DRAFT GUIDANCE DOCUMENT FOR THE DERIVATION OF AN
ACUTE REFERENCE DOSE

The objective of the document is to provide stepwise approach for a harmonized guidance on how to use optimally all available toxicological data, how to refine the exposure calculation for the acute risk assessment and what to do if more data is needed for derivation of Acute Reference Dose.

The aim of this guidance document is not to provide a new test guideline and does not aim to encourage additional animal testing, but provides guidance, how to perform and tailor a single exposure test, what are the minimum parameters, depending on all available data. If in exceptional cases, a single exposure study is necessary; this study should be performed according to a harmonised OECD test procedure.

The focus of this guidance is only on acute oral exposures, whereas general principles and concepts which can be applied to dermal and inhalation exposure routes should be addressed in separate OECD guidance documents.

A. Background

Regulatory requirements or legislations relating to the protection of human health have led to the need to consider the establishment of an Acute Reference Value (ARV) for all potentially acutely toxic substances with relevant acute human exposure scenarios. This applies mainly to pesticide, biocide, and veterinary drug residues in food and drinking water for which an Acute Reference Dose (ARfD) has to be considered (1, 2, 3, and 4). Regulatory authorities are required to protect the general population against effects induced by acute oral exposure to hazardous substances, if the Tolerable Daily Intake (TDI) is substantially exceeded for short periods of time (6, 7).

The Acute Reference Dose of a chemical is an estimate of the amount of a substance in food and/or drinking water, normally expressed on a body weight basis, that can be ingested in a period of 24 hours or less, without appreciable health risk to consumer, on the basis of all the known facts at the time of the evaluation (2).

Various guidance documents are available for setting Acute Reference Values (1, 2, 3, 4, 5, and 7). The WHO panel of the Joint Meeting on Pesticide Residues (JMPR) adopted general
considerations in setting of ARfDs for pesticide chemicals (1, 2). Solecki et al. (4) described in detail a step-wise process for establishing ARfDs, as well as specific considerations and guidance regarding the identification of the most appropriate critical effects for selected toxicological endpoints.

For a critical effect, a no-observed-adverse-effect-level (NOAEL) that is typically determined from laboratory animal studies has been traditionally used as a Point of Departure (PoD) when deriving an ARfD. An alternative method to derive an improved PoD is the use of the Benchmark Dose (BMD) modelling approach response1. The BMD is defined as the dose producing a predetermined level of change in response (such as a 10% increase in the incidence of a particular toxic effect) compared with the background. A BMD is derived by fitting a mathematical model to the dose-response data, and is often accompanied by an estimate of the statistical lower confidence limit (BMDL) on the BMD2.

The JMPR guidance acknowledged that endpoints from a repeat dose toxicity study could be used for setting an ARfD if the critical effect of the compound has not been adequately evaluated in a single exposure study. This approach is likely to be conservative.

A retrospective analysis of ARfD values of 198 active pesticide substances which have been evaluated and peer-reviewed in the European Union (Appendix to Annex 2) has shown that only for less than 10% of the pesticides the ARfD was based on repeated dose toxicity or multigeneration studies. The majority of ARfD values were based on studies in which specific acute alerts were investigated and only for a small portion of pesticides (4 %) special acute studies were submitted for the ARfD derivation. These special studies to evaluate the acute toxicity as a basis for the ARfD derivation were mostly performed additionally to the basic data requirements in a process of informal discussions between notifiers and authorities on specific test protocols. These studies were performed, if the acute intake estimation was exceeding a potentially conservatively established ARfD. However, in some cases such submitted studies were not acceptable by the authorities because of quality deficiencies as a result of a missing guidance.

In other cases, e.g. if monitoring data showing an exceedance of a conservatively derived ARfD, a refinement of a not adequately established NOAEL may be addressed in an appropriate special single exposure study to perform a realistic human health risk assessment or to justify the lowering of MRLs or the deletion of an authorisation of a Plant Protection Product.

