for these inconsistencies. Only in few cases, different judgements as to whether an ARfD was unnecessary were the reasons for deriving different ARfD between some agencies. The following gives a short description of main areas of inconsistencies identified in this comparison.

A clear and consistent basis for the decision not to establish an ARfD was identified as being necessary to harmonise the practice of ARfD setting. The criteria for not establishing an ARfD differed between the regulatory bodies. Depending upon the agency, the conclusion that an ARfD is unnecessary varied between 14% and 54% of the substances for which ARfD establishment was considered by an agency. One of the main reasons was that different cut-off values for not setting an ARfD were applied.

It was also apparent, that the current database of toxicological studies is not optimal for the derivation of the ARfD. More specific information on the acute toxicity other than lethality is often needed for setting an adequate ARfD. The development of an acute study design that produces more comprehensive toxicological and toxicokinetic data for setting ARfDs was considered to be of high value.

Of the 533 ARfD values from the 387 pesticides, for which an ARfD value was established on animal studies, only 23% of the ARfD values were based on single dose studies. These single dose studies included a high percentage of acute neurotoxicity studies in rats. Another, 39% of these ARfD values were based on maternal and/or developmental effects in developmental toxicity studies in rats or rabbits. Only a low percentage of the ARfD values summarised in the inventory were actually based on observed acute findings in these repeat dose toxicity studies, since in the majority of the subchronic and chronic studies no such acute findings were reported.

The toxicological data sets used to derive ARfDs were not always relevant to a single day's exposure and where this was the case, a conservative value was derived to make the best use of the available data and avoid further animal testing. In some cases, if acute effects were predicted but there were no good acute data, the ADI value was chosen as a conservative surrogate.

Dosing technique can play a crucial role in the derivation of a NOAEL. Where gavage dosing was used, a lower no-effect level was often observed compared to dosing by incorporation of test material into the diet.

More guidance was considered necessary on ethical and scientific considerations for the use of human data. The policy on the use of human studies for ARfD setting differed between the regulatory agencies considered in this analysis. The number of substances for which the ARfD was based on a human study was comparable (8–13 pesticides, mostly organophosphates) in all regulatory bodies, with the exception of the Netherlands and US-Environmental Protection Agency, which had at the time of the inventory only one ARfD based on a human study.

More guidance was also considered necessary on the use of safety factors as well as the introduction of the concept for chemical-specific adjustment factors (IPCS, 2001c) so that they are applicable in a consistent manner. The majority of ARfDs were derived using default safety factors, in analogy to the derivation of ADI values. Of the 473 ARfD values based on animal studies, 404 were derived using the default 100-fold safety factor, while the use of a higher or lower safety factor occurred for 63 and 6 ARfDs respectively. Of the 52 ARfDs based on human studies, 48 used the default safety factor of 10, two used higher values (50- and 100-fold) and two used lower values (5- and 7-fold).

More guidance was also considered necessary for establishing more than one ARfD (i.e., which subpopulations could be protected by different values, based on whether exposure data for these subpopulations were available). For most pesticides, the different regulatory bodies established only one ARfD. The percentage of pesticides which had two separate ARfD values was between 0% and 4.2% for most regulatory agencies. However, one of the agencies established two separate ARfDs for 36% of the pesticides which were considered for ARfD derivation viz. one for the general population and one for females of child-bearing age.

3. General guidance on the derivation of ARfD

3.1. General considerations in setting an ARfD

The decision to set an ARfD should be based on toxicological grounds because an ARfD is a toxicological limit value. In considering whether an ARfD is necessary for agricultural pesticides, it is not advisable to take into

account current agricultural practice and related residues for existing crop use because, with different application rates or new applications on other crops (or other crop groups), higher residue values and/or higher dietary intakes may arise. The decision as to whether an ARfD is necessary should be based on the hazard profile of a pesticide, as well on specific endpoints which may be particularly relevant to acute effects.

Most of the scientific concepts applying to the setting of acceptable daily intakes (ADI, as guidance value for chronic toxicity), apply equally to the setting of ARfDs (e.g., consideration of the scientific quality of studies). The repeatability of effects and the appropriateness of dosing regimen are basic elements in the consideration of the overall toxicological database, including toxicokinetic and mode of action information, when determining the relevance of an endpoint for setting an ARfD. When assessing the need for an ARfD, the entire database should be reviewed with a weight-of-evidence approach used to determine whether adverse effects seen in repeat dose toxicity studies might be relevant to single exposures.

Before results from short-term repeat dose studies can be used to set an ARfD, clarification of the following questions is needed:

- Which endpoints indicative of toxicity on target organs in standard short-term studies can be used for the setting of an ARfD?
- Which effects on these endpoints could possibly occur after a single dose exposure?

In performing an acute risk assessment of a pesticide, the most appropriate studies and safety factors have to be used to derive one, or in exceptional cases two, acute reference values. In some cases it may be necessary to set an additional ARfD for main metabolites, if they occur on crops and are therefore included in the residue definition (e.g., if they are likely to show a different acute toxicity profile or when they are not observed in animal metabolism studies).

In establishing 'threshold' levels, the application of Benchmark Dose approaches could be considered wherever possible, in order to make best use of available dose–response data (Slob et al., 2005; IPCS, in preparation). The JMPR will consider developing further guidance on the application of these approaches as more practical experience becomes available.

The upper limit for a relevant ARfD (i.e., cut-off value for setting an ARfD on practical grounds) was considered with reference to the potential range of dietary exposures to acutely toxic pesticides. A rough estimate of such exposures could be produced, assuming:

- A 50 kg person consuming 500 g of fruit in a single sitting. The fruit consists of a single large item (e.g., small melon) and has been treated with a pesticide having a maximum residue level (MRL) of, for example, 20 mg/kg. Trial data show a variability factor[1] of 5 is applicable.
- The estimated maximum exposure could be [20 mg/kg (MRL) × 5 (variability) × 0.5 kg (mass)]/50 kg bw = 1 mg/kg bw. Another estimate using grapes confirmed the order of the estimate.

However, further issues need consideration when deciding on a practical cut-off value for ARfDs:

- A small number of pesticide commodity combinations have MRLs in excess of 20 mg/kg, though they might not have a toxicity profile.
- Infants and small children might have a higher rate of consumption relative to body weight.
- For certain commodities, a variability factor greater than 5 might be applicable.

This estimate indicates that any cut-off for ARfDs should be at a value greater than 1 mg/kg bw. A value of 5 mg/kg bw is proposed as a conservative value to cover all eventualities for agricultural pesticides, based on practical considerations on consumption and maximum residue levels in foods. An ARfD cut off at 5 mg/kg bw would equate to a NOAEL of 500 mg/kg bw/d in an animal study, when default uncertainty factors are applied.

By analogy, relevant upper limits might be considered for other chemicals, e.g., for non-agricultural pesticides or drinking water contaminants.

If, during the derivation of an ARfD it becomes apparent that a previously derived ADI is higher than the ARfD, the ADI should be reconsidered. Such a situation can occur for a number of reasons, e.g., the availability of additional studies, or compounds producing more severe effects when given by gavage than in the diet (JMPR, 2001b).

3.2. Biological and toxicological considerations

The following are key points for consideration when evaluating the database regarding the potential for acute toxicity:

- In the absence of data to the contrary all indications of acute toxicity observed in repeat dose studies should be considered as potentially relevant to setting an ARfD.
- Particular weight should be given to observations and investigations at the beginning of repeat dose studies.

[1] The variability factor is defined as the ratio of the 97.5th percentile of the distribution of pesticide residue per unit to the mean residue for the lot ($v = 97.5$th percentile divided by the mean). **Reference:** Manual on the Submission and Evaluation of Pesticide Residues Data, 2002, http://www.fao.org/ag/agp/agpp/Pesticid/p.htm.

- The NOAEL from the most sensitive species should be used unless there is evidence to demonstrate it is not appropriate for a human risk assessment.
- Isolated findings, showing no specificity or clear pattern are not necessarily indications of acute toxicity.

In determining the appropriateness of using doses and endpoints from subchronic or chronic toxicity studies to establish an ARfD, a weight-of-evidence evaluation should be conducted that considers all relevant data. This includes what is known about the toxic mode of action and the pertinent biology of the system that is affected. One of the main challenges is to evaluate whether those effects are likely to occur at the observed dose levels also following an acute exposure.

The substance and/or metabolite may raise concerns for acute toxicity by compromising the capacity of the organism to compensate or maintain homeostasis, as a result of effects on:

- A critical period of vulnerability or a critical step in a sequence of events.
- A small reserve of a target protein or molecule.
- The synthesis or production of a protein or molecule with a rapid turnover.
- A biological system with limited redundancy.
- A target cell population with limited reparability (e.g., neuron cell loss from central nervous systems).

Toxicological information on interim results and the consideration of progression of a lesion (where relevant) in repeated dose studies may provide insight into the relevance of endpoints for setting acute dietary limits. For example, if interim data indicate that the response is minimal and becomes pronounced or severe after increasing exposure duration, then repeated exposures are probably the determining factor in the response. Interpretation of the relevance of endpoints, should consider toxicokinetic information (e.g., slow elimination kinetics or C_{max} dependent toxicities would raise concern for acute toxicity), as well as information on the acute toxicity of structurally similar chemicals.

3.3. Stepwise process for setting ARfDs

The following stepwise process for setting ARfDs for agricultural pesticides is proposed:

I. Evaluate the total database of the substance and establish a toxicological profile for the active substance.

II. *Consider the principles for not setting an ARfD*
- No findings indicative of effects elicited by an acute exposure are observed at doses up to about 500 mg/kg bw/day; AND/OR.
- No substance-related mortalities are observed at doses up to 1000 mg/kg bw in single dose oral studies.
- If mortality is the only trigger, the cause of death should be confirmed as being relevant to human exposures.

If an ARfD is not set, the reasons must be justified and clearly explained.If the above criteria do not exclude the setting of an ARfD, then one should be set using the most appropriate endpoint.

