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GUIDANCE FOR THE SETTING OF AN ACUTE REFERENCE DOSE (ARfD)

(does not necessarily represent the views of the Commission services)

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FOREWORD

The World Health Organisation 1989 publication "Guidelines for predicting the dietary intake of pesticide residues" (WHO, 1989) had formed the basis of consumer risk assessments of pesticide residues for a number of years. Concerns have been recently expressed that acute toxic effects may sometimes be elicited following consumption of food containing residues of certain pesticides. The 1994 JMPR (FAO/WHO, 1994) considered situations in which the ADI derived from subchronic or long-term studies was probably not an appropriate toxicological benchmark for assessing risk posed by short-term exposure to acutely toxic residues.

In 1997, the ECCO meeting discussed in the section Metabolism and mammalian toxicology the Acute Reference Dose (ARfD) as a new concept and it was agreed that more guidance was needed on the setting and the use of this new threshold value. At the ECCO meetings in 1998, a first German draft proposal for setting ARfD-values was discussed. This draft was based on a revised document from ACP (AAHL/3/98) and the recommendations of the 1998 JMPR (FAO/WHO, 1998) in Rome. A revised version of the German draft proposal for setting ARfD-values was submitted by the ECCO-team to the Member countries for comments in 1999. The issue of ARfDs has again been raised by the CCPR during its Thirty-second Session and 2000 JMPR (FAO/WHO 2000) has been requested to develop this area further. National regulatory agencies and the European Union have also addressed the issue.

The proposal is based on the recommendations made to a meeting of the SCP on 11 May 1999 and includes comments from several EU Member countries and recommendations of the 2000 JMPR in Geneva as well as the experience of the ECCO meetings in 1999 and 2000 in which ARfDs were discussed for more than 30 pesticides. The document represents a consensus of present knowledge and will be updated on the basis of new data and/or policy. Therefore, this document is not intended to be legally binding.

This guidance document is intended for use by the European Commission and Member States in the framework of the Standing Committee on Plant Health for setting of acute reference dose at inclusion of active substances on Annex I of the Directive 91/414/EEC. The guidance is also intended to be useful to the applicants for the submission of scientifically reasoned proposals for acute reference dose levels on the basis of all relevant toxicological information as required in Directive 91/414/EEC. The use of an ARfD, however, is part of the risk assessment and decision-making process for the registration of Plant Protection Products at Member State level and is therefore not covered in the scope of this document.

The following points for setting an ARfD needs further discussion by the SCP:

- Some formal EC position on the use of human data should be developed.
- The needs to perform not only a toxicological risk assessment of the full data package, but also an appreciation of the acute dietary intake based on residue data and dietary intake.
- A specific OECD-Guideline should be developed on the basis of the draft study protocol for an appropriate animal study to set an ARfD (Appendix 1)

Chapter 1 INTRODUCTION

1.1 For the assessment of health risk after subchronic or chronic exposure to pesticides, the ADI has been established. Because the ADI is usually based on NOAELs from lifespan or subchronic studies and because the gap between the NOAEL and LOAEL may be large this can introduce an additional safety margin, a single exposure above the ADI for may not represent a real human health risk, if the ADI is not exceeded over a long period of time.

1.2 Certain pesticides might present an acute hazard, however so that such excesses are of toxicological concern. As a matter of standard practice in the risk assessment of residues in food and drinking water, the case for setting an acute reference dose (ARfD) should be considered for all compounds. Since acute effects may occur after only a single dose, exposure above the ARfD will result in a decreased margin of safety.

1.3 The ARfD of a chemical was defined by the 1998 JMPR as „an estimate of a substance in food or drinking water, expressed on body weight basis, that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risk to the consumer on the basis of all known facts at the time of evaluation“ (FAO/WHO, 1998).

1.4 The following categories of toxicological alerts should suggest the need to establish an ARfD (FAO/WHO, 2000):

i. Lethality after administration of a single low dose orally
ii. Developmental effects, except when these are clearly a consequence of maternal toxicity
iii. Clinical signs, other pharmacological effects, or effects on target organs observed early in studies with repeated doses, including effects on behaviour or on the gastrointestinal, cardiovascular or respiratory system
iv. Acute neurotoxicity, including that due to exposure to organophosphates and carbamates.
v. Hormonal or other biochemical alterations observed in studies with repeated doses, which might conceivably be elicited by a single dose.

1.5 When there is no toxicological alert for acute effects, and it is concluded that establishment of an ARfD is unnecessary, the basis for this decision should be clearly stated.

1.6 Although establishment of an ARfD relies heavily on expert judgement, there is the need for a flexible approach, which needs to be reported as transparently as possible. ARfDs may need to be reassessed in the light of new data.

Chapter 2 HAZARD CHARACTERISATION

General considerations on the derivation of an ARfD

2.1 The aim of this chapter is to provide a short reference to the general aspects of hazard characterisation. The fundamental steps in hazard characterisation are:

- description of the toxicological profile of the substance;
- identification of the relevant critical effects in the most relevant and sensitive species; and
- dose-response evaluation and identification of the threshold dose levels for the critical effects.

2.2 Precise information on the toxicological and metabolism data requirements and test methods for the inclusion of an active substance in Annex I and for an application for the authorisation of a plant protection product is provided by Commission Directive 94/79/EC. An ARfD is derived from the information on the toxicological properties of the active substance. The toxicological and metabolism studies required for inclusion of the active substance in Annex I of Directive 91/414/EEC are given in its Annex II.

2.3 For most toxicological endpoints it is generally agreed that there is a threshold below which no toxic effect occurs. The highest dose level at which no statistically and/or biologically significant increases in frequency or severity of adverse effects are observed between the exposed population and its appropriate controls is usually defined as the NOAEL (no observed adverse effect level). Typically, an ARfD is based primarily on a NOAEL.

2.4 Within the standard package of mammalian toxicology studies available for a pesticide active substance there is a wide range of study types investigating numerous end points after different treatment periods. Most of the studies available will have used the oral route. The ARfD is an oral limit value and should therefore be derived from oral data.