1 http://www.who.int/ipcs/methods/harmonization/draft_document_for_comment.pdf
The ILSI Health and Environmental Sciences Institute (HESI), through its Agricultural Chemical Safety Assessment (ACSA) Committee designed a toxicity testing scheme for agricultural chemicals that incorporates current understanding of pesticide toxicology and exposure and provides relevant toxicity parameters that would be used in a tiered approach (10), which should contribute to moving away from paradigms that involve extensive animal testing for ‘every possible adverse outcome’ to a more science-based tiered approach and to reduce dog studies (i.e. in the one year study) and other testing requirements. The ILSI HESI Task Force devised a set of studies in a tiered approach which could provide information for the most relevant human exposure periods and outlines a draft protocol for an single dose test in dogs or rodents as an optional step five (11). Doe et al (11) proposed that the ARfD could be based on a repeated dosing study (28 or 90 day), and if the ARfD derived from the repeated dosing study indicates an adequate margin of exposure, then the requirement to perform a single-dose study could be waived. The authors emphasized that if a single exposure study is considered necessary, existing data/knowledge should be considered to determine the relevant endpoints and the most appropriate species (rat or dog). It should never be necessary to perform both the rat and the dog single-dose study. If such a study is done, then it should conform to the design outlined in the publication (11) to avoid repetitions of such a study related to an insufficient test protocol.

The tiered approach proposed by ILSI HESI to determine the need for a single exposure study is consistent with the stepwise approach outlined by Solecki et al. (4). As emphasized by Solecki et al. (4) and by Doe et al. (11), results of existing toxicity data combined with knowledge of potential human exposure should be used to determine the need for this single day study. The available toxicity studies may also guide whether the dog or the rat should be used for the assessment of a single day exposure, taking into account the relevant endpoints for acute exposure. Considering existing knowledge of toxicity and exposure before the conduct of this study is consistent with a more science/hypothesis-based approach to determine what specific in vivo testing is appropriate, thus meeting an important goal for risk assessment to be based on greater efficiency and fewer animals.

The single exposure study design proposed by the JMPR (2) and Solecki et al. (4) and adopted by the ILSI HESI Task Force (11) forms the basis of the guidance, how to perform and tailor a single exposure test, in Annex 2 of this document.

The results of the single exposure study should (i) clarify whether a substance poses an unacceptable acute risk and (ii) allow the derivation of a refined ARfD for acute intake of residues in food and drinking water.
At the 13th Meeting of the OECD Working Group on Pesticides in 2002, the JMPR presented a proposal for an OECD Test Guideline animal study, designed to establish an ARfD for dietary risk assessment for human health (8). The EC, Spain and Crop Life International supported this proposal, suggested improvements and recommended that JMPR should proceed by approaching the Working Group of National Co-ordinators to the Test Guidelines Programme (WNT). The 19th WNT meeting then agreed that an improved stepwise approach for a harmonised guidance should be developed for the derivation of an ARfD. The WNT agreed to include the project in the work plan for 2007 (9).

B. Purpose

The objective of this document is to provide a stepwise approach for a harmonized guidance on how to set the ARfD based on all appropriate existing toxicological and exposure data. The general considerations in setting an ARfD in a an enhanced step-wise process, as well as specific considerations and guidance regarding the identification of the most appropriate critical effects for selected toxicological endpoints are described in detail. 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 these selected relevant endpoints. Special emphasis is placed on evaluating whether toxic effects observed in the standard package of repeated dose toxicity studies may also occur after single doses.

Data are not often available for many types of effects under acute exposure conditions and it is possible that the NOAELs and endpoints that will be critical for setting an ARfD may differ from those for setting chronic RfDs, or ADIs. The general principle is agreed that the ARfD should be equal to or greater than other long-term reference values of the same chemical (i.e. an individual can generally tolerate a higher amount of a substance with an acute exposure than with a repeated exposure). This is important because ARfDs are typically coupled with high-end exposure values rather than the average exposure values that are employed in risk assessments involving repeat exposures.

If the acute intake estimation exceeds such a potentially conservatively established ARfD in a first step, reassessment of the risk assessment may be addressed as a second step in a refinement of the exposure assessment and as a last resort in a third step a single exposure study may be needed for the generation of toxicological data to establish and refine Acute Reference Dose values.
This guidance document is intended to promote a harmonised scientific basis for the
derivation of ARfDs suitable for refined acute risk assessment in a range of acute human oral
exposure scenarios.

This proposed guidance document will:

- replace the need to conduct unnecessary tests on animals by introducing an extended
  stepwise approach for human health risk assessment, including a refined exposure
  assessment,
- reduce the need to repeat animal tests which have not been performed in a way which
  adequately satisfy the requirements of different regulatory agencies, and
- refine a harmonised procedure for determining an ARfD of a compound in situations
  where available data do not adequately characterise the acute hazard.