III. Selection of appropriate endpoints for setting an ARfD (see below)
- Select the toxicological endpoints most relevant for a single (day) exposure.
- Select the most relevant or adequate study in which this/these endpoints have been adequately determined.
- Identify the NOAELs for these endpoints.
- Select the most relevant endpoint providing the lowest NOAEL.

An endpoint from a repeat dose toxicity study should be used if the critical effect of the compound has not been adequately evaluated in a single dose study. This is likely to be a more conservative approach and should be stated. This does not mean that a safety factor other than the default value should be applied. A refinement of such a NOAEL (e.g., in a special single dose study) may be necessary, if the acute intake estimation (see Section 3.8) exceeds such a potentially conservatively established ARfD.If after consideration of all the endpoints, an ARfD is not set, then the reasons must be justified and explained.

IV. *Selection of appropriate safety factors for setting an ARfD (see Section 3.5 below)*
- Derive the ARfD using appropriate safety factors.

The term 'safety factor' is based on current JMPR terminology and applied in this paper as a synonym for uncertainty, adjustment and assessment factors used by other bodies.

3.4. Toxicological endpoints relevant for ARfD derivation

A number of effects could be due to a single exposure. The relevance of these effects should be considered on a case-by-case basis. The route of substance administration should be considered carefully with regard to available toxicokinetic data, in order to minimise influences which are not relevant for the intake of residues (e.g., effects induced by gavage or a specific vehicle or formulation used).

The following list of target effects is not a comprehensive listing of all possible relevant endpoints, but these toxic mechanisms are taken into account as acute toxic alerts, relevant for the consideration of ARfDs for pesticides:

- Haematotoxicity including methaemoglobin formation and haemolytic anaemia.
- Immunotoxicity, which might conceivably be elicited by a single exposure.
- Acute neurotoxicity, including delayed neuropathy, behavioural effects and inhibition of acetylcholinesterase.
- Liver and kidney toxicity, observed in single dose studies or early in repeated dose studies.
- Endocrine effects with hormonal or other biochemical alterations observed in single dose studies or early in repeated dose studies.
- Developmental effects, e.g., resorptions, malformations, other effects on the offspring.

Direct effects on the GI tract/stomach should be assessed carefully to determine the relevance to human exposure e.g., are they due to irritation or a pharmacological action; are they related to the method of administration (present with bolus dosing but not by dietary admixture). For example, diarrhoea and vomiting in dogs should be considered not relevant for setting an ARfD if these effects are related to high concentrations following specific dosing methods (e.g., capsule administration or gavage) and local (irritant) effects.

3.5. Safety factors

3.5.1. Background

The process of deriving ARfDs is essentially the same as that for deriving an ADI, involving the determination of the most appropriate NOAEL and a safety factor. The safety factors are used to extrapolate from animal data to the average human and to allow for variation in sensitivity within the human population. The default factors for extrapolating from animals to humans are composed of 2.5 for toxicodynamics and 4 for toxicokinetics; the human variability factor is composed of identical factors of 3.2 for both toxicokinetics and toxicodynamics. This subdivision permits the use of specific data on a chemical to derive chemical-specific adjustment factors (CSAFs). A detailed description of this process is in the report of an IPCS workshop (IPCS, 2001c; Meek et al., 2002). More recent work by Renwick's group has indicated that for certain compounds or certain human subgroups a value greater than the default would be appropriate for one of the factors since actual variability is greater than the default (Dorne et al., 2003, 2004; Walton et al., 2004). However, at this stage it is unclear whether this would translate into greater overall variation when all the factors are considered together.

The 2001 JMPR considered that the derivation of acute reference doses was well suited to the use of CSAFs to inform the determination of the overall uncertainty or safety factor. In addition to addressing the components that are to be changed from the defaults, there must also be a consideration of the other components to demonstrate that these do not need to be refined.

3.5.2. Data availability

For most compounds evaluated to date there is insufficient information to permit a scientifically based CSAF approach. Only if additional substance-specific experimental data (e.g., a comparison of toxicokinetics between rats and humans for a given substance) are available, default assessment factor values would be reduced or raised accordingly (Meek et al., 2002). However, for pesticides, such data are rarely available.

Data on the mechanism of toxic action are available for certain chemicals, most frequently veterinary medicines and pesticides that have a common mechanism against both the target species and non-target mammals. These data, together with information on the time-course of effects, can provide an indication as to whether the action is reversible. Data on absorption, excretion and toxicokinetics are often available. Together with information on the mechanism of action this may help to evaluate whether effects are likely to be related to peak concentrations (C_{max}) or plasma concentration integrated over time (area-under-the-curve, or AUC). Human toxicity data are available for a small number of chemicals and can either be used directly to derive ARfDs or as part of the overall consideration of interspecies sensitivity. The overall database on a compound can be used to determine the overall quality of the available data and identify areas of increased or decreased uncertainty.

3.5.3. Data interpretation

Where data exist that can be used to derive a CSAF, these should be used in preference to the existing defaults. However, if there is insufficient information, the default approach should be applied.

A number of situations could justify the use of safety factors higher or lower than the default values of 100 and 10 on the basis of animal and human data, respectively (JMPR, 2001a). This paper expands on these and divides them into kinetic/dynamic factors and other factors.

3.5.4. Kinetic and dynamic factors

When the effect under consideration is due to reversible interaction of the compound with a pharmacological

target (e.g., a receptor or ion channel) or due to direct irritation, then the concentration of the substance rather than total intake should determine the magnitude of the effect i.e., the C_{max} is likely to be more relevant than AUC. A reduction in toxicokinetic variation by 2-fold may be justified, leading to an overall default factor of 25 for animal studies (i.e., 5×5 instead of 10×10 for inter- and intraspecies factors) and 5 (instead of 10) for human studies.

JMPR has used CSAFs in the derivation of ARfDs for several carbamate insecticides that inhibit acetylcholinesterase. These compounds do not require metabolic activation, they react reversibly with a pharmacological target (acetylcholinesterase), the magnitude of the pharmacological effect is proportional to the C_{max} rather than the AUC and the excretion is rapid. In such circumstances, the determining factor is the C_{max}, which has been shown to have lower variability than clearance, as it depends mainly on the rate and extent of gastrointestinal absorbance. A 2-fold reduction in the toxicokinetic factor(s) is then applied by JMPR. It appears that this approach has not been adopted by any risk assessment bodies other than the JMPR.

If reductions are proposed in one or more of the default factors, evidence should be adduced to indicate that none of the other factors need to be increased above the default (e.g., if the production or detoxification of a toxic metabolite is involved, there may be a large variation in metabolic capacity).

If human data are available but these are not used directly to derive the ARfD, they might be sufficient to demonstrate that the findings in experimental animals are qualitatively and quantitatively similar to humans, thereby supporting the use of a reduced factor (e.g., data on the production and degradation of a toxic metabolite). Similarly, if data from a wide range of species exhibit similar qualitative and quantitative effects, it could be possible to conclude that the variation between the most sensitive of these and humans would be <10.

3.5.5. Additional safety factors

When a NOAEL has not been identified for the most appropriate endpoint, the LOAEL can be used in exceptional cases as the basis for the ARfD. In such a situation, the selection of an additional safety factor up to 10 will depend upon the magnitude of the effect and the steepness of the dose response curve.

An extra safety factor has often been adopted for the severity of the effect. However, the degree of severity of an effect may be somewhat subjective and it would not be feasible to grade all possible toxicological effects by their severity. Therefore, if a toxicological effect is judged to be irreversible or particularly severe, this should be a trigger to consider the finding in more detail before choosing an appropriate safety factor (see following point).

Depending on a detailed consideration of the following questions, it may be appropriate to include an additional safety factor:

- Has the study shown an adequate margin between the NOAEL and the LOAEL?
- Is the finding supported by data from other studies or by knowledge of the mechanism of action of the compound?
- Is there is a high level of uncertainty in the database?
- Have measurements been taken at appropriate times and have they used appropriately sensitive methods?
- Has the study on which it is proposed to base the ARfD used adequate group sizes?

3.5.6. Reduced safety factors

A reduced factor might be appropriate if the endpoint used to derive an ARfD is of minimal adversity and the critical NOAEL is from a repeat dose study e.g., reduced food consumption and body weight gain (i.e., observed in the first days) or increased organ weight with minimal pathological change. When considering body weight changes considerations need to be given to potential problems of palatability of the feed.

There is a large margin between the LOAEL and the NOAEL and an appropriate benchmark dose, or similar, evaluation indicates that the benchmark dose would be significantly higher than the actual NOAEL dose used in the study. Alternatively, a benchmark dose or other Point-of-Departure procedure could be used, with the application of default safety factor(s).

3.5.7. Conclusions

In determining the appropriate safety factor for deriving an ARfD, a stepwise approach is proposed:

- Determine if it is adequate to support the derivation of scientifically based assessment factor(s) (i.e., CSAF).
- If a specific adjustment factor cannot be derived, consider if there is any information to indicate reduced or increased uncertainty. If not, the 10-fold or 100-fold default should be used.
- Whenever a safety factor other than the default is used, a clear explanation of the derivation of the factor must be provided.

3.5.8. Future directions

The use of physiologically based pharmacokinetic modelling to provide more information on human tissue doses relative to experimental animals would improve the derivation of CSAF. The use of in silico analysis techniques and bioinformatics can provide information on the likely variation in response in different species and subgroups.

3.6. Different ARfDs for population subgroups

It is preferable to set a single ARfD to cover the whole population, in particular for risk management and enforcement purposes. It is important to ensure that any ARfDs established are adequate to protect the embryo/fetus from possible in utero effects. While an ARfD based on developmental (embryo/fetal) effects would necessarily apply to women of childbearing age, it is recognised that such an ARfD may be conservative and not relevant to other population subgroups. This may be the case for children 1–6 years of age for whom specific acute consumption data are available, and thus can be separately modelled with respect to acute dietary intake of pesticide residues. The use of a sensitive ARfD for children could lead to an unreasonably conservative short-term dietary risk assessment. Thus in those situations in which a developmental endpoint drives an ARfD for a compound exhibiting no other toxicity at the developmental NOAEL, it may be considered to set a second value based on another, non-developmental, endpoint.