2.5 For the identification of the critical effect after single exposure, the quality of the experimental design, the types, site, incidence and severity of effects as well as the treatment period should be taken into account. The critical acute effect may be defined as the most relevant adverse effect occurring after single exposure at the lowest dose in the target organ for acute effects.

2.6 The data package was required originally by Article 13 (1) of Directive 91/414/EEC to derive an ADI and an AOEL. Unfortunately, the available study protocols do not cover both end points and treatment period directly relevant to setting a specific ARfD. It was recognised that current toxicological databases are not designed for this purpose and does not contain a specific study designed to address the end-points and treatment periods relevant to determine an ARfD accurately.

2.7 With regard to the available or required studies for registration of pesticides it is difficult or impossible to estimate the critical acute effects after one or only a few doses, which should be applied for the estimation of a specific ARfD. Therefore, with the exception of a few specific compounds (namely

cholinesterase inhibitors and a few others) ARfDs have usually been established on the basis of multiple dosing studies, with the result that most of them are "conservative".

2.8 In general, an ARfD can only derived, if an ADI is already allocated and the "Criteria for inclusion of Active Substances in Annex I of Council Directive 91/414/EEC" can be fulfilled. In general, it will not be possible to set an ARfD if a threshold dose does not exist.

2.9 With an animal study, it is necessary to consider whether the effects on which a NOAEL is based are relevant to humans. Relevance to humans is sometimes difficult to assess and it has to be assumed that the NOAEL for the most sensitive animal species is relevant to humans in the absence of data against this.

2.10 In general, most data will be obtained from animal studies, but some human data may be available. From the scientific perspective, sound human data might take precedence over animal data. From an ethical point of view, it is necessary to take the ethical aspects into considerations regarding the use of human studies for the authorisation of any pesticide very seriously. However, the ethical status of studies in humans must be established before they are taken into consideration.

Toxicological end-points in Annex II of Directive 91/414/EWG for setting an ARfD

2.11 Directive 91/414/EEC does not require a study specifically designed for deriving an ARfD. For all active substances, all studies in the toxicology database have to be considered in this process but the most relevant studies are likely to be repeated-dose short-term (28-day or 90-day studies) and developmental studies. Acute neurotoxicity studies are not required under 91/414/EEC but when they are available, they are often the most relevant study for setting an ARfD.

AIIA5.1: ADME studies

2.12 These toxicokinetic studies can provide important basic information about oral absorption, distribution, accumulation, metabolism and excretion of a substance. Therefore these background data (e.g. plasma peaks, oral absorption, bioaccumulation data) might be of value for justification, if there is a concern with regard to acute intake of one or few doses for the parent compound or of some metabolites.

2.13 Data on comparative toxicokinetics in animals and humans might be useful with regard to an experimentally based refinement of the assessment factor for inter-species variability.

AIIA5.2: Acute oral toxicity

2.14 These single dose studies are aimed to identify levels producing death or serious signs of toxicity with a main purpose for classification and labelling. In general these studies will have limited applicability for estimation of target organs. Although with some frequency, acute oral lethality studies have included data on relevant clinical signs and or specific effects, they are usually not appropriate to determine NOAELs for critical effects as currently performed. Therefore, no really appropriate studies are

available to assess acute hazard from the database for acute effects after one or only a few doses as the most appropriate basis for an estimation of an acute reference dose.

2.15 In future, acute oral studies with a reduced number of dose levels and animals according to OECD-guidelines 420, 423 or 425 will be preferred for estimation of an „approximate LD50". These LD50 alternatives rely more on observation of signs and effects than body counts so that they may be of more use in setting ARfDs.

AIIA5.3: Subacute studies

2.16 Before initiating 90-day studies, subacute oral range-finding studies will often be performed. These studies will use dosing for short-term periods such as 14 days to mostly 28 days and aim to identify particularly a maximum dose and sometimes also a NOAEL, which could be used in the follow-up studies. Therefore, small animal numbers and large dose intervals not appropriate for setting of threshold values are normally used.

2.17 Subacute studies may represent one of the most adequate sources of data for setting ARfDs, particularly if the relevant acute toxic endpoints have been measured. However, they must be of adequate design (e.g., compliant with OECD 407).

AIIA5.3: Subchronic studies

2.18 Mostly two different species (rat, dog) are investigated after repeated dosing via gavage or diet for commonly 90 days. Extensive histopathological, clinical chemistry and haematology examinations are performed, that these studies are likely to cover key end points. But the effects noted are results of a repeated dose exposure (much more than one or only a few doses).

2.19 Subchronic toxicity studies are most commonly used, but are not the most appropriate types of 'standard' study for setting ARfDs. Sometimes the same dose levels are used in the studies from which the ADI is estimated/derived. Such studies should be used for setting ARfDs, if it can be justified, that there is no relevant difference between acute and subchronic effects or when there is an absence of acute data, as this provides a conservative first tier assessment. In addition, there may be instances where findings seen at the start of a repeat dose study are due acute exposure.

AIIA5.4: Genotoxicity studies

2.20 These studies are aimed to identify genotoxic effects - mostly in vitro - that they are generally of limited value in setting ARfDs.

2.21 There are a small number of in vivo tests (e.g. a micronucleus study) in which single high doses are administered, but only a limited number of endpoints with regard to setting ARfDs are investigated. If the appropriate end point has been determined with a NOAEL, such in vivo genotoxicity studies may be considered helpful for setting an ARfD.

AIIA5.5: Chronic/carcinogenicity studies

2.22 Chronic toxicity studies are the most commonly used types of 'standard' study for setting ADIs. Such studies could be used for setting ARfDs, if it can be justified, that there is no difference between acute, subchronic and chronic effects. Generally, all aspects relevant to setting an ARfD will have been investigated in more appropriate shorter-term studies.

2.23 Although an ARfD is routinely based on the relevant NOAEL from a particular set of short-term toxicity studies, it may sometimes be more appropriate to use a higher NOAEL, especially when a chronic study may indicate a higher NOAEL than a short-term study because of differences in dose level selection.