This document presents specific guidance

- how to refine the exposure calculation for the acute risk assessment in Annex 1, and
- how to perform a tailored single exposure study and what are the minimum parameters,
  depending on all available data, which allows the derivation of a NOAEL/LOAEL or
  benchmark dose for the most relevant acute effect(s) in the most appropriate species, but
  not intended to become a routine data requirement in the Annex 2.

C. Basic Considerations

The appropriateness of all available endpoints from subchronic and chronic studies to
establish ARfDs needs to be carefully considered in a first step. The pertinent biology of the
system affected should be considered to determine whether an acute exposure may
compromise the ability of the organ to compensate and maintain homeostasis. Particular
weight should be given to observations and investigations at the beginning of repeat dose
studies. Isolated findings, showing no specificity or clear pattern are not necessarily
indications of toxicity. 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.

The NOAEL from the most adequate study in the most sensitive species should be used unless
there is evidence to demonstrate it is not appropriate for a human risk assessment.

After reviewing the available toxicological database, the possible exposure scenarios should
be considered in a second step, based on the guidance in Annex 2. A tiered human health risk
assessment should be conducted that includes a comparison of the Acute Reference Value
with the potential acute oral exposure (or internal body burden), based on a worst-case
assumption. If this worst-case assessment does not indicate unacceptable health risks, no
further refinement of the acute risk assessment may be warranted. But, if this risk assessment indicates a borderline or a clear concern, then the next tier should focus on a further refinement of the exposure assessment (from refined acute intake estimation). If the health risks are now acceptable, no further refinement is warranted. If the refined exposure assessment still shows unacceptable health risks and if conservative assumptions were used in setting the ARfD, then in a third step it should be considered, if a single exposure study may be warranted to establish a refined Acute Reference Dose and how to perform and tailor this single exposure test, based on the guidance in Appendix to Annex 2. This may only be necessary for a very limited number of substances.

D. Extended Tiered-Approach for the Derivation of an appropriate ARfD

STEP ONE

1. Evaluate the total database of the substance and establish a toxicological profile for the relevant exposure periods to this substance.

2. Consider the principles for not setting an ARfD

- No findings indicative of adverse effects elicited by an acute exposure are seen at doses which are relevant for the acute risk assessment (e.g. up to about 500 mg/kg bw/day for residues of pesticides, justification see 1 and 4) AND/OR
- No substance-related mortalities are observed at doses up to 1000 mg/kg bw in single dose oral studies (i.e. limit dose for acute testing).
- If mortality is the only trigger, the cause of death should be confirmed as being relevant to human exposures.

If the above criteria do not exclude the setting of an ARfD, then further consideration should be given to setting a value, using the most appropriate endpoint in the most relevant species.

3. Selection of appropriate endpoints for setting an ARfD

- Select the toxicological endpoints most relevant for a single (day) exposure in the most relevant species.
- Select the most relevant or adequate study in which 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.

If after consideration of all the endpoints in appropriate available studies, an ARfD is not set, then the reasons must be justified and explained.

4. Selection of appropriate safety factors for setting an ARfD

- Derive the ARfD using an appropriate safety factor (SF)

- In determining the appropriate safety factor, a stepwise approach is proposed.

  - Determine whether the database is adequate to support the derivation of a chemical-specific adjustment factor (CSAF) (14). IPCS recommended “default sub factors”, i.e. 4-fold and 2.5-fold for inter-species toxicokinetic and toxicodynamic differences, respectively, and 3.16 for each of human interindividual toxicokinetic and toxicodynamic differences.

  - Some reduction for human toxicokinetic differences from its default value of 3.16, would be justified (16). JMPR suggested that a 50% reduction would be appropriate for compounds whose effects are dependent on Cmax, and which are rapidly eliminated, the combined adjustment factor would be 25.