3.7. Use of human data in acute pesticide risk assessments

Human data on a pesticide, whether from volunteer studies or other investigations of human exposures, can be extremely valuable in setting the animal data into context and, when available, should always be evaluated even if they are not used to derive an ARfD.

Not only may a human study allow identifying endpoints (NOAELs/LOAELs) for use in risk assessment, other important information may be gained such as the nature of the effect and its pattern of onset and duration. The human data could be available from a number of sources: epidemiology studies of acute effects in human populations exposed to the chemical under evaluation; direct administration to volunteers; monitoring of those exposed following normal use of the chemical; exposures from accidental or deliberate poisonings; the use of the same compounds as human pharmaceuticals. Such studies often involve single or short-term exposures that can be of relevance, directly or indirectly, to the derivation of ARfDs.

3.7.1. Accidental or deliberate poisonings

These will often involve acute, relatively high exposures. Although in many cases the true exposure is not known with any precision, investigations of such cases can provide information about the nature of the human response compared to the laboratory animal toxicology. In cases where there is reliably documented exposure, it may be possible to compare dose response relationships in humans with the experimental animal dose response relationship.

3.7.2. Epidemiology studies

Epidemiology studies are occasionally conducted on acute effects in a manner documented well enough to associate exposure with effects. There are various types of studies or study designs that are well described and defined in various text books and other document's (Coggon et al., 1997; IPCS, 1983).

In most epidemiology studies, exposures are not measured directly but estimated in a semi-quantitative manner from surrogates such as usage or residence near to a source. The absence of precise exposure information should not prevent such studies from being used to inform the overall consideration.

3.7.3. Monitoring studies

Information from biomarker studies can be of value in determining if predictions of human exposures appear to be realistic. Investigations using biomarkers of effect (e.g., cholinesterase activity) allow the possible evaluation of a human response to a chemical exposure in the manner in which it occurs and can provide information on whether responses in exposed humans are in line with those found in the animal database. If these studies involve single or short-term exposures, they can inform ARfD assessments, particularly if such values are based on repeat dose animal studies. However, also monitoring studies often lack reliable quantitative information on exposures.

3.7.4. Clinical trials on the same or similar compounds used as pharmaceuticals

Some pesticides (e.g., fungicides) that can give rise to residues in food are the same or similar to drugs used as human pharmaceuticals or veterinary medicines (e.g., antibiotics). Data from the clinical trials performed as part of the registration process for pharmaceuticals might be relevant to ARfD assessments e.g., are effects reported in repeat dose animal studies seen in human subjects after a single dose. In some countries the strict data protection laws applying to pharmaceuticals can restrict access to such data, but this would not apply if the company marketing the veterinary medicine or pesticide also produced the pharmaceutical.

3.7.5. Volunteer studies

The use of human volunteer data in chemical risk assessments is currently a controversial issue with a wide range of views held by national authorities and individuals. The use of human data reduces the level of uncertainty when extrapolating from animal models and is seen as a valuable contribution to science-based decision making. An alternative view is that human volunteers should never be exposed deliberately to a chemical that provides them with no benefit. One of the main concerns of both sides is the ethical issues surrounding the

performance of studies in human volunteers. Guidelines for the ethical performance of human studies have been developed at an international level (Declaration of Helsinki, 1997) and national levels (e.g., UK Royal College of Physicians, 1990) with a view to ensuring any human trials are performed to minimum standards. A recent report prepared by the National Academy of Sciences for the US EPA (2004) recommended that intentional dosing studies in humans can be used for regulatory purposes only if scientific and ethical standards are met and the societal benefits of the study outweigh any anticipated risks to participants.

The JMPR has considered human data at many of its meetings. General discussions on the use of human data took place at the 2001 and 2002 JMPR Meetings (JMPR, 2001b; JMPR, 2002). The 2002 JMPR meeting reaffirmed the principle that endpoints from human studies could be used for setting dietary guideline values if they had been conducted in accordance with relevant ethical guidelines. Because scientific controversy sometimes surrounds the interpretation and significance of results, the quality of the data, as well as the consistency of responses among different human studies (if several studies are available) should be considered. Additionally, because the designs of human studies have some limitations in comparison with those in experimental animals their use should always be considered in the context of the overall toxicological database.

The following factors should be considered when determining whether to use a human study in the derivation of an ARfD:

- Recent studies in humans should include clear statements that they were performed in accordance with internationally accepted ethical standards. For older studies, ethical considerations should take into account both current standards and the standards pertaining at the time the study was performed (JMPR, 1999b).
- The study should be assessed for the quality and integrity of the data and provide adequate documentation of the methods (including statistics and control values) and results. A poorly designed or conducted study in humans (as with experimental animals) should not be used for risk assessment.
- The number of subjects for each group should be large enough to allow adequate statistical power for a reliable analysis of the response. The acceptable group size will depend on factors such as inter-individual variation in response and the level of change not considered to be adverse. The IPCS Guidance for the use of chemical-specific adjustment factors proposed a minimum group size of 5 (IPCS, 2001c). Studies using small group sizes might be useable, e.g., by combining results from two or more dose levels or applying an increased safety factor.
- The critical endpoints identified in animal studies should have been investigated appropriately in human studies when ethically acceptable.
- If only one sex or a particular age group has been used, the general applicability of the results should be ascertained (e.g., animal studies can be used to determine whether males and females are likely to respond differently to the test material).
- ARfDs based on studies in humans should provide a sufficient margin of safety for toxicological endpoints that cannot readily be addressed by such studies (e.g., developmental toxicity).
- Studies that have not been performed in accordance with ethical principles but are scientifically valid should be used only if the findings indicate that acceptable human exposure is lower than the level that would be determined without the use of such a study.

Many studies in volunteers are short-term or involve regular measurements of biomarkers of effect and are thus directly relevant to considerations of ARfDs.

3.8. Intake considerations in relation to ARfDs—dietary risk assessment

For risk management purposes, the ARfD of a compound is compared to the estimated short-term intake of a pesticide through various foods. This allows to identify for which crops and pesticide applications regulatory actions are necessary for public health protection.

The first step in estimating the short-term intake of pesticide residues is to determine if the commodity is homogenous or not in relation to consumption. For commodities that are basically 'homogeneous' when consumed because they are centrally processed like cereals or because there are a large number of individual units per portion (e.g., cherries and berries), individual unit variation is not considered to be of concern. However for commodities like fruits and vegetables that are consumed whole or in large pieces (3 or fewer commodity units per large portion), individual unit variability with respect to pesticide residues needs to be considered. Three different cases are considered, depending on the type of commodity.

- The first case covers those foods in which the available composite residue data reflect the residue levels in the food portion consumed.
- The second case covers foods in which the available composite residue data may not reflect the residue levels in the food portion consumed i.e., individual units which may provide a significant portion of the meal, could have a significantly higher residue level than that measured in a composite sample of the commodity.

- The third case relates to processed commodities where bulking and blending mean that the median residue level derived from supervised trials (and adjusted for processing) represents the highest likely residue.

The models for calculating acute dietary exposure to pesticide residues as performed by JMPR consider the relevant of the three scenarios above and two sets of populations: the general population and the population 1–6 years. At the international level there is only limited short-term consumption data—the only population groups for which acute intake values are available are the general population and children from 1 to 6 years. Although the population group of 1–6 years is considered to have high food consumption on a body weight basis, examination of the available data sets shows that there are some groups of food commodities for which the general population has a higher consumption per body weight basis e.g., cereals, berries, exotic fruits, oils, dried pulses and a range of 'strong flavoured' fruits and vegetables. Thus, the general assumption that children will always be the critical subgroup when considering acute toxic effects of pesticide residues, is not correct for all commodities. Therefore, both consumer groups are considered within the work of JMPR.

Current dietary models maintain a degree of conservatism in the calculations in order to ensure consumer safety. Where assumptions have to be made, defaults may be higher than necessary but refinements can be made where appropriate data exist. The fact that for the international evaluation the consumption data are recorded on a daily basis does restrict some further refinements that could be possible in matching the intake scenarios and the appropriate dosing period in experimental studies used in determining the acute reference dose. Further work is required in both areas to match the dosing used in toxicology studies and the acute consumption scenarios.

4. Specific guidance on the derivation of ARfDs

Particular toxicological endpoints which are relevant to ARfD establishment are considered below. Note that this guidance is not intended to comprehensively cover all potentially relevant endpoints but focuses on the interpretation of those which have proved to be problematic in reaching a decision as to whether an effect is relevant to an acute exposure to residues of agricultural pesticides in foods.

4.1. Haematotoxicity

4.1.1. Methaemoglobin formation

4.1.1.1. General background. A range of chemicals is able to induce methaemoglobin (MetHb) formation, thereby inhibiting normal oxygen transport and oxygenation of tissues. MetHb is formed when the iron atom in haemoglobin is oxidised from the ferrous to the ferric state by the superoxide ion. Under normal physiological conditions, MetHb is formed spontaneously in very low quantities. The amount of MetHb present results from the equilibrium between the formation of MetHb and the activity of the reducing systems.

Reducing capacity varies between species as does the background level of MetHb. In general, rat/mouse/rabbit/guinea pig/monkey have a higher reducing capacity than dog/cat/humans. Species with high reducing capacity tend to have lower background levels of MetHb. This does not necessarily mean that the dog is the most relevant species for studying MetHb formation since metabolism of xenobiotics, with formation of key metabolites, may be necessary for MetHb formation (Calabrese, 1991). Within the human population, there are several groups with increased susceptibility for MetHb formation (Griffin, 1997).

An increase in MetHb formation can only occur when the available reducing capacity is overwhelmed. Any increase in MetHb indicates that residual reducing capacity has been depleted. For repeated exposure, this is an undesirable effect since it affects the fitness of the individual.