AII5.6: Multi-generation studies

2.24 The observed adverse effects on parental toxicity and reproductive performance are related to a repeated intake and may influence the ARfD conservatively. However, some fertility effects can be due to a single dose at a critical time.

2.25 The studies are unique in investigating certain end points especially in the offspring and sometimes pregnant/lactating animals. Therefore, this only type of 'standard' study, which investigates any evidence of increased effects/susceptibility in this sub-group, should be considered carefully for use in setting an ARfD.

AIIA5.6: Developmental/teratology studies

2.26 These studies generally use gavage dosing for approximately 10 to 15 days during gestation. The main aim is to investigate effects on the foetus and the dam. Any effects on the foetus can be considered as being caused by a single or few doses, but this is usually not justified by standard studies. Given the relatively short dosing period any effects reported could be relevant to an ARfD assessment, especially if developmental toxicity is the most critical effect.

2.27 The parameters investigated for non-reproductive end points in the dams are often very limited and only estimated in one sex. Sometimes, there is a dose free period of approximately 4 to 6 days before sacrifice. The critical effects may be influenced by an increased susceptibility in pregnant animals, which refers to a specific sub-population.

2.28 If the critical end point has been investigated and an NOAEL was determined, these studies may be applicable to derive an ARfD for a sensitive sub-population (e.g. pregnant women) and the unborn offspring.

AIIA5.7: Delayed neurotoxicity studies

2.29 These single or multiple dose studies are aimed to identify critical neurotoxic effects for specific substances (e.g. organophosphates). According OECD test Guideline 418 & 419 these tests are

performed on hens. For most pesticides, the delayed neurotoxicity is not the acute critical effect; therefore these tests are mostly negligible for setting an ARfD.

AIIA5.7: Neurotoxicity studies

2.30 Various pesticides have specific neurotoxic properties, which may become apparent as specific behavioural changes (see also clinical signs) or specific neuropathological lesions. If neurotoxicity is the most critical effect, acute or developmental neurotoxicity studies on rats will probably be available and are highly relevant for setting an ARfD.

2.31 Acute neurotoxicity studies are extremely useful even for non-neurotoxic agents, as they look at behaviour, signs and a range of organs in considerable detail.

AIIA5.8: Mechanistic studies

2.32 These tests (e.g. disruption of hormonal status, enzyme induction profile) are performed to clarify mechanisms of target effects. Mostly repeat intakes are used and several examination times will often be present with a view to understanding temporal effects and identifying NOAELs. The significance of the investigations to man will need to be assessed on a case-by-case basis as there is no standardised design applied for such studies.

AIIA5.9: Human volunteer studies

2.33 Investigations on humans might be very useful for the derivation of the ARfD. However, the performance of new studies is only acceptable, if they fulfil the respective national and international ethic conventions (Anonym, 1998).

2.34 These may be available for compounds with a well-understood and reversible mechanism of toxicity (e.g. organophosphorus compounds and carbamate insecticides), which can be monitored by non-invasive techniques or blood sampling. Studies on closely related compounds with medicinal uses may be of value. Repeat dosing, 2 weeks or more, is often used and multiple investigation times will provide time course information and data on reversibility. Especially, if the critical effects are investigated after one or only a few doses these studies are of great importance for setting ARfDs. However, some older studies are of questionable quality.

2.35 Human volunteer studies can remove the uncertainty of inter species extrapolation and usually measure effects over short periods. However, it is not acceptable exposing humans to pesticides, only for the sake of lowering a safety factor. If the appropriate end point has been determined after one or only a few doses these could be considered the most appropriate study for setting an ARfD.

Chapter 3 EXTRAPOLATION FROM TOXICITY DATA TO AN ARfD

Single and multiple exposures

3.1 The food item of concern could be a fruit that is eaten within a short time and the acute dietary intake can occur at single sitting (one meal or serving) or be spread over one day. Therefore, it could be necessary for specific effects of concern to compare a single treatment with the same dose administered in a repeated-dose study over one day. Especially, if rapid reversibility is playing an important role, a specific study design (administration by repeated gavage on the same day or by diet over 24 h) for the specific exposure scenario may be necessary.

3.2 For certain targets, it is possible, that the critical effect observed after single exposure might be changed after multiple exposures by the induction of adaptive mechanisms, e.g. induction of metabolising enzymes or of repair mechanisms. Such mechanistic considerations must be taken into consideration, if effects after single dose exposures should be extrapolated to multiple exposures and vice versa.

Gavage versus Diet

3.3 Gavage administration has been criticised with respect to its relevance for human exposure because this way of dosing results in a bolus dose in the GI-tract, whereas human exposure through the diet will show a more gradual exposure within a certain time frame. For most substances, however, gavage administration might be considered as a 'worst case' acute exposure compared to dietary exposure. On the other hand, eating a 'hot apple' may be considered a 'gavage-like' exposure.

3.4 For some organophosphate pesticides, which induce a prolonged AChE inhibition, it may be argued that a certain dose fractionated over several dosages within one day might result in a larger AChE inhibition than that resulting from a single bolus dose. In these cases, gavage dosing can possibly not be considered as a 'worst case' condition.

3.5 The possible difference in the ARfD value based on differences in gavage vs. dietary exposure is considered to be relatively small compared to other uncertainties such as the accuracy of the NOAEL and the use of assessment factors. Therefore, gavage dosing is considered a relevant treatment for setting an ARfD, unless the available information indicates otherwise (e.g. kinetics or the use of irrelevant/peculiar vehicles).

Different species

3.6 In different species, the same substance can show different critical effects or targets after single and multiple exposures. Most toxicological studies required for pesticides are performed in rats. Dogs must be tested as second species after a subchronic exposure period. Mice are normally tested only in long-term bioassays and the rabbit is only tested in developmental toxicity studies as second species. Acute delayed neurotoxicity can be tested only in hens.