  - If a specific factor cannot be derived, consider if there is any information to indicate reduced or increased uncertainty. A combined SF may be based (16) on

    
    \[(AKAF \text{ or } AKUF) \times (ADAF \text{ or } ADUF) \times (HKAF \text{ or } HKUF) \times (HDAF \text{ or } HDUF)\]

    
    - where AK represents inter-species toxicokinetic variability
    - AD represents inter-species toxicodynamic variability
    - HK represents human interindividual toxicokinetic variability
    - HD represents human interindividual toxicodynamic variability
    - AF represents a chemical-specific adjustment factor
    - UF represents a default uncertainty sub-factor

  - If not, the 100-fold (or 10-fold) default should be used. When using data obtained from experimental animals, the default safety factor is 100. This comprises a factor of 10 to allow for inter-species differences and a factor of 10 for intra-species (human inter-individual) differences. The overall safety factor is the product of these two factors, i.e. \(10 \times 10\).
Whenever a safety factor other than a default is used, a clear explanation of the derivation of the factor must be provided.

**STEP TWO (Annex 1)**

5. Application of the ARfD for the acute risk assessment
   - Determine whether the acute exposure estimate is exceeding the ARfD.
   - If the acute intake estimation does not exceed the ARfD, no further refinement is necessary.

If the risk assessment indicates a borderline or a clear concern, then a refinement of the exposure assessment should be performed.

6. Refinement of the exposure calculation for the acute risk assessment
   - In determining a refined exposure calculation, a stepwise approach is proposed.

If the risk assessment indicates still a clear concern, then a refinement of the ARfD could be performed.

**STEP THREE (Annex 2)**

7. Experimental refinement of the ARfD derivation
   - As a last resort a single exposure study according to the test design in the Annex of this guidance may be needed for the generation of data to establish and refine more appropriate ARfD.

**E. Specific Guidance on the Derivation of ARfDs**

Particular toxicology end-points which are relevant to ARfD establishment are considered in the JMPR publication (2) and by Solecki et al. (1). Note that these documents are not intended to comprehensively cover all potentially relevant endpoints but focuses on the interpretation of an extended number of selected endpoints which have proved to be problematic in reaching a decision as to whether an effect is relevant to an acute exposure.

- **Haematotoxicity**: The induction of methaemoglobinaemia is considered to be a critical effect in consideration of acute responses to chemical exposure. For acute exposure to methaemoglobin-inducing xenobiotics, a level of 4% methaemoglobin (or higher) above background in dogs or a statistically-significant increase in rodents cf. background is considered to be relevant to set an ARfD. Haemolytic anaemias induced by mechanical damage, immune mediated anaemia, oxidative injury to RBCs and non-oxidative damage
are considered to be less relevant for ARfD derivation since the severity of such effects appear to generally depend on prolonged exposure. If changes in haematological parameters are observed early in a repeated-dose study and do not appear to progress during the course of the study, then such effects can be considered as relating to acute exposure to the substance. 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.

- **Immunotoxicity**: Immunotoxicity data derived from subchronic studies are not likely to be appropriate for setting a reference dose for acute exposure limits. It is unlikely that an acute exposure will produce persistent effects on immune function because the immune system cells are constantly replaced and because of the inherent redundancy in the system (e.g. alternative mechanisms to resist infection).

- **Neurotoxicity**: The nervous system has limited capacity for repair and regeneration. Therefore, 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. 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. Delayed neurotoxicity following single chemical exposures can occur and thus any acute exposure study should have an adequate period of investigation. In functional observation batteries (FOB) a large amount of data is produced; interpretation of such studies should include a consideration not only of the statistical significance of results but the nature, severity, persistence, dose-relationship and pattern of the effects. Isolated findings showing no specificity or clear pattern do not necessarily indicate neurotoxicity.

The most common neurotoxic end-point 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; these apply equally to the setting of ADIs and ARfDs. For inhibition of acetylcholinesterase a specific cut off (20%) is used routinely to differentiate between adverse and non-adverse effects.

- **Kidney and liver effects**: If 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
these 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. When
interpreting data on liver and kidney toxicity in repeat-dose studies, one has to consider
two important aspects, firstly, the type of effect observed and secondly, any information on
correlations between exposure duration and effect.

For liver toxicity it is considered that findings of increased serum cholesterol, cirrhosis,
induced activity of metabolising enzymes, regenerative hyperplasia, hepatocyte
hypertrophy, fibrosis, or sclerosis in repeat-dose studies are, in isolation, either adaptive or
the result of prolonged exposure and therefore are not applicable for deriving an ARfD.

For kidney toxicity it is considered that the following findings of kidney toxicity in repeat
dose studies are, in isolation, 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 liver and kidney toxicity should be considered as potentially relevant
to the derivation of an ARfD.