4.1.1.2. Data availability. MetHb levels are not an obligatory measurement in the regular set of haematological examinations (test guidelines no. 407, 408, 409; OECD, 2005). MetHb is measured only when there are indications for its formation. When included, MetHb is measured in those studies were regular haematological investigations are performed. MetHb is mostly not measured in acute studies (test guidelines no. 401, 420, 423 or 425; OECD, 2005) or in special studies like developmental- or reproduction studies. For setting an ARfD, measuring MetHb levels in acute studies may however be highly valuable.

Routine observations in acute toxicity studies might identify signs of MetHb production e.g., blue extremities but such signs could also be produced by other anomalies of tissue oxygenation.

4.1.1.3. Interpretation of data. Depending on the kinetics of a compound, MetHb formation after a single dose can occur rapidly (maybe maximal after only 1–4 h), with MetHb levels returning to control levels within a few hours. Thus the effect is relevant for acute dietary risk assessment. In addition, it is known that adaptation occurs upon repeated exposure to MetHb-inducing substances. Thus, the absence of increased MetHb levels in a repeated dose study does not necessarily mean that there is no effect on MetHb formation in a single dose study with the same dose. When evaluating MetHb

levels, especially in relation to setting an ARfD, the timing of measurements should be considered because of the time course of MetHb formation and reduction. If it is clear that the measurements were not performed at the most appropriate time, an additional safety factor may be considered.

MetHb formation can be indicated by clinical signs such as blue/grey appearance of the extremities. Coleman and Coleman (1996) reported that in some humans cyanotic signs can already be observed at MetHb levels of below 6% although most individuals can tolerate levels of 10%. Therefore, levels of 6% MetHb seems to be a threshold for the occurrence of clinical signs due to MetHb formation in sensitive individuals. For practical purposes and as an aid to improving consistency and as a consensus approach, for acute exposure to MetHb inducing substances it is proposed to consider MetHb levels of about 4% above background level or higher as being adverse.

4.1.1.4. Conclusions. For acute exposure to methaemoglobin-inducing xenobiotics, a level of about 4% or more above background level in dogs and a statistically significant increase by comparison with controls in rodents is considered to represent a conservative approach to setting an ARfD.

4.1.2. Acute haemolytic anaemia

4.1.2.1. General background. Haemolysis can occur by different mechanisms, including: (1)mechanical injury of red blood cells (RBCs), (2) immune mediated anaemia, (3) oxidative injury of the cell membrane (including Heinz Body formation), and (4) non-oxidative injury of the cell membrane, (5) effects on circulating and precursor cells. Some mechanisms are relevant for acute exposure, others less so. In most cases, the mechanism will not be known. In that case, results after different exposure periods may be the key guidance to decide whether or not haemolysis is relevant for acute exposure (see below).

4.1.2.1.1. Mechanical damage. Some forms of haemolytic anaemia (also called microangiopathic anaemia) are caused by red cell mechanical damage incurred during their passage through an occluded microvascular bed. The small vessels may be narrowed by platelet clumps and fibrin clots, by metastatic cancer, or by the endothelial proliferation in severe vasculitis or hypertension. Sometimes these effects can be induced by chemicals, the most well known being bacterial toxins and chemotherapeutic agents. Some of these anaemia's are restricted to damage to the renal vascular system, closely linking acute renal effects with haemolysis (e.g., after exposure to phenylhydrazine). Although these effects can sometimes occur after single doses, and are therefore potentially relevant for setting an ARfD, this mechanism is probably less relevant for pesticides.

4.1.2.1.2. Immune-mediated anaemia. Some substances, especially known for human drugs, are able to induce an immune mediated haemolytic anaemia. Antibody-coated RBCs may be destroyed in the circulation by complement activation (intravascular immune haemolysis) and/or within the reticuloendothelial system, that is, the spleen and liver (extravascular immune haemolysis).

If exposure to such a substance occurs for the first time, the minimal time required for the development of immunocytopenias is several days (primary immune response). In sensitised individuals, reactions can occur at any time during a continuous or an intermittent administration, or immediately after re-exposure to the substance (secondary immune response). Various substances of different types have been claimed to induce immune mediated haemolysis. However, only a few substances have been shown to induce immune mediated haemolysis in large groups of patients (primarily antibiotics). For other compounds only individual case reports exist. Diagnosis of immune mediated haemolysis is difficult and it is doubtful whether this is an important feature for pesticide safety evaluation.

4.1.2.1.3. Oxidative damage of the RBCs. A range of substances, including pesticides, can produce oxidative stress in RBCs. Haemolysis occurs because the oxidative insult causes denaturation of haemoglobin into insoluble deposits that attach to the red cell membrane (Heinz bodies). It is not known whether the active metabolites that produce MetHb are also involved in the production of Heinz bodies by a mechanism unrelated to MetHb production.

This reduces the flexibility and functioning of the erythrocytes. The spleen usually removes damaged RBCs from the blood. However, they are demonstrable for a longer period in the blood than are increased MetHb concentrations. In general the presence of Heinz bodies nearly always points to MetHb formation, though not in all cases (Russell et al., 1982). In addition, the lipid and protein components of the membrane also suffer direct oxidative damage.

After exposure to the oxidative insult, Hb concentrations can decrease rapidly. As the bone marrow responds to the anaemic stimulus, the reticulocyte count begins to rise within a few days and reaches a peak by 7–10 days in humans.

Species differences in susceptibility for Heinz body formation have been reported. RBCs of rabbit, monkey, chicken, and guinea pig are the least sensitive, followed by man, mouse, and dog, and finally the cat (Blom, 2001). This susceptibility ranking is not substantiated by firm data.

Although oxidative damage and Heinz body formation can occur directly after a single exposure, observation of Heinz bodies in repeated dose studies is very likely to result from repeated exposure because the

oxidation, denaturation and precipitation of haemoglobin may increase over time. Nevertheless, the observation of Heinz bodies can be considered as a continuum from oxidative stress and haemoglobin denaturation, and is therefore a sign of adverse effects following a single exposure.

4.1.2.1.4. Non-oxidative damage. Some non-agricultural pesticide compounds (e.g., some metals (As, Pb, Cu) and natural venom's) are able to produce haemolysis through other types of mechanisms such as enzyme inhibition or inhibition of ion-transport mechanisms. In the case of enzyme inhibition, such as inhibition of haemoglobin synthesis, the effects will gradually appear and are probably less relevant for acute exposure. In cases of ion-transport inhibition, the osmotic conditions in the red blood cell can change quite quickly resulting in e.g., cell swelling and lysis. These types of mechanisms are considered less relevant for agricultural pesticides.

4.1.2.1.5. Effects on circulating cells and precursor cells. Several chemical substances are able to induce haematotoxic effects through other mechanisms than MetHb formation and acute haemolysis. Benzene (although not a pesticide) is a well-known example, and the most extensively investigated substance, of this group.

Changes in circulating blood cells can be the consequence of direct action of a chemical on that particular cell type or of actions on precursor or stem cells. The present routine measurements of cells in the blood cannot easily discriminate between the underlying causes of a change in circulating cells.

Direct effects on circulating blood cells can be relevant for a single day exposure. However, a reduction in specific cell types can be counteracted by increased activity of precursor cells or stem cells in order to regenerate new cells. It is possible therefore, that after some weeks a certain action of a chemical on circulating cells cannot be monitored because the haematopoetic system has undergone some adaptive changes. Changes in circulating cells due to loss of stem cells (and precursor cells) are irreversible and therefore highly important. However, such effects cannot be determined directly from the basic haematological measurements normally performed in regular toxicity studies. Additional types of determinations are needed to cover such effects if they are suspected.

4.1.2.2. Data availability. Measurements of haematological parameters are obligatory investigations in repeated dose toxicity studies. In studies with durations of 90 days or longer, often interim measurements are also available. Essentially, data on some haematological parameters are always available in a pesticide dossier. MetHb, carboxyHb and reticulocytes are not investigated routinely but are often included when haematological changes have been identified in an earlier study.

A range of primary and secondary haematological parameters is measured and reported. Various parameters, however, are closely linked to each other or directly calculated from other parameters. Therefore, when evaluating data on haematological parameters one has to look for patterns of effects rather than effects on single parameters.

4.1.2.3. Interpretation of data. In some repeat dose studies, measurements of haematological parameters are performed at various time points. In order to acquire an insight into the time course of haematological effects in relation to their relevance for a single day exposure, interim results can be very helpful.

If changes in haematological parameters can, for example, already be observed early in the study (e.g., after 2, 4 or 8 weeks) and do not appear to progress during the study duration, it is possible that these effects are induced early on in the exposure period. In that case, the effects can be considered relevant for acute exposure to the substance. In such a case one could argue that brief exposure to the test substance may already result in haematological effects.

If however, the interim results show less effect on the haematological parameters, or when effects seem to get worse over the exposure duration, the repeated exposure to the substance is probably the determining factor in causing the effects. The haematological effects as such can then be considered not relevant for a single day exposure. If for example, mild anaemia is observed after 28 days exposure but not at 14 days, one could argue that the repeated exposure is the most important aspect for inducing haematological effects. The effects should in that case not be used for setting the ARfD.

Therefore, the time course of effects is an important aspect in the selection of haematological endpoints for setting an ARfD.

4.1.2.4. Conclusions. In evaluating haematological effects such as anaemia, the evaluator has to look at the total pattern of haematological parameters instead of focussing on the effect on a single parameter. Haematological parameters are very closely interrelated.

In assessing whether effects observed in repeated dose studies should be used for setting an ARfD, one has to evaluate the mechanism of action. If known, this could provide arguments for selecting or not selecting the endpoint for setting an ARfD. In addition, one has to evaluate any information available on the relation between exposure duration and haematological effects in order to decide whether or not the effects could have been induced by a single exposure.

4.1.2.5. Future directions. One of the future directions to improve the database for derivation an ARfD for substances with acute haematotoxic effects could be

the inclusion of blood sampling and haematological investigations during the early stages of a repeat dose study.

4.2. Immunotoxicity

4.2.1. General background

Immunotoxicity has been increasingly recognised as an important endpoint in the overall toxicity evaluation of chemicals. Laboratory experiments in rodents have indicated that certain pesticides are able to induce alterations in immune function (Barnett, 1997; Rogers, 1996).