3.7 Ideally, an ARfD should be based on a specific study in the most sensitive species. If a specific ARfD cannot be set and is based on a study in another species, this must be taken into consideration and explained as part of the decision making process.

Different population sub-groups

3.8 For an acute dietary risk assessment different population sub-groups might be considered. But in most cases, only toxicity studies on adult or sometimes pregnant animals are available. In the standard data package for pesticides, there is no appropriate database for the determination of different ARfDs with regard to different population sub-groups. Therefore, it is not desirable from a regulatory point of view to have more than one ARfD per substance and, conform the ADI concept; only one ARfD should be derived, and is meant to cover all sub-population groups.

3.9 If consideration of specific sub-populations is required based on the toxicological profile, specific subgroups might be investigated (e.g. juvenile vs. adult or pregnant vs. virginal). At the 1998 York meeting, there was a plenary discussion as to whether an extra safety factor needed to be applied for children < 6 years old. It was concluded that as long as the database was adequate, no additional factors should be routinely applied (Anonym, 1998).

Assessment factors

3.10 To translate the critical NOAEL into an ARfD, assessment factors accounting for uncertainties in extrapolation from toxicity data to the exposed human population have to be applied. For the sake of clarity in this guidance document, the term assessment factor is used and is meant as a general term to cover all factors designated in the literature as safety factor, uncertainty factor, extrapolation factor, adjustment factor, etc.

3.11 At present risk assessment for non-cancer toxic endpoints is based on the assumption of a threshold and makes use of standard default uncertainty factors. These are based on a 10-fold factor for interspecies variability and a 10-fold factor for intra-individual variability when considering risks to the general population. Indeed, an overall assessment factor of 100 has been proposed for the majority of ARfDs set at ECCO peer review meetings on Mammalian Toxicology.

3.12 In order for either toxicokinetic or mechanistic data to contribute quantitatively to risk assessment, in the absence of a full biologically based dose-response model, it was recommended that the current procedure of applying 10-fold factors for inter-species differences and human variability be refined (IPCS, 1994). Subdivision of each 10-fold factor into toxicokinetic and toxicodynamic components would allow part of the default to be replaced by relevant, chemical-related, specific data when these were available. Therefore, these values have been further subdivided on the basis of a separation between toxicokinetics and toxicodynamics for both extrapolation from animals to humans (2.5 and 4.0 respectively) and for intra-human variability (3.16 and 3.16 respectively). Full details as to how these were derived are provided in IPCS EHC 210 (IPCS, 1999).

3.13 Physiologically based pharmacokinetic modelling may be of value in determining the appropriate factor to use. It should be considered that when the end-point of concern for an acute effect is due to reversible interaction of the compound with a pharmacological target, such as a receptor or an ion

channel, the concentration of the substance, rather than total exposure, should determine the magnitude of the effect. It is conceivable that it would be possible to reduce the assessment factor from the normal default of 100. The establishment of ARfDs might be particularly well suited to the derivation and application of chemical-specific adjustment factors to replace the default uncertainty factor. However, the extent to which the uncertainty factor could be reduced under such circumstances has yet to be determined (FAO/WHO 2000).

3.14 Discussion and weighing of all available data is an important element for the final choice of the overall assessment factor. The basis for the proposed overall assessment factor needs to be clearly stated. Key factors are:

(a) interspecies differences in toxic response to a chemical

(b) intraspecies (interindividual) differences in toxic response to a chemical

(d) quality/extent of toxicity data

(a) Interspecies differences

3.15 Where the critical NOAEL is based on animal data, a factor of 10 is normally applicable. If the critical NOAEL is based on reliable human data, a factor of one may be applicable; if there are significant limitations to the human data that determine the critical NOAEL, a factor higher than one should be used. In special cases, where there are data, which permit a more reliable comparison of animal versus human sensitivity for the critical toxicological effect of the substance, consideration should be given to refining the standard interspecies factor of 10.

3.16 Renwick (1993) analysed data on inter-species differences and human variability in toxicokinetics and toxicodynamics for a limited number of compounds. These data indicated greater potential variability within kinetics than in dynamics, so that a larger factor was suggested for kinetic variability. Based on this analysis, it was proposed that the 10-fold standard interspecies factor could be subdivided with a factor of 4 ($10^{0.6}$) for toxicokinetics and 2.5 ($10^{0.4}$) for toxicodynamics (IPCS, 1999).

(b) Intraspecies (interindividual) differences

3.17 It is recommended that the current precautionary approach followed at ECCO Mammalian Toxicology meetings of applying a 10-fold factor continues, unless a convincing scientific case can be made for applying a lower factor.

3.18 In subsequent review for a WHO Task Group on Environmental Health Criteria for Guidance Values for Human Exposure Limits (IPCS, 1994), it was concluded the database analysed by Renwick (1993) was insufficient to justify an uneven subdivision of the 10-fold factor for human variability, and therefore this factor was divided evenly into two sub-factors each of $10^{0.5}$ (3.16 or 3.2). This equal subdivision of the human variability factor was supported by a subsequent more extensive analysis (Renwick & Lazarus, 1998), of appropriate kinetic parameters for 60 compounds in humans, and concentration-effect data for 49 compound-related effects.

(c) Quality/extent of toxicity data

3.19 If there are limitations in the available short-term toxicity data, it may still be possible to set an ARfD but in this case the ARfD is at the same level as the ADI. Such a situation might arise, for example, where no appropriate study has evaluated the critical acute effect of concern and further data are considered necessary.

Chapter 4 RECOMMENDED DETERMINATION OF THE ACUTE REFERENCE DOSE

Determination of the Acute Reference Dose

4.1 It is recommended that an ARfD is derived in a stepwise approach considering what specific toxicological data are provided by manufacturers for the calculation of ARfDs. The following logic steps are proposed:

- Consider the toxicity profile and determine, if acute effects are likely to be relevant.
- If acute effects are not relevant, allocation of an ARfD is not necessary.
- If acute effects are relevant, derive an ARfD as best possible using available data.
- If subchronic , chronic or other multiple dose studies are used to derive the ARfD, comment that the value may be conservative.
- If an ARfD is derived from the same study as ADI and any acute exposure might be higher than the ADI, further data may allow refinement.