Endocrine effects: In general, effects on the endocrine system other than those affecting
female reproduction are considered to be unlikely to arise as a consequence of acute
exposure.

- Developmental effects: Any treatment-related adverse effect on fetuses 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. ARfDs based on reductions in fetal
bodyweight gain may be conservative and should be evaluated in the context of all
pertinent data, including other developmental effects. Consideration should be given to the
degree of maternal toxicity when considering whether fetal effects may be occurring as a
direct effect of the chemical; severe maternal toxicity means a direct effect is less likely.

- Direct effects on GI tract / stomach: Occasionally a chemical can cause adverse effects on
  the gastro-intestinal tract. These effects may be exerted through three different modes of
action.

  When gastro-intestinal effects occur, they are most commonly observed only after a bolus
administration of a compound (by gavage or capsule) in fasted animals and administration
of similar doses in food does not cause the same effects. In this case, the gastro-intestinal
effects are most likely due to a local irritant effect of large amounts of the compound in the
gastrointestinal tract. Since the ARfD applies to ingestion of a compound in food or
drinking water, local gastro-intestinal effects exerted by bolus administration are not considered to be relevant for setting an ARfD.

Secondly, a chemical administered in food may exert a local toxicological effect on the gastro-intestinal tract. Since the ARfD applies to chemicals in food, such an effect is likely to be relevant for setting an ARfD. For these direct effects, the application of inter- and intraspecies toxicokinetic considerations can be modified. Thus it would be appropriate to reduce the toxicokinetic fractions of the inter- and intraspecies assessment factors. Furthermore, in terms of toxicodynamics, it could be reasonably be assumed (in the absence of other information) that animals and humans will respond to such an insult in the same way. Thus, in deriving an ARfD based on local gastro-intestinal effects exerted by a substance administered in food, a reduction of the assessment factors would be appropriate. However, such a reduction in the assessment factors should always be justified by explanatory text in the hazard and risk assessment document.

Thirdly, chemicals may exert an effect on the gastro-intestinal tract through a systemic action. For instance, it is known that the dopamine agonist apomorphine causes vomiting in humans and dogs (not in rodents), through a direct stimulation of the chemoceptor trigger zone for emesis in the area postrema of the medulla oblongata of the CNS. Such an indirect effect on the GI tract is considered to be relevant for setting an ARfD. For such indirect gastro-intestinal effects, inter- and intraspecies differences in the toxicokinetics as well as the toxicodynamics of the substance should be taken into account. If it has been determined that the indirect effect on the gastro-intestinal tract is the result of a pharmacological (receptor-mediated) action of a compound, a reduction of the default 10 x 10 assessment factors may be appropriate (16). The reasons for establishing (or not establishing) an ARfD on the basis of gastro-intestinal effects observed after single or short-term dosing, and the assessment factors applied should always be justified by appropriate explanatory text.
Other findings indicative of adverse effects elicited by an acute exposure:

- Clinical signs observed in acute (LD50) Toxicity studies
- Clinical signs and mortality in developmental neurotoxicity studies
- Behavioural abnormalities and clinical signs in the first days of repeated dose studies, which are not indicative of neurotoxicity
- Decreased body weight gain, reduced food and/or water intake signs in the first days of repeated dose studies, which are indicative of general toxicity and not based on palatability of the feed.

F. Animal Welfare Consideration

For reasons of animal welfare, the request for additional experimental animal data should always be a last resort in the risk assessment process; additional single exposure study should NOT be performed:

- if the derivation of an Acute Reference Dose is considered unnecessary for toxicological reasons (e.g. see criteria recommended by the 2004 JMPR as detailed in Solecki et al. (1),
- if adequate acute toxicity studies are available which indicate relevant effects after single exposure, e.g., developmental toxicity and acute neurotoxicity studies,
- if adequate repeated dose studies are available which indicate acute effects shortly after exposure,
- if a compound has negligible residues such that refined dietary exposure estimates indicate an adequate margin of safety even if measured against a conservative Acute Reference Dose derived from a repeated dose study, or
- if exposure estimates indicate levels of exposure which provide an adequate margin of safety even when measured against a conservative Acute Reference Dose derived from a repeated dose study.

In the single exposure study, a minimum but sufficient number of animals of the most appropriate species should be utilised to produce the required additional data. Dogs should be used only when it has been demonstrated that they are the most sensitive species to the test substance if a single exposure study needs to be conducted.