Classification of immune system effects include:

- *Immunosuppression*: Decreased immune system responsiveness that may range from mild to marked severity, and may be expressed as changes in function or, with more severe suppression, an increased incidence of infections.
- *Hypersensitivity/allergy*: One of several types of inappropriate immune responses to chemicals or antigens, the responses are harmful, rather than protective (e.g., asthma, allergies).
- *Autoimmunity*: An inappropriate immune response to components of the body that can result in tissue damage and in some cases organ failure.
- *Immune system dysregulation*: Misdirection of the immune response due to altered antigen processing or mediator production.

4.2.2. Data availability

Pathological and histopathological examination of the immune system organs and tissues and differential white cell counts are performed in the standard subchronic and chronic guideline studies. Serum immunoglobulin levels may also be measured. The 28-day repeated dose study in rats (test guideline no. 407; OECD, 2005) includes organ weights (thymus, popliteal and mesenteric lymph nodes), histopathology (thymus, spleen, popliteal and mesenteric lymph nodes, Peyer's patches and bone marrow), and serum IgM levels. Based on two international ring studies, additional measures have been proposed for purposes of screening for immunotoxicity (Schulte et al., 2002). Although not a data requirement, EPA has published a test guideline for a 28 day rat immunotoxicity study (OPPTS test guideline no. 870.7800; OPPTS, 1998a) that includes histopathology of the immune organs/tissues, an assessment of natural killer cell activity and/or enumeration of splenic or peripheral blood total B cells, total T cells, and T cell subpopulations, and an assessment of immune system function (i.e., response to T-cell-dependent antigen, sheep red blood cells). A skin sensitisation study (test guideline no. 406; OECD, 2005) also is available for labelling purposes. Indicators of immunotoxic effects that may be commonly available from standard guideline tests include histopathologic effects in immune tissues and organs, changes in serum immunoglobulin levels, altered spleen and lymph node weights, and altered peripheral blood counts.

4.2.3. Data interpretation

Even though effects on the immune system can be induced by a single exposure, typically a high dose may be required to cause immunotoxicity following a single exposure except for chemicals that are eliminated slowly. Changes in immune function at high doses may be the result of overt toxicity (decreased food intake, irritation or inflammation, increased glucocorticoid release, or a general decline in fitness) rather than a direct effect on the immune system. Nevertheless, it is unlikely that a single exposure to a chemical will produce persistent immunotoxicity in adults, since immune system cells are constantly replaced and because of the inherent redundancy in the system (i.e., alternate mechanisms to resist infection). The only potential concern would be for a compound that induced an auto-immune response.

4.2.4. Conclusions

In general, immunotoxicity data derived from repeated dose studies are not appropriate for setting an ARfD for acute adult exposure limits. There may be exceptions, and thus a case by case weight of evidence analysis should be conducted that considers elimination kinetics and affinity for lymphoid tissues.

4.3. Neurotoxicity

4.3.1. General background

Neurotoxicity is the production of an adverse change in the structure or function of the central or peripheral nervous systems due to exposure to a chemical. Structural changes can be identified preferably by histopathological investigation. Functional effects (transient or irreversible) can be identified by neurochemical, neurophysiological or behavioural investigations. Neurotoxicity can be produced by direct effects on the nervous system or can be secondary to general toxicity or toxicity to other organ systems e.g., hypoxia or reduced intracellular energy production.

The nervous system differs from most other organ systems in its limited capacity for repair and regeneration, which reduces the potential for recovery when cell death or damage has occurred. Therefore, any neurotoxicity seen in repeat dose studies could be the result of a single exposure that is not reparable. Although the nervous system has a reserve capacity that can be considered to provide a mechanism for compensation following a neurotoxic event, this can also mask low level neurotoxicity

that can become evident following subsequent discrete exposures or in the future. The process of nerve degeneration can be either rapid or prolonged.

Delayed neurotoxicity, where an adverse effect is not seen until some time following a single exposure, has been found with a number of chemicals e.g., tri-ortho-cresyl phosphate. There is thus a need to consider if an adequate period of investigation has been used in studies using acute exposure or whether there is sufficient mechanistic information to show that differentiated cells in the central nervous systems are not damaged irreparably.

A detailed consideration of neurotoxicity risk assessments can be found in Environmental Health Criteria document 223 (IPCS, 2001a).

4.3.2. Data availability

Information on the chemical structure, the relationship to known neuroactive compounds and, where available, the mechanism of action can provide useful information on the acute neurotoxic potential of a chemical.

Acute toxicity studies used primarily for classification (test guidelines no. 401, 420, 423 or 425; OECD, 2005) include some investigations for signs of neurotoxicity (e.g., tremors, convulsions or salivation) but minimal pathological investigation of nervous tissue.

A wide range of functional tests are available for evaluating neurotoxicity, including:

- *Sensory effects*: Auditory startle, visual discrimination, pain sensitivity, pupil response, sensory-evoked potentials.
- *Neuromuscular effects*: Grip strength, hindlimb splay, motor activity, righting reflex, tremors and spasms, acetylcholinesterase activity.
- *Learning and memory*: Conditioned behaviour, operant behaviours, maze tests.

In common with most scientific procedures, careful experimental design is necessary to control potential confounding effects. For example, toxicant-induced alterations in growth rate can indirectly impact hind limb splay due to the confounding of body size on this measure. Motor dysfunction can impact assessments of learning and memory where the performance is dependent on intact motor function.

A range of these tests are included in the standard acute and repeat dose neurotoxicity test protocol (test guideline no. 424; OECD, 2005). Histopathological examinations of nervous tissues, including those preserved using perfusion fixation, is also included in test guideline 424.

General examinations of clinical signs and behaviour, plus investigations of response to stimuli, grip strength and motor activity as well as histopathological examination of brain, spinal cord, eyes and peripheral nerves are requirements of repeated dose 28 day/90 day oral toxicity studies in rodents (test guidelines no. 407, 408; OECD, 2005). Specific investigations of primary toxic endpoints (e.g., cholinesterase inhibition) are often added to the test protocols when the mechanism of toxicity is known or suspected.

4.3.3. Data interpretation

In modern studies, particularly those incorporating functional observation batteries (FOB) a large number of data points are generated, which may produce some statistically significant results due purely to chance. Interpretation of such studies should include a consideration not only of statistical significance of individual results but the nature, severity, persistence, dose-relationship and pattern of the effects in relation to concurrent and appropriate historic control animals. The presence of a few apparently unrelated or non-specific, but statistically significant, effects are not necessarily indicative of neurotoxicity.

The nervous system of mammals, and to a lesser extent other vertebrates, have many common elements and thus any effects seen in experimental animals should be considered directly applicable to human risk assessments unless there is convincing evidence that the findings are not relevant to human exposures. Negative results in neurotoxicity investigations in an animal study do not necessarily mean there will be no effect in humans—for example, the Parkinson like syndrome seen in humans exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is not reproduced in rats. Therefore, unless there are robust data to the contrary, the most sensitive species should be used in deriving ARfDs for neurotoxic compounds.

In addition to long-term or irreversible effects associated with acute exposure, attention should be paid to transient neurotoxic effects, as these could be considered as adverse under a range of circumstances, e.g., impaired motor performance could have safety implications for those operating machinery. Some neurotoxins such as cholinesterase inhibitors, ethanol and volatile solvents can produce improved performance in some investigations using low level exposures but adverse effects at higher concentrations. Both types of effects are associated with direct interaction with the nervous system and the beneficial effects might parallel adverse effects on other variables that were not investigated in a particular study.

Neurotoxic endpoints can normally be assessed during the in-life phase of a study. This has benefits in terms of deriving ARfDs when there are no acute exposure studies available. For example, with a cholinesterase inhibitor checking for clinical signs such as salivation, tremors or abnormal behaviour in the first few days of

dosing it may be possible to determine if the effects are relevant to a single exposure. These observations can provide information on whether the observed effect (e.g., salivation) is acute in nature but if a more sensitive endpoint (e.g., acetylcholinesterase inhibition) is associated with the observed effect but was only determined at the end of the study, then the more sensitive endpoint should also be considered in the acute assessment. Knowledge of the mechanism of neurotoxicity either in mammals, or in the case of insecticides against the pest, can be of benefit in deciding on appropriate endpoints for investigation and as a method of identifying any links between effects seen in the whole animal and the molecular target.

The most common neurotoxic endpoint used to date in the derivation of ARfDs for pesticides is inhibition of acetylcholinesterase. The JMPR has previously defined criteria for the assessment of cholinesterase inhibition (JMPR, 1999c). These criteria apply equally to the setting of ARfDs and ADIs. For inhibition of acetylcholinesterase a specific cut off of 20% is used routinely to differentiate between adverse and non-adverse effects. For other endpoints, no such commonly accepted cut-off values are available and the decision on whether or not an effect is adverse will be determined based on statistical considerations and deviations from normal biological ranges.

4.3.4. Conclusions

As a default assumption in deriving ARfDs, all nervous system effects are considered as evidence of neurotoxicity. This means that in addition to long-term or irreversible effects associated with acute exposure, attention should be paid to transient effects, as these could be considered as adverse under some circumstances.

Interpretation of neurotoxicity studies should include a consideration of the nature, severity, persistence, dose-relationship and pattern of the effects. Isolated findings showing no specificity or clear pattern do not necessarily indicate neurotoxicity.

In acute studies on compounds showing repeat dose neurotoxicity, the extent and duration of investigations must be shown to be adequate before it can be concluded that the compound does not have any acute neurotoxic potential.

Delayed neurotoxicity following single chemical exposures can occur and thus any acute exposure study should have an adequate period of investigation. Otherwise there should be sufficient mechanistic information to show that differentiated cells in the central nervous systems are not damaged irreparably.

The most common neurotoxic endpoint used to date in the derivation of ARfDs for pesticides is inhibition of acetylcholinesterase. A specific cut off 20% inhibition is used routinely to differentiate between adverse and non-adverse effects.