4.2 The following are three principal possibilities:

i. Not allocated because not necessary;
ii. Derived from an appropriate study characterising a specific acute hazard;
iii. Derived from the same study as ADI.

(i) No allocation of an ARfD

4.3 If, on the basis of its toxicological profile, a pesticide is considered unlikely to present an acute hazard, an ARfD will not be established. This should be clearly expressed in the evaluation report. In such cases, an acute intake of residues might exceed the ADI for several days. It seems reasonable to attempt to identify categories of pesticides for which an ARfD would normally not be necessary. But, a clear guidance cannot be given on the current experience in setting such values.

4.4 The following reasons may be of importance, that it can be concluded from the submitted toxicological data, that there is no concern with regard to acute intake of one or few doses and an acute reference dose might not be allocated or necessary:

- the pesticide has shown a very low acute oral toxicity (e.g. no adverse clinical signs and deaths have been observed at the limit dose for acute LD50 testing), and
- the toxicological profile was based only on e.g. mild effects, which are not relevant for acute intake (e.g. adaptive liver enlargement, chronic body weight reduction, reduced food intake), and
- the toxicological profile has no other alerts for acute toxicity.

4.5 In the list of end points regarding „Impact on Human and Animal Health“ it should referred as follows:

ARfD	Not allocated (not necessary regarding ...[see above].)

4.6 The following reasons may be of importance, that it can be concluded from the submitted residue data, that there is no concern with regard to acute intake of one or few doses and an acute reference dose might not be necessary regarding the current uses:

- supported uses cannot result in residues in food or feed (e.g. rodenticides), or
- available data confirm that no residues in food or feed will occur from the use categories, or
- no residues will be detectable for all possible intended uses (e.g. herbicides), or
- the toxicity profile suggests that an ARfD derived from appropriate acute or repeat-dose studies would be substantially in excess of the „best estimate“ figure, which could be taken in with normal food.

4.7 In the list of end points regarding „Impact on Human and Animal Health“ it should referred as follows:

ARfD	Not allocated (not necessary regarding current uses)

(ii) Determination of a specific ARfD

4.8 If specific acute toxic effects have been observed and short-term intakes above the ADI are likely, an estimation of a specific ARfD might be necessary. In this case the ARfD is be related to a specific effect.

4.9 When it has been determined that an ARfD should be established but data do not permit establishment of an accurate value, it will be allocated with a more conservative value on the basis of the available information with an indication of the type of study considered to be necessary to refine the estimate.

4.10 For animal welfare reasons, new acute studies to refine the estimate should not be required when it has been determined that intake does not exceed an ARfD based on short or long-term studies. However, if short-term intakes are clearly estimated above the ARfD, additional studies may be required and/or performed.

4.11 A specific study designed to enable an accurate ARfD to be set will only be undertaken after all other studies required have been conducted. In this situation, the toxicology profile of an active substance will be fully documented and understood. Therefore, the most sensitive species and relevant toxicology end-point(s) for an active substance would be known and a specific, focussed study could be designed to investigate this end-point(s).

4.12 A draft study protocol for such an appropriate animal study to set an ARfD was proposed by the 2000 JMPR (FAO/WHO 2000). For this purpose, a specific OECD-Guideline should be developed on the basis of the proposal, which is presented in Appendix 1.

4.13 In the list of end points regarding „Impact on Human and Animal Health“ it should referred as follows:

ARfD	x.xx mg/kg bw, based on ABCD study

(iii) Using of the ADI as appropriate for ARfD

4.14 Where a specific ARfD cannot be derived from the available toxicology data base, and an acute dietary risk assessment is judged necessary, the ADI should be used e.g. to set a more conservative ARfD

based on long-term effects. For animal welfare reasons, a specific acute study for the determination of a specific ARfD will not be required, if short-term intakes are not estimated above the ADI.

4.15 If there are no significant / substantial differences between the critical endpoints identified in the acute and repeated dose toxicity studies, the ARfD should be derived from the lowest relevant dose level from the subchronic or chronic toxicity study with the relevant endpoint on which the ADI would normally be based.

4.16 If the lowest NOAELs of the acute, short-term and long-term studies are at comparable dose level and it can be concluded, that there are no relevant differences in the critical effects, then the ADI should be used for pragmatical reasons as an estimate of risk from acute intake. In such cases, an exceeding of the ADI is not allowed, also for a short-term period or single ingestion.

4.17 In the list of end points regarding „Impact on Human and Animal Health“ it should referred as follows:

ARfD	Likely to be in the same order as the ADI (x.xx mg/kg bw)

Chapter 5 SUMMARY AND OVERALL CONCLUSION

5.1 The ARfD of a chemical was defined by the 1998 JMPR as „an estimate of a substance in food or drinking water, expressed on body weight basis, that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risk to the consumer on the basis of all known facts at the time of evaluation“ (FAO/WHO, 1998).

5.2 Derivation of an ARfD should be considered for all pesticides. However, this needs not only a toxicological risk assessment of the full data package, but also an appreciation of the acute dietary intake based on residue data and dietary intake. This information from the residue evaluation is essential for an efficient estimation of an ARfD.

5.3 The determination of the most relevant end point in the best applicable study using the most appropriate safety factor needs consideration on a case-by-case basis. The appropriate end point for setting the ARfD should be based on expert judgement following a thorough review of the ADME and mammalian toxicology package together with information on the likely mechanism of toxicity. In some instances a potentially relevant end point may be identified from the mechanism of action. Human in vivo data can be extremely useful when appropriately and ethically conducted (Anonym, 1998).

5.4 The standard mammalian toxicology package for pesticides might contain no studies, which are really appropriate to set an ARfD. In many instances the available study protocols do not cover both end points and timings directly relevant to setting a rigorous ARfD (AAHL/3/98). However, where possible, an attempt to derive a „best estimate“ ARfD on the basis of available data should be made; only in exceptional cases additional studies should be required.