If the rabbit is the most sensitive species, i.e. in a developmental study, no additional experimental animal data should be submitted from a single exposure study in rabbits.
G. Consideration of the Route of Exposure

The focus of this guidance is on acute oral exposures as the most likely exposure route for humans for pesticides, since in general, oral administration is the route most often considered also in repeated dose studies for pesticides and other chemicals.

General principles and concepts which can be applied to dermal and inhalation exposure routes should be addressed in separate OECD guidance documents. If for example the most likely exposure route of a chemical for humans is inhalation, studies with inhalatory exposure would be the preferred route of administration. This would eliminate the need for a route-to-route extrapolation, with all its uncertainties, assumptions and limitations. Additional guidance for dermal and inhalation studies can be found in appropriate OECD test guidelines. However, if appropriate pharmacokinetic studies are available and/or port of entry effects data are available route-to-route extrapolations might be performed to avoid additional route specific animal testing. Therefore, an ARfD may be transformed into an internal value considering the extent of absorption of the substance along the respective route of application, if appropriate data are available. This acute internal value can be compared to the different routes of exposure without additional animal testing. This approach is equivalent to the acute systemic AEL applied in different regulatory frameworks, e.g. for biocides (5).

H. Consideration of Human Data

Human data from accidental or deliberate poisonings, biomarker monitoring studies, epidemiology studies, volunteer studies, and clinical trails on the same or structurally-similar compounds can provide useful data to help establish ARfDs. The use of human volunteer data in chemical risk assessment is a controversial issue, with a range of views held by different countries and individuals. Therefore, the portion of ARfD values derived from human studies varies in a wide range between different authorities. In a retrospective analysis of EU ARfDs only 0.5% of the values were derived from human studies. In an older retrospective analysis not restricted to Europe approximately 10% of the ARfD values were derived from human studies (1). However it is recognised that the use of such data can reduce the level of uncertainty inherent in extrapolating from animal models, if such data exist from the past or from studies in nutritional physiology and medicine. For some substances like copper which is used as a pesticide but which is also an essential nutritional compound the results from human studies may be indispensable. There needs to be adequate consideration of both scientific and ethical issues. The JMPR has considered human data at many of its meetings. The JMPR
reaffirmed the principle that endpoints from existing human volunteer studies could be used for setting health intake standards if they had been conducted in accordance with relevant ethical and scientific guidelines (2).

The PPR Panel has also published the opinion (12) that human data on a pesticide, whether from volunteer studies or from other investigations of human exposures in the workplace or environment, can be extremely valuable in placing the animal data in context and, when available, should always be evaluated even when they are not used to derive a reference value.

Due to the ethical implications of studies in humans, they must be conducted in accordance with principles such as those expressed in the Declaration of Helsinki (13) or equivalent statements prepared for use by national and/or multinational authorities (4).

For existing studies, both current standards and the standards pertaining at the time the study was performed should be taken into account. The results of tests involving humans when ethically and scientifically acceptable should be used to derive reference values, including ARfDs, and not be considered simply supportive of reference values derived from animal data. The use of data from existing scientifically valid studies that are not compliant with ethical principles might be justified if the findings indicate that human risk would be underestimated without the use of these findings. Scientific considerations for the use of studies in humans for the derivation of an ARfD were published by OECD (15).

I. Consideration of Different Subpopulations

It is important that the ARfD is adequate to protect the whole population (e.g., general, prenatal, postnatal, and older child).

The single exposure study in the Annex is based on testing in adult animals and thus intended to provide a health base value for the general population.

However, it is also important to ensure that the ARfD is adequate to protect the embryo/foetus from possible in utero effects. Therefore, use of data from developmental studies for the derivation of Acute Reference Values is considered, as a more conservative approach. 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 (1, 2). There are several OECD test guidelines that serve to evaluate potential developmental toxicants following prenatal and postnatal exposures, including prenatal toxicity (OECD 414), reproductive (e.g. OECD 416) and developmental neurotoxicity (OECD 424) studies.
While an Acute Reference Dose based on developmental (embryo/foetal) effects would be appropriate for women of child-bearing age, it is recognised that the same value may be overly conservative with respect to other subgroups in the population. For example, children aged 1 to 6 years; the use of a refined Acute Reference Dose based on \textit{in utero} effects could be inappropriate as they unlikely to be at risk for the developmental toxicity observed. In this situation, separate modelling with respect to acute dietary intake of residues can be performed taking into account age-specific acute consumption data. Alternatively, it might be necessary to address higher sensitivity of children to other forms of acute toxicity by testing during early life-stages. Therefore, in some situations it may be necessary to set an Acute Reference Dose for the general population and another value for other populations of concern.