4.3.5. Future directions

The incorporation of relevant investigations based on the (neuro)toxicological profile of a compound at the beginning of repeat dose studies and a better recording of clinical signs or the performance of specific acute studies should provide information that would permit a more refined assessment of the relevance for ARfD derivation of neurotoxicity seen after repeated exposure.

4.4. Liver and kidney toxicity

The liver and the kidney are organs that are often affected by exposure to toxic substances. Compared with other organs, toxic responses in rodent studies occur relatively frequently in liver and kidney. Reasons for this include the high metabolic capability and the portal blood supply of the liver and the concentration of xenobiotics in the kidney in the process of excretion.

4.4.1. Hepatotoxicity

4.4.1.1. General background. The liver is a major site for metabolism of exogenous chemicals (xenobiotics), resulting in the formation of metabolites which may be more or less toxic than the parent compound. Organs that have lower or even lack such metabolic capabilities are less susceptible to chemicals requiring metabolic activation to exhibit toxicity. The liver is also, apart from the GI-tract, the first major organ to be exposed to ingested toxins due to its portal blood supply and toxins may be, at least partially, removed from the circulation during the first pass, providing protection to other organs while increasing the likelihood of hepatic injury (Zimmermann, 1978; Moslen, 1996; Miyai, 1991). For these reasons, the observation and interpretation of effects on the liver may be relevant for the setting of acute reference doses.

4.4.1.2. Data availability. Liver toxicity is monitored in standard toxicity studies by a range of investigations including organ weights, clinical biochemistry parameters (enzymes, proteins, lipids, etc.), urinalysis, and histopathology. These analyses are regularly performed in repeated dose toxicity studies.

The following endpoints are considered to be mainly related to liver toxicity:

- Pathology (liver weight; gross necropsy and histopathology).
- Haematology (blood clotting time/potential).
- Clinical biochemistry (total cholesterol, albumin, total bilirubin) more than two enzymes indicative of hepatocellular effects (such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, and sorbitol dehydrogenase), additional enzymes (of hepatic or other origin) and bile acids.

Histopathological investigation plays an especially important role in making risk assessment decisions. The following non-neoplastic liver lesions may be observed as a result of liver toxicity: necrosis of hepatocytes, apoptosis of hepatocytes, hepatic lipidosis, cholestasis, pigment deposition, hypertrophy of hepatocytes, necrosis of bile duct, hyperplasia of bile duct, necrosis of non-parenchymal cells (endothelial cells, Kupffer cells), hepatitis, cirrhosis. The most severe injury results in necrosis. However, lesser injury may also result in degenerative changes or in adaptation.

Depending on the dose, most of the above mentioned morphological lesions as well as all of the afore-mentioned effects on parameters of clinical chemistry and haematology may occur after a single application of liver toxins. Necrosis and apoptosis are typical results of acute high dose intoxication. In addition, lipidosis, cholestasis and pigment deposition may also occur as a result of an acute intoxication and may be observed in short-term studies.

4.4.1.3. Data interpretation. Although there are some exceptions (e.g., cholinesterase inhibition by carbamates), it is a general principle in toxicology that effect levels (and no effect levels) decrease with increasing exposure duration, certainly for systemic effects on liver and kidney. This means that—in general—effect and no-effects levels in repeated dose studies may be lower than effect and no-effect levels in single or repeated dose studies (Kramer et al., 1996; Groeneveld et al., 2004).

In general, liver and kidney effects are investigated mostly in repeated dose toxicity studies and mechanistic studies. In many cases, a risk assessor should evaluate whether effects observed in such studies are a relevant starting point for setting an ARfD.

An important consideration is the assessment of adaptive responses of the liver following exposure to xenobiotics. Effects like increased liver weight, hypertrophy of hepatocytes and increased activity of metabolising enzymes are mostly adaptive responses and a reaction of the liver after repeated exposure (Schulte-Hermann, 1979). Therefore, such responses of the liver following exposure to xenobiotics are not considered to be relevant effects for the setting of an ARfD.

Other effects that can occur in the liver when exposed to chemicals for a few days include increased fat accumulation (steatosis) (Zimmermann, 1978; Klaassen, 1996). Maximal effect is often reached after several days of exposure in laboratory animals. Therefore, these effects are of less relevance for the setting of an ARfD.

Other effects essentially occur only (or primarily) after prolonged exposure. Examples of such effects are liver cirrhosis, fibrosis, sclerosis, hepatic congestion, and hepatitis (Zimmermann, 1978). Cirrhosis is the typical result of a chronic liver intoxication resulting from repeated hepatocellular necrosis, regeneration and fibrosis. Further possible effects of chronic liver intoxication are liver tumours. However, neoplastic lesions are usually not observed in short-term studies. Therefore, cirrhosis and neoplastic lesions are not considered to be relevant effects for the setting of an ARfD.

4.4.1.4. Conclusions. When interpreting data on liver toxicity in repeated dose studies, two important aspects should be considered. First, the type of effect observed and second, any information on correlations between exposure duration and the effect.

It is considered that the following findings of hepatotoxicity in repeat dose studies, in isolation, are either adaptive or the result of prolonged exposure and are not applicable for deriving an ARfD: increased serum cholesterol, cirrhosis, neoplastic lesions, induced activity of metabolising enzymes, regenerative hyperplasia, hepatocyte hypertrophy, fibrosis, sclerosis.

All other findings of hepatotoxicity should be considered as potentially relevant to the derivation of an ARfD. In assessing the relevance to acute toxicity the evaluator should consider the general factors on a case by case basis, e.g., the dose level producing effects and the progression of the effect with increased duration of dosing.

4.4.2. Nephrotoxicity

4.4.2.1. General background. The kidney is an organ that is often affected by exposure to toxic substances. Compared with other organs, toxic responses in animal studies occur relatively frequently in kidney. The kidney is particularly susceptible to the toxic effects of drugs and environmental chemicals due to its high blood flow to mass ratio and its unique function of concentrating urine including xenobiotics and their metabolites. As such, the kidney is typically exposed to a higher concentration of chemicals than other tissues (Klaassen, 1996).

For these reasons, the observation and interpretation of effects on kidney are especially relevant for the setting of acute reference doses. Evidence that kidney toxicity occurs after acute exposure to xenobiotics (including pesticides) comes from various studies (Abend, 1994; Agostini and Bianchin, 2003; Hong et al., 2001; Tada et al., 1992).

4.4.2.2. Data availability. Kidney toxicity is monitored in standard toxicity studies by a range of investigations including organ weights, clinical biochemistry parameters, urinalysis, and histopathology. These analyses are regularly performed in repeated dose toxicity studies.

The following endpoints are considered to be related to kidney toxicity:

- Pathology (kidney weight, gross necropsy, and histopathology).

- Clinical biochemistry (blood creatinine, total serum protein, potassium).
- Urinalysis (volume, appearance, osmolality or specific gravity, pH, protein—analysis of marker proteins of glomerular or tubular injury, glucose, cell debris—red and white blood cells, epithelial cells).

4.4.2.3. Data interpretation. Effects and the mechanisms involved in kidney toxicity may be far more complex than in liver (Diezi, 1983). In acute renal failure, several types of mechanisms may be involved such as vascular damage, vasoconstriction, glomerular or tubular injury resulting in haematuria (erythrocyturia, leucocyturia). Measurements of serum creatinine, urea and potassium as well as cell debris in urine are indicators for such effects. Some of the most important pathological changes of the kidneys which may be observed in short-term toxicity studies are:

- Necrosis (cortical necrosis, papillary necrosis).
- Degenerative changes (tubular epithelial vacuolation, hyaline droplets, intracellular inclusion bodies, cytoplasmic pigmentation, tubular dilatation, crystal formation, mineralisation, calculi).
- Inflammatory changes (glomerulonephritis, pyelonephritis, interstitial nephritis).
- Vascular changes (vascular thrombosis, infarction, periarteritis).

The mechanisms contributing to chronic renal failure may be diverse (Klaassen, 1996). Chronic effects in the kidney can be correlated with inflammatory reactions, ischaemia or hyperplasia following regenerative necrosis. Measurement of calcium, phosphate and immunological analysis of antibodies are mostly related to subchronic or chronic effects and therefore of less relevance for the derivation of an ARfD. However, in general, it is difficult to divide effects on the kidney in the categories of 'acute' and 'chronic' because of the various mechanisms involved, both direct and indirect. The kidneys have a substantial capacity for compensation upon loss of renal function due to chemical insult. This indicates that changes in renal function due to chemical exposure may not be detected until the levels at which compensatory mechanisms occur are exceeded. However, if such compensatory mechanisms remain operative for a considerable period of time, these alterations can be maladaptive in the long term.

When kidney toxicity is the most critical endpoint available for setting an ARfD, it is difficult to rule out specific effects that are in principal not relevant for setting an ARfD. Although there are some exceptions, it is a general principle in toxicology that effect levels (and no effect levels) decrease with increasing exposure duration, certainly for systemic effects on liver and kidney (Groeneveld et al., 2004; Kramer et al., 1996). This means in general that effect and no-effects levels in repeated dose studies may be lower than effect and no-effect levels in single or repeated dose studies. In those studies where kidney toxicity has been observed after acute exposure, the dose levels are often relatively high (Tada et al., 1992; Hong et al., 2001). The evaluator should therefore, on a case-by-case basis, consider the relevance and appropriateness of selecting kidney toxicity effects for setting an ARfD.

4.4.2.4. Conclusions. It is considered that the following findings of kidney toxicity in repeat dose studies, in isolation, are the result of prolonged exposure and are not applicable for deriving an ARfD: increased organ weight, regenerative hyperplasia, altered serum calcium and phosphate. All other findings of kidney toxicity should be considered as potentially relevant to the derivation of an ARfD.

4.4.2.5. Future directions. There are not enough data on acute liver and kidney effects of pesticides based on the available repeated dose toxicity studies and mechanistic studies. Incorporation of relevant investigations at the beginning of repeat dose studies should provide information that would permit a more refined assessment of the relevance for ARfD derivation of neurotoxicity seen after repeated exposure.