5.5 It is recommended that an ARfD is to derive in a stepwise approach considering what specific data are provided by manufacturers for the calculation of ARfDs. Principal possibilities likely to be either:

i. Not allocated because not necessary;
ii. Derived from an appropriate study characterising a specific acute hazard;
iii. Derived from the same study as ADI.

5.6 If a pesticide was shown not to present an acute hazard or if its uses cannot result in residues, or data show no residues in food or feed, an ARfD needs not be derived. In such cases, the reasoning must be clearly indicated.

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Appendix 1 PROPOSED TEST GUIDELINE - SINGLE ORAL DOSE TOXICITY STUDY

(Only for use in setting acute RfDs for pesticide residues in food and drinking water)

A. Background

Dietary exposure to a substance in food or drinking water may occur over a short period of time, e.g. , during a single meal or over one day. The consumption of pesticide residues remaining on treated agricultural commodities represents such an exposure. Sometimes, the toxicological profile of the chemical substance raises concerns about the potential for risks of concern following such exposures-particularly to infants and children. As a matter of standard practice in the risk assessment of residues in food and drinking water, the case for setting an acute reference dose (ARfD)[1] should be considered for all compounds. The decision to proceed, however, must be made on a case-by-case basis.

A specific study designed to enable an accurate ARfD to be set will only be undertaken after all other studies required have been conducted. In this situation, the toxicology profile of an active substance will be fully documented and understood. Therefore, the most sensitive species and relevant toxicology end-point(s) for an active substance would be known and a specific, focussed study could be designed to investigate this end-point(s).

This study would be conducted only after it has been determined from the existing toxicological database that acute effects may occur. Overall, there is the need for a flexible approach, depending upon the species and the observed and/or expected effect(s) with a given compound. The study would be tailored to include the evaluation of endpoints that have been identified as targets in acceptable repeated dose and other key studies with the test substance. This targeted evaluation would assure the greatest efficiency in study design and execution, and would reflect refinement of the use of animals and other resources.

Several categories of toxicological alerts have been identified which support the need to establish an acute RfD (FAO/WHO 2000). They include:

- Lethality after administration of a single low dose orally
- Developmental effects, except when these are clearly a consequence of maternal toxicity
- Clinical signs, other pharmacological effects, or effects on target organs observed early in studies with repeated doses, including effects on behaviour or on the gastrointestinal, cardiovascular, or respiratory system
- Acute neurotoxicity, including that due to exposure to organophosphates and carbamates.
- Hormonal or other biochemical alterations observed in studies with repeated doses,, which might conceivably be elicited by a single dose.

B. Purpose

This study provides information on the possible health hazards that may arise following a single exposure to the

[1] The acute reference dose is defined as " the estimate of the amount of a substance in food or drinking water, expressed on a milligram per kilogram body weight basis, that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risk to the consumer on the basis of all the known facts at the time of the evaluation" (WHO, 1997)

test substance. The data from this study provide information useful for the setting of an acute reference dose to be used in estimating acute dietary risk for infants, children and other members of the population.

The test method incorporates relevant elements of the acute neurotoxicity screening battery which incorporates single exposures to the substance and of the basic repeated dose toxicity studies in rodents and nonrodents (i.e., 28-day, 90-day and chronic) that may be used for chemicals when such information is needed to understand the consequences of longer-term exposures. It includes single exposures at multiple dose levels which are either minimally toxic or NOAELs. The goal of the study is to identify the most appropriate NOAEL to which safety factors are applied to derive an ARfD.

C. Principle of the Test

The test substance is orally administered as a single dose to several groups of experimental animals, one dose level per group. A control group is also maintained. The animals are followed closely each day for signs of toxicity, with termination of the subgroups at one of two time periods (24-hours and at least 14 days post-treatment). Animals that die or are killed during the test are necropsied and at the conclusion of each subgroup's test period, the remaining animals are sacrificed.

D. Description of the method

1. Selection of animal species

Rodent: The preferred rodent species is the rat, although occasionally, it may be that the mouse is shown to be more sensitive than the rat or a better model for the human. Commonly used strains of healthy animals should be employed. Females should be nulliparous and non-pregnant. When adult animals are to be studied, dosing should occur when the animals are between 8 and 10 weeks of age. There may be circumstances, however, in which it is desirable to determine if there are age-related differences in sensitivity of response to the substance. In these cases, there should be more than one group of animals studied-one group for which dosing occurred at age 8-10 weeks, the other(s) for which dosing occurs at one or more time periods earlier in postnatal life. At the commencement of the study, the weight variation of the animals used should be minimal and not exceed $\pm$ 20% of the mean weight of each sex. Preferably the animals used in this study and from the same strain and source as those animals used in the repeated dose and other key studies that make up the toxicological database for the test substance.

If the existing toxicity database, including, at a minimum, a 90-day, subchronic repeated dose study, indicates that the dog (or mouse) is significantly more sensitive than the rat, and no other information exists to indicate which species is more appropriate for human health hazard assessment, then groups of dogs or mice may be used. If the mouse is the preferred rodent species, the principles employed for the rat should be applied.

Nonrodent: If the dog is identified as the species of choice, a defined breed should be selected; the beagle is frequently used. Young adult animals should be used. Dosing should commence after a period

of acclimation (at least 5 days is recommended), preferably at 4-6 months of age, but not later then 9 months of age. At the commencement of the study, the weight variation of the animals used should be minimal and not exceed ± 20% of the mean weight of each sex. Females should be nulliparous and non-pregnant.