\textbf{J. References}


(5) ECB Ispra (2008) TNsG on Annex I Inclusion Chapter 4.1: Quantitative Risk Characterisation; (currently in public consultation)


World Medical Association Declaration of Helsinki, 1964; amended most recently in 2000


ENV/JM/MONO (2000) 7


REFINEMENT OF THE EXPOSURE CALCULATION FOR THE ACUTE RISK ASSESSMENT

Acute exposure calculation and risk assessment (IESTI equation) currently follows the recommendations by the WHO/FAO Joint Meeting on Pesticide Residues (JMPR) as laid down in the FAO manual on the submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed (17).

The JMPR had recently discussed the uncertainties in the calculation and interpretation of international estimated short-term intake (IESTI) (18, 19). In characterizing the risks associated with the short-term dietary exposure to a pesticide from the consumption of a certain food, the IESTI is compared with the established acute reference dose (ARfD) of the compound, and the intake expressed as a percentage of the ARfD. This value can then be used to make a judgment about the potential risk associated with the consumption of that food commodity. In a case where an IESTI calculation, for a crop/pesticide combination, results in an intake higher than 100% ARfD, the Meeting will state according to current practice: “The information provided to the JMPR precludes an estimate that the short-term dietary intake would be below the ARfD for the consumption of the commodity”. Due to the uncertainties in the assessment, arising from the uncertainties in each of the parameters or assumptions used, an exceedance of the ARfD does not necessarily represent a health risk to the consumers. The establishment of an ARfD which is necessarily conservative and/or a conservative assessment of exposure will lead to an overly conservative estimate of acute dietary risk. Some governments, regional authorities, the CCPR and the JMPR have discussed the possibilities for improvement in the methodology currently used by the JMPR in assessing the short term dietary intake of pesticide residues. In this context, the 2007 JMPR Meeting also welcomed the publication of an Opinion by the European Food Safety Authority (EFSA) on ‘Acute dietary intake assessment of pesticide residues in fruit and vegetables’ (20).

Further approaches are under discussion but are not yet implemented.

Calculations of intake recognize four different cases (1, 2a, 2b and 3). Case 1 is the simple case where the residue in a composite sample reflects the residue level in a meal-sized portion.
of the commodity. Case 2 is the situation where the meal-sized portion as a single fruit or vegetable unit might have a higher residue than the composite. Case 2 is further divided into case 2a and case 2b where the unit size is less than or greater than the large portion size respectively. Case 3 allows for the likely bulking and blending of processed commodities such as flour, vegetable oils and fruit juices.

The following abbreviations are used in the equations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>LP</td>
<td>Highest large portion reported (97.5th percentile of eaters)</td>
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<tr>
<td>HR</td>
<td>Highest residue in composite sample of edible portion found in the supervised trials used for estimating the maximum residue level</td>
</tr>
<tr>
<td>bw</td>
<td>Mean body weight</td>
</tr>
<tr>
<td>U</td>
<td>Unit weight of the edible portion</td>
</tr>
<tr>
<td>v</td>
<td>Variability factor - the factor applied to the composite residue to estimate the residue level in a high-residue unit</td>
</tr>
<tr>
<td>STMR</td>
<td>Supervised trials median residue</td>
</tr>
<tr>
<td>STMR-P</td>
<td>Supervised trials median residue in processed commodity</td>
</tr>
</tbody>
</table>

**Case 1**

The residue in a composite sample (raw or processed) reflects the residue level in a meal-sized portion of the commodity (unit weight is below 0.025 kg).

\[
\text{IESTI} = \frac{\text{LP} \times (\text{HR})}{\text{bw}}
\]

**Case 2:**

The meal-sized portion, such as a single fruit or vegetable unit might have a higher residue than the composite (whole fruit or vegetable unit weight is above 0.025 kg).