4.5. Endocrine effects

This section is on endocrine disruption which is not considered a toxicological endpoint per se but rather a mode of action that may lead to adverse effects. The report of the International Programme on Chemical Safety on the 'Global Assessment of the State of the Science of Endocrine Disruptors' provides background information on the endocrine system and potential mechanisms of endocrine disruption by chemicals (IPCS, 2002).

4.5.1. General background

Hormones regulate a broad range of biological processes including the development and function of the reproductive and nervous systems, and carbohydrate and protein metabolism. The pituitary, thyroid, adrenal, pancreas, and gonads are major glands/organs of the endocrine system. Each neuroendocrine axis is comprised of a complex feedback pathway.

The complexity and sensitivity of the endocrine system provides the potential for the chemical induction of adverse effects through a number of mechanisms and at many of the organs within the endocrine axis. A single chemical can have multiple effects on an organism, and may disrupt endocrine function through one or more mechanisms. In addition to multiple mechanistic target

sites, endocrine disrupting chemicals may also alter the neuroendocrine axis by modifying the function of one or more of the major organs involved. Such multiple effects may occur at a single dose or may vary with the dose administered. A chemical may also act by one mechanism but cause different effects depending on the life stage of exposure. Most is known about the chemical disruption of the hypothalamic-pituitary-gonadal axis and the hypothalamic-pituitary-thyroid axis, and thus will be the focus of this guidance.

4.5.2. Data availability

At the present time, there are no test guidelines for endocrine toxicity per se. However, the standard developmental and reproduction toxicity guidelines include evaluation of a number of parameters sensitive to perturbation of endocrine function. Test protocols for 90-day and chronic studies (test guidelines no. 408/409 and 452/453; OECD, 2005) include histopathological examination of a range of endocrine tissues (e.g., pituitary, thyroid, pancreas, adrenals, gonads, accessory sex organs, uterus, mammary glands). Circulating hormone levels can be measured during animal studies, usually to investigate the mechanism of changes to endocrine function seen in other studies with the same or closely related compounds and they are not normally part of the routine clinical chemistry evaluations. The OECD and several regulatory bodies (e.g., US Environmental Protection Agency, European Commission) are currently developing and validating a tiered screening and testing approach designed to detect chemicals capable of affecting estrogen, androgen and thyroid hormone activity.

In rat reproduction toxicity/developmental studies, antiandrogenic chemicals typically induce alterations in sexual differentiation in male offspring. Commonly observed effects include changes in anogenital distance, hypospadias, ectopic testes, vaginal pouches, agenesis of the ventral prostate, delay in preputial separation, and nipple retention in male rats. Estrogenic and antiestrogenic chemicals can affect anogenital distance, vaginal patency and ovarian cyclicity, and lead to urogenital malformations in the reproductive tract or interfere with pregnancy in female rats.

4.5.3. Data interpretation

In general, adverse effects on the endocrine system observed in routine toxicological testing for regulatory purposes—other than those agents that disrupt the precise timing of endocrine events involved in the control of the ovarian cycle and maintenance of pregnancy, kill important hormone secreting cells, and affect development of the offspring—are considered to be unlikely to arise as a consequence of acute adult exposure. However, there may be exceptions, therefore a case by case analysis should be conducted.

4.5.4. Developmental effects

Aspects of normal development are highly dependent on the proper timing, presence, and function of steroid hormones, and any interference with the normal sequence of endocrine events may produce permanent alterations in reproductive tract morphology and/or function. At later stages, hormonal activity regulates growth and puberty. As with any developing system, a single dose of a chemical during a critical window can potentially lead to abnormal development (see Section 4.5.4). Thus, it should be assumed that an acute exposure to an endocrine disrupting chemical can have an adverse effect to the fetus, unless there is information to indicate otherwise.

4.5.5. Reproductive function

When interpreting endocrine effects on reproduction, one must consider the basic biology of the animal, gender differences in the hormonal control of reproduction, as well as the unique features of the hormonal control of pregnancy. This is particularly true for interpreting the appropriateness of setting ARfDs on data routinely obtained after repeated or chronic dosing. There is general support that acute interruption of the hormonal control of testicular function and male reproductive physiology does not result in an adverse outcome. However, there are exceptions to this generalisation. Leydig cell toxicants, such as ethane disulfonate, will destroy these cells within the testes after a single dose and the resulting decrease in testosterone availability can persist for prolonged periods. Similarly, in the female, where there are a series of critical endocrine events that occur at key times over the ovarian cycle which when altered by chemical exposure, a single exposure may result in adverse outcome. It has been shown that a brief exposure to certain pesticides (e.g., triazines, dithiocarbamates) administered during a sensitive window for neural regulation of ovulation, will block the preovulatory surge of LH, delay ovulation and result in impaired oocyte viability, polyspermia, altered viability of the embryo/fetus and reduced litter size (Stoker et al., 2003). Thus, the observation of an adverse effect on female reproductive physiology and pregnancy after a chronic study should raise the concern that a similar effect may be seen after a single dose.

4.5.6. Thyroid function

A single dose of a chemical that perturbs thyroid homeostasis would not be expected to impact tissue function due to the buffering of thyroid hormone concentrations by homeostatic mechanisms. Thus, it would not be appropriate to establish an ARfD based on thyroid effects in the adult rat. Furthermore, the rat is a sensitive model to thyroid perturbation. A number of quantitative differences between rats and humans explain this increase in sensitivity including a longer

half-life and larger reserve of thyroid hormone in humans compared to rats (Dohler et al., 1979). Most antithyroid pesticides operate by increasing hepatic metabolism and excretion of thyroid hormone. This mode of action is not plausible at realistic levels of human exposure to food use pesticides. Thorough consideration should be given to the appropriateness of deriving of an ARfD based on thyroid effects following repeated dosing to rats.

4.5.7. Weight of evidence analysis

Endocrine processes governing development and adult reproductive function are dynamic events and can have critical windows of sensitivity. If the critical level of hormone or critical level of receptor activation, is altered at critical windows of susceptibility, there is no chance for the endocrine system to compensate. Single doses could result in permanent, adverse effects in such situations. Thus, in determining the appropriateness of setting acute dietary doses based on repeated-dosing studies of endocrine effects, a case by case analysis should be conducted. This analysis should consider kinetics, the components and key events involved in the endocrine mechanism, the redundancy in the system, as well as the ability of the organism to compensate. For example, if the chemical decreases the circulating or tissue concentration of a hormone by interfering with its synthesis or increasing its clearance, it is important to consider the biological half life of the hormone relative to the length of the critical window, as well as the concentration of free hormone and hormone binding protein. Changes in plasma hormone levels in the absence of morphological effects need to be interpreted with caution because such changes alone may not necessarily signal an adverse event. Dose response assessment of in vivo effects data should aid in determining potency of the endocrine disrupting chemicals. Finally, any change in reproductive or thyroid function must be considered in light of the overall response observed in the animal as well as age and sex differences. As these hormones may also be influenced by excessive general stress and systemic toxicity, these potentially confounding factors must be taken into consideration when interpreting the results of studies on suspected endocrine disruptors.

4.5.8. Conclusions

Developmental effects found after exposure to an endocrine disrupting chemical should be assumed to be relevant for establishing acute reference doses, unless there is information to indicate otherwise. It should be assumed that effects on female reproductive function and development of their offspring can arise if there is a single well-timed exposure. These effects should be considered relevant for setting an ARfD. Effects on male reproductive function are more likely to occur with repeated or chronic exposures.

Thyroid effects in the rat are not considered appropriate for derivation of ARfDs given the buffering capacity of the human thyroid system compared to the rat.

Because there are exceptions to generalisations, in interpreting the appropriateness of endocrine toxicity endpoints from repeated dosing studies for derivation of ARfDs, a case-by-case weight of the evidence analysis is needed that considers all pertinent information including the basic biology of the endocrine system that is perturbed.

4.5.9. Future directions

Endocrine toxicity is a rather new discipline and as such the guidance given is considered to be interim.

4.6. Developmental effects

4.6.1. General background

The period of development is defined as ranging from the formation of the gametes (of either parent) through prenatal development, and postnatally to the time of sexual maturation. The range of adverse effects which may arise from exposure to a chemical during development include death (pre- or postimplantation loss/resorption, fetal, stillbirth, or postnatal), malformations or variations of a structural nature, growth retardation or specific developmental delays, and altered postnatal functional or behavioural capabilities. These effects may be manifested anytime during the lifespan of the organism. When identifying the appropriateness of selecting endpoints and doses from studies with developmental exposures for derivation of an ARfD, it is important to consider critical or sensitive windows of exposure that impact on the developing organism (Manson and Wise, 1991). Compared to the adult, the developing organism undergoes rapid and complex changes in relatively short period. It is well established that a single dose of a chemical can induce developmental effects.

4.6.2. Data availability

Because development occurs over a relatively prolonged period, several protocols are used to address different potential periods of susceptibility and endpoints of developmental toxicity (IPCS, 2001b). The commonly used protocol for evaluating developmental toxicity in laboratory animals is the prenatal developmental toxicity study that involves administration of the test compound to pregnant animals (usually rats or rabbits) during gestation (test guideline no. 414 OECD, 2005). Another protocol is the multi-generation reproduction toxicity study (test guideline no.416; OECD, 2005) and the one-generation reproduction study (test guideline no. 415; OECD, 2005). These reproduction studies are unique in that they produce a F_1 generation that is exposed during the entire period of development (i.e., prior to conception through sexual maturation). The

parameters measured in these studies evaluate several key developmental effects including viability and growth, endpoints of reproductive development (e.g., anogenital distance, vaginal patency, preputial separation), structural malformation of reproductive tissues, external morphology, as well as reproductive function as an adult. A third study, designed to evaluate critical windows of nervous system development is the developmental neurotoxicity study (OPPTS, 1998b). In this protocol, pregnant rats are dosed with the chemical from gestation day 6 through to postnatal day 10 or 21, and offspring are evaluated for neurobehavioural and neuropathological effects.