2. Housing and feeding conditions

Rodents: The temperature in the experimental animal room should be ± 22 degrees C (± 3 degrees C). Although relative humidity should be at least 30% and preferably not to exceed 70% other than during room cleaning, the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Rodents may be housed individually, or be caged in small groups of the same sex; for group caging, no more than five animals should be housed per cage.
Dogs: It is recommended that each animal be caged individually. In any case, the number of animals per cage must not interfere with a clear observation of each animal. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

3. Preparation of animals

In the standard study, healthy young adult animals are randomly assigned to the control and treatment groups. Cages should be arranged in such a way that possible effects due to cage placement are minimised. The animals are identified uniquely and kept in their cages for at least 5 days prior to the start of the study to allow for acclimatisation to the laboratory conditions.
When the standard study is expanded to evaluate exposures to subjects pre-weaning, then the animals (dams and offspring) must be handled in a manner consistent with OECD TG 416 (the multigeneration reproduction study).

4. Preparation of the doses

a. For technical reasons, the test compound (and control vehicle, if one is needed) should administered by gavage to the rodent, by capsule to the dog. However, an ARfD is designed to protect consumers of food and therefore the option to prepare a test substance in an animals' food might be more relevant than use of a bolus gavage dose to the potential acute toxicity to humans by exposure through food residues. In addition, the dog will consume a palatable daily ration completely within one hour. Therefore, in cases where the palatability of the diet for dogs has been established, administration via the feed is essentially comparable to a bolus capsule dose.

b. Where necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that, wherever possible, the use of an aqueous solution be considered first, followed by consideration of a solution/emulsion in oil (e.g., corn oil) and then by

possible solution on other vehicles. For vehicles other than water, the toxic characteristics of the vehicle must be known. The homogeneity of the test substance in the vehicle should be assured.

E. Procedure

1. Number and sex of animals

Rodent: At least 20 animals (ten males and ten females) should be used at each dose level, including a control group. A minimum of ten animals per sex per dose group should be used for the 24-hour evaluation. A minimum of five males and five females from each dose level should be used for the 14-day post-treatment evaluation.

Dog: At least 8 animals (four males and four females) should be used at each dose level, and including a control group. A minimum of four animals per sex per dose group should be used for the 24-hour evaluation. At least two males and two females from each dose level should be used for the 14-day post-treatment evaluation.

If existing data show that one sex is *clearly and con*sistently more sensitive than the other, the study design may be modified to test only in that sex, with the attendant reduction in the total number of animals required.

2. Dosage

a. Generally, three test groups and a control group should be used. Dose levels should be selected taking into account any existing toxicity and (toxico-) kinetic data available for the test compound or related materials. At a minimum, results from a 90-day, subchronic, repeated dose study should be available. Results from prenatal developmental and multigeneration reproductive toxicity studies also would be useful in dose selection. The highest dose level should be chosen with the aim of inducing toxic effects, but not death or severe suffering. Thereafter, a descending sequence of dose levels should be selected with a view to demonstrating any dose-related response and NOAEL at the lowest dose level.

b. Except for treatment with a vehicle instead of the test substance, the animals in the control group should be handled in an identical manner to the test group subjects. If a vehicle is used in administering the test substance, the control group should receive the vehicle in the highest volume used.

c. When there is evidence in the repeat dose studies that the toxicodynamic effect of the test substance is cumulative, (e.g., irreversible inhibition of acetylcholinesterase), the use of a split dose regimen (i.e., two or three dose increments over 24 hours) may be appropriate.

3. Administration of doses

Non- fasted rodents are dosed with the test substance by gavage or feed. This should be done in a single dose to the animals using a stomach tube or a suitable intubation cannula The maximum volume of liquid that can be administered at one time depends upon the size of the test animal. The volume should not exceed 1 ml/100g body weight, except in the case of aqueous solutions where 2 ml/100g bw may be used. Except for irritating or corrosive substances which will normally reveal exacerbated effects with higher concentrations, variability in volume should be minimized by adjusting the concentration to ensure a constant volume at all dose levels. If administration is via feed, the single dose should be consumed as a total ration completely within at least 4 hours [validation necessary]..

Non-fasted dogs should be dosed by gelatin capsule or in feed as a palatable daily ration, which will be consumed completely within one hour..

4. Clinical Observations

a. Depending on the toxicokinetic and toxicologic profile of the substance, the observation period might be up to 14 days. Animals in the interim sacrifice group will be terminated at latest 24 hours post applicationem or after the full dose is consumed via feed [validation necessary].

b. General observations should be made at least once a day, preferably at the same time(s) each day. The health condition of the animals should be recorded. At least twice daily, all animals are observed, if morbidity and mortality may occur.

c. Once before exposure to the test substance (to allow for within-subject comparisons) and at specific times thereafter, detailed clinical observations should be made in all animals. Full clinical evaluations should occur at: time of peak effect and 0.5, 1, 2, 4 and 24 hours after dosing. The 14-day subgroup should have clinical observations carried out on them daily after the first 24 hours. These observations should be made outside the home cage in a standard arena and preferably at the same time each day. They should be carefully recorded, preferably using scoring systems, explicitly defined by the testing laboratory. Effort should be made to ensure that variations in the test conditions are minimal and that observations are preferably conducted by observers unaware of the treatment. Signs noted should include, but not be limited to, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation,

piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture, response to handling as well as the presence of clonic or tonic movements, stereotypes (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backwards) should also be recorded.

d. Functional observations

(1) If the test species used is the rat, sensory reactivity to stimuli of different types (e.g., auditory, visual ands proprioceptive stimuli), grip strength and motor activity should be assessed unless existing data from acceptable repeated dose studies with the test substance *definitively* indicate that these parameters are not affected by the test substance.

(2) This evaluation should be conducted at the estimated time of peak effect, and just before interim termination of the 24-hour treatment subgroup.) If the peak effect is sufficiently close to 24 h then either the 24 h observation or the peak effect are sufficient.If effects are observed additionally just before final termination of the reversibility subgroup.

(3) The elements described in this Guideline may be combined with the acute neurotoxicity screening battery study, as long as none of the requirements of either are violated by the combination.

5. Body weight and food/water consumption

All animals should be weighed on the day of treatment and daily thereafter. Measurements of food consumption and drinking water intake should be made daily for the first week, and at the end of the study at 14 days.