**Case 2a:** Unit edible weight of raw commodity is less than large portion weight.

\[
\text{IESTI} = \frac{U \times (\text{HR}) \times v + (\text{LP} - U) \times (\text{HR})}{\text{bw}}
\]

The Case 2a formula is based on the assumption that the first unit contains residues at the [HR × v] level and the next ones contain residues at the HR level, which represents the residue in the composite from the same lot as the first one.

**Case 2b:** Unit edible weight of raw commodity exceeds large portion weight.
IESTI = \frac{LP \times (HR) \times v}{bw}

The Case 2b formula is based on the assumption that there is only one consumed unit and it contains residues at the [HR \times v] level.

Case 3

Case 3 is for those processed commodities where bulking or blending means that the STMR-P represents the likely highest residue.

IESTI = \frac{LP \times STMR-P}{bw}

It has to be noted, that not always a HR for the edible portion can be derived, because only data on the whole commodity are available. Then the first step acute exposure calculation would be based on the highest residue in the whole raw agricultural commodity (RAC). First refinement option here would be to generate supervised trials residue data referring to the edible portion (e.g. citrus fruit, banana, kiwi fruit or pineapple without peel or mango, peach without stone) and to derive a HR from those trials.

Acute exposure calculations based on the HR might still result in an exceedance of an ARfD and require further exposure refinement.

The HR is usually derived from supervised field trials that have been conducted according to the maximum GAP. It is based on the edible part of the raw commodity in most cases. However, some RACs are always processed before consumption by the public (e.g. potatoes, sugar beet, rape seed). The refined dietary exposure assessment refers to “food as eaten” and takes into account processing factors and residues in the edible portion as appropriate. HR values in the equations are replaced by the corresponding HR-P values (with “P” being the processing factor). More guidance on processing studies and processing factors can be found in OECD Test Guideline 508 “Magnitude of Pesticide Residues in Processed Commodities”.

Another refinement option is the more detailed analysis of consumption data and the refinement of LP. In many consumption surveys individual intakes of commodities arising from various food items are aggregated over the day based on the RAC. Due to this combination, information about the processing state of the food is lost: e.g. the intake of raw apples, apple juice and apple pie are combined to a total figure for apples based on the RAC.
This aggregation normally results in an overestimation of the exposure and should be taken into account, if further information is available.

Another important factor is the selection of the appropriate subgroup for the dietary risk assessment. Several ARfDs refer to specific subgroups (e.g. women in child-bearing age) and thus do not allow for the use of all consumption data available (especially not those for children).

A further refinement option is the replacement of the default variability factor $v$ by experimental data. Though on FAO/WHO level a default factor of 3 is already used, which can not be reduced much further by using experimental data, EU Member States on the other hand still use factors of 5, 7 and 10, depending on the commodity. In those cases it might be appropriate to conduct a supervised residue study to determine the unit to unit variability. Data should be representative for different fruit sizes and fruit exposure situations. For statistical reasons, at least a total of 120 single units should be analyzed. According to Hamilton et al. (21) at least 119 samples are needed to estimate the 97.5 percentile with a 95 % confidence interval.

It was concluded by the JMPR (18, 19) that the IESTI and the ARfD values are not absolute numbers but are associated with uncertainty and variability. While it is possible to reduce uncertainty, biological variability can only be characterized. Both are set conservatively and the degree of conservatism reflects the level of uncertainty and variability in the data. The IESTI calculation should assist the decision making process rather than be the sole determinant of acceptable or unacceptable risk. The calculation takes into account only the parameters presented to it. At present, the decision making process does not take into account important qualitative influences, e.g. the nature of the toxicological endpoint. In order to improve the estimation process the uncertainty of the individual components of the estimation should be examined and possible ways of improvements be identified.

It is recommended that the main objectives in the exposure refinement would be the improvement of the estimation of the short-term dietary intake of pesticides and that the refinement should include inter alia the following specific issues:

- Uncertainty and variability of the parameters used in the estimation;
- Ways to improve the consumption, unit weight and body weight data provided to the JMPR;
- Identification of additional subgroups of the population for which the assessment should be conducted, e.g., toddlers;
- The adequacy of the IESTI equations when residues from monitoring/enforcement data are used or the need of a specific methodology for this application;
Annex 2

GUIDANCE FOR CONDUCTING A SINGLE EXPOSURE TOXICITY STUDY

This is not a test guideline, only an advise, how to perform and tailor a single exposure test, what are the minimum parameters, depending on