4.6.3. Interpretation of data

Typical developmental toxicity study designs generally do not provide information to distinguish whether a developmental effect resulted from a single dose or only after multiple doses. Unless information indicates otherwise, it should be assumed that treatment-related adverse effects on the fetus or offspring resulting from exposure during any phase of development (i.e., from gametes through sexual maturation) are appropriate to use in acute dietary risk assessment, despite the fact that the treatment generally involved repeated dosing.

Although there have not been rigorous investigations, ARfDs based on reductions of fetal body weight gain arising from multiple dose studies are generally thought to be conservative. A recent analysis (van Raaij et al., 2003) compared results from a small number of chemicals tested in a developmental toxicity study and found that the NOAELs for fetal weight reductions following acute exposures tended to be higher than the NOAELs from multiple dose studies. The authors, however, do not rule out the possibility that such body weight changes would not occur after a single dose and there are examples in the literature. For example, a single dose of 5-fluorouracil on gestation day 14 in a rat model resulted in significant fetal body weight change, which was the most sensitive developmental effect (Lau et al., 2001). Thus, any reductions in fetal body weight should be evaluated in the context of all pertinent data including other developmental effects as well as maternal toxicity.

Decisions concerning the appropriateness of endpoints from developmental studies must be based on a case-by-case scientific judgement, and the selection of any endpoint for acute dietary risk assessments requires a consideration of all pertinent information including data on metabolism/toxicokinetics and mechanism of toxicity. For example, the assumption that an observed effect is due to a single dose may be conservative if developmental effects data correlate with the AUC rather than C_{max} as determined in pharmacokinetic studies.

Maternal toxicity following repeated dosing in developmental toxicity studies may not be appropriate for setting an ARfD unless the clinical observations or other toxicity in the dams are observed after a single dose of the test substance.

In selecting endpoints and doses for derivation of reference doses, Environmental Health Criteria document nr. 225 provides guidance on the evaluation of developmental and reproduction toxicity data (IPCS, 2001b). Very briefly, it is important to look for the pattern of responses and the relationship of responses across different studies, and to carefully interpret the toxicological significance of some developmental effects (e.g., altered incidence of structural variations) by taking into account background incidence and strain or species specific factors. It is also important to distinguish a developmental effect from a secondary response. Because standard study designs require that the highest dose tested cause some minimal indication of maternal toxicity, it is important to distinguish whether developmental effects seen at doses causing maternal toxicity are a direct cause of the chemical or an indirect result of altered maternal homeostasis. It should be assumed that the observed developmental effect is the primary consequence of the test chemical (even when maternal toxicity is observed), unless there are data demonstrating that the fetal effects likely occur as a consequence of maternal toxicity which is the result of repeated dosing and are causally liked through a defined mode of action, or the fetal effects are found only at doses that produce excessive maternal toxicity.

4.6.4. Conclusions

Because of critical windows of sensitivity for developmental effects, it should be assumed that most developmental endpoints from repeated dosing studies are relevant for setting acute dietary doses, unless there is evidence to the contrary (e.g., the fetal effects are found only at doses that produce excessive maternal toxicity). The conservatism of this assumption is influenced by a number of factors including the length of the critical window, the kinetics of the substance (e.g., half life, build-up in tissues), and the mechanism of action (e.g., whether it is C_{max} or AUC dependent).

Developmental effects which are limited to reductions in fetal body weight gain or occur only at doses that produce excessive maternal toxicity may not be considered relevant for ARfD setting.

5. Guidance for a single dose study protocol

5.1. Background

Currently available data sets usually do not allow the accurate evaluation of the acute toxicity of compounds. JMPR experts therefore developed a single dose study protocol, with the goal to enable more accurate

derivation of ARfDs with a targeted study for compounds with a reasonably well-known toxicity profile but an inadequate database for derivation of an ARfD. The goal is to submit guidance for this study protocol to the OECD Test Guidelines program.

5.2. Rationale for a targeted single dose study

If the derivation of an ARfD is considered unnecessary, such a single dose study need not be performed. Moreover, if a compound has negligible residues such that dietary intake calculations indicate an adequate margin of safety even when measured against a conservative ARfD derived from a repeated dose study, then it could be considered unnecessary to perform a single dose study.

A specific study designed to enable an accurate ARfD to be set should only be undertaken after the toxicological profile of an active substance is reasonably well documented and understood (i.e., at least the short-term toxicity has been evaluated in rats and dogs). Therefore, at this stage the most sensitive species and relevant toxicology endpoint(s) for an active substance should be known and a specific, focussed study could be designed to investigate this endpoint(s).

Regarding the study protocol, a flexible approach is necessary, depending upon the species and the observed and/or expected effect(s) with a given compound, with use of the minimum number of animals necessary for a thorough safety assessment of the chemical of interest, whilst ensuring the minimum amount of distress in the animals in the test. The study would be tailored to include the evaluation of toxicology endpoints that have been identified in acceptable repeated dose and other key studies with the test substance. This targeted evaluation would ensure efficient study design and execution, with conservative use of animals and other resources.

5.3. Data interpretation

The acute study is only for use in setting ARfDs for chemical residues in food and drinking water and intended to provide data to refine acute risk assessments relevant to acute human dietary exposures. The information should be considered with a view to possible refinement of safety factors used in the derivation of the ARfD.

The aim of the single dose study is to identify the most appropriate NOAEL and LOAEL to derive an ARfD, to provide further information on the dose–response curve and time to peak effects and reversibility for the acute toxic effects after single exposure, and to provide a flexible approach for an adequate characterisation of relevant acute effects. The aim of the single dose study is not to identify any lethal doses or provide data on mortality or morbidity after acute exposure to a chemical. It is also not intended that such a study would be required routinely.

5.4. Principle of the test

The test substance is orally administered as a single dose at several dose levels to groups of experimental animals. A control group is also maintained. The animals are followed closely for signs of toxicity, with termination of subgroups at one of two time periods (within 24 h and up to 14 days post-treatment). Dose levels and study design will be influenced by the quantitative and qualitative outcome of the repeated dose studies, findings in existing high dose acute studies and will be supported by relevant data on toxicokinetics.

5.5. Conclusion

The proposed draft test protocol of the single oral dose toxicity study is not intended to be required routinely. The purpose is rather to give detailed guidance in case there is an indication from the standard tests that there is concern about acute toxicity, but the data do not allow for the derivation of an ARfD. It is intended to be used for compounds with relevant residues in food and drinking water, with a reasonably well-defined toxicity profile and relevant concern about acute toxicity, but insufficient data to reliably derive an ARfD.

5.6. Future directions

The purpose of this single dose study is an additional test in justified cases, specifically tailored to the compound where there is already some information such as target organ toxicity of concern. The publication of this protocol at the JMPR web site would provide harmonised guidance for performance of such a study.

As a next step, it is recommended, that this draft protocol is reviewed and endorsed as a guidance document within the OECD test guidelines programme to avoid unnecessary testing of the same compound by different protocols required by different agencies in the absence of an harmonised test protocol.

6. Overall conclusions

The establishment of an ARfD should be considered for all chemicals, the uses of which may lead to residues in food and drinking water. The appropriateness or otherwise of using doses and endpoints from subchronic and chronic studies to establish ARfDs needs to be carefully considered. Particular weight should be given to observations and investigations at the beginning of repeat dose studies. In the absence of information to the contrary, all toxic effects seen in repeat dose studies

should be evaluated for their relevance in establishing an ARfD.

This guidance proposes a stepwise approach for setting ARfDs for agricultural pesticides, the principles of which are also applicable to other chemical residues in food and drinking water. In particular the guidance outlines some toxicological endpoints which may be particularly relevant as key acute toxicity alerts:

- *Haematotoxicity*: The induction of methaemoglobinaemia is considered to be a critical effect in consideration of acute responses to chemical exposure. A level of about 4% or more above background level in dogs and a statistically significant increase by comparison with controls in rodents is considered to represent a conservative approach to setting an ARfD. Haemolytic anaemia is considered to be less relevant for ARfD derivation since the severity of such an effect appears to generally depend on prolonged exposure.
- *Immunotoxicity*: Immunotoxicity data derived from subchronic studies are not likely to be appropriate for setting an ARfD for acute adult exposure limits because the immune system cells are constantly replaced and because of the inherent redundancy in the system.
- *Neurotoxicity*: Any neurotoxicity seen in repeat dose studies could be the result of a single exposure that is not reparable, i.e., any evidence of neurotoxicity should be considered relevant to an ARfD assessment unless it can be demonstrated that the effects are produced only after repeated exposures.
- *Kidney and liver effects*: If the effects on these organs cannot be discounted as being either adaptive or as the result of prolonged exposure, an ARfD can be derived on the basis of such effects. Such an ARfD is likely to be conservative and it may be possible to subsequently refine it using an appropriately designed single dose study.
- *Endocrine effects*: In general, adverse effects on the endocrine system observed in routine toxicological testing for regulatory purposes—other than those affecting female reproduction and development of the offspring—are considered to be unlikely to arise as a consequence of acute exposure. However, exceptions may occur and a case-by-case analysis is required. These exceptions may include exposure during critical periods of development, exposure during sensitive times in the estrous cycle in which the hormonal control of follicular development and ovulation is altered, and in response to toxicants that are lethal to the hormone secreting cells of the ovary after a brief exposure.
- *Developmental effects*: Any treatment-related adverse effect on foetuses or offspring which has resulted from exposure during any phase of development should be considered as potentially appropriate to use in acute dietary risk assessment, despite the fact that the treatment period typically consists of repeated dosing.

Currently available data sets usually do not allow the accurate evaluation of the acute toxicity of compounds. A single dose study, with the goal to enable more accurate derivation of ARfDs for compounds with a reasonably well-known toxicity profile but an inadequate database for derivation of an ARfD, is not intended to be required routinely. It is intended to be used for compounds with relevant residues in food and drinking water, with a reasonably well-defined (repeated dose) toxicity profile and relevant concern about acute toxicity, but insufficient data to reliably derive an ARfD. If an ARfD is deemed necessary on toxicity grounds then this should be a trigger for performing a short-term (acute) intake assessment in order to identify critical food crops that contribute most to acute exposure and to advise risk management on targeted actions.

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