6. Hematology

a. Unless existing data from acceptable repeated dose studies with the test substance *definitively* indicate that the hematopoetic system is not a target site, the following hematological examinations should be made just prior to or as part of the procedure for killing the animals (i.e. interim termination and at the end of the test period): hematocrit, hemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count and a measure of blood clotting time/potential.

b. Blood samples should be taken from a named site just prior to or as part of the procedure for killing the animals, and stored under appropriate conditions.

7. Clinical biochemistry

a. Clinical biochemistry determinations to investigate major toxic effects in tissues, and specifically, effects on kidney and liver, should be performed on blood samples of all animals just prior to or as part of the procedure for killing the animals (apart from those found moribund and/or intercurrently killed). Overnight fasting of the animals prior to blood sampling is recommended. Unless existing data from acceptable repeated dose studies with the test substance *definitively* indicate that the parameter is not affected by the test substance, the following investigations of plasma or serum shall include: sodium, potassium, chloride, glucose, total cholesterol, urea, creatinine, total protein and albumin, at least two enzymes indicative of hepatocellular effects (such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, and sorbitol dehydrogenase). Measurements of additional enzymes (of hepatic or other origin) and bile acids may provide useful information under certain circumstances.

b. Urinalysis determinations should be performed just prior interim termination and if effects are observed at the end of the study, using timed urine volume collection. Unless existing data from acceptable repeated dose studies with the test substance *definitively* indicate that the parameter is not affected by the test substance, the following parameters should be evaluated: appearance, volume, osmolality or specific gravity, pH, protein, glucose, blood and blood cells.

c. In addition, studies to investigate serum markers of general tissue damage should be considered. Other determinations that should be carried out if the known properties of the test substance may, or are suspected to, affect related metabolic profiles include: calcium, phosphate, fasting triglycerides, specific hormones, blood methemoglobin and cholinesterase(s). These need to be identified for chemicals in certain classes or on a case-by-case basis.

d. If a specific effect of the test substance has been observed using special techniques in other studies, then these techniques should also be used in this study. For instance, cholinesterase inhibition in plasma, red blood cells, brain and peripheral nervous tissue should be measured for compounds known to inhibit these enzymes.

e. Consideration should be given to determination of hematological and clinical biochemistry variables before dosing begins.

9. Pathology

a. Gross necropsy

(1) All animals in the study shall be subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. Unless existing data from acceptable repeated dose studies

with the test substance *definitively* indicate that the tissue is not affected by the test substance, the liver, kidneys, adrenals, testes, epididymides, ovary, uterus, thymus, spleen, brain and heart of all animals (apart from those found moribund and/or intercurrently killed) should be trimmed of any adherent tissue, as appropriate, and their wet weight taken as soon as possible after dissection to avoid drying.

(2) Unless existing data from acceptable repeated dose studies with the test substance *definitively* indicate that the tissue is not affected by the test substance, the following tissues should be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination:
all gross lesions, brain (representative regions including cerebrum, cerebellum and pons), spinal cord, stomach, small and large intestines (including Peyer's patches), liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea and lungs (preserved by inflation with fixative and then immersion),ovaries, testes, epididymides, accessory sex organs (e.g., prostate, seminal vesicles), ovary and uterus, urinary bladder, lymph nodes (preferably one lymph node covering the route of administration and another one distant from the route of administration to cover systemic effects), peripheral nerve (sciatic or tibial) preferably in close proximity to the muscle, eye and a section of bone marrow (or, alternatively, a fresh mounted bone marrow aspirate). The clinical and other finding may suggest the need to examine additional tissues. Also, any organs considered likely to be target organs based upon the known properties of the test substance should be preserved.

b. Histopathology

(1) Unless existing data from acceptable repeated dose studies with the test substance *definitively* indicate that the tissue is not affected by the test substance, full histopathology should be carried out on the preserved organs and tissues of all animals in the control and high dose groups of the main study and the satellite interim sacrifice groups. These examinations should be extended to animals of all other dosage groups, if treatment-related changes are observed in the high dose group.

(2) All gross lesions shall be examined.

F. Data and reporting

1. Individual data should be provided. Additionally, all data should be summarized in tabular form showing, for each test group, the number of animals at the start of the test, the number of animals found dead during the test or killed for humane reasons and the time of death or humane kill, the number showing signs of toxicity, a description of the signs of toxicity observed, including time of onset, duration, and severity of any toxic effects, the number of animals showing lesions, the type of lesions and the percentage of animals displaying each type of lesion.

2. When possible, numerical results should be evaluated by an appropriate and generally acceptable statistical method. The statistical method should be selected during the design of the study.

G. Test report

The test report must include the following information:

1. Rationale for specific study design

(e.g., choice of species and sex, dose selection, endpoint selection)

2. Test substance

a. Physical nature, purity and physicochemical properties

b. Identification data

3. Vehicle

(if appropriate): Justification for choice, if other than water

4. Test animal

a. Species/strain used

b. Number, age and sex of animals

c. Source, housing conditions, diet, etc.

d. Individual weights of animals at the start of the test

5. Test conditions

a. Doses

b. Details of test substance formulation

c. Details of administration of the test substance

d. Details of food and water quality

6. Results

a) Body weight/body weight changes

b) Food consumption

c) Toxic response data by sex and dose level, including signs of toxicity

d) Nature, severity and duration of clinical observations (whether reversible or not)

e) Neurological assessment (as appropriate for species tested)- e.g., sensory activity, grip strength and motor activity assessments in the rodent

f) Hematological tests with relevant baseline values

g) Clinical biochemistry tests with relevant baseline values

h) Body weight at 24 hours (all animals), 7 days (both satellite groups) and at 14 days (for remaining satellite group) or at time of unplanned death.

i) Necropsy findings

j) A detailed description of all histopathological findings

k) Statistical treatment of results, where appropriate

l) Analyses to confirm concentration of test substance in dosing solution

7. Discussion of results

8. Conclusions

H. References

The following references should be consulted for additional background material on this test Guideline *[Others to be added*]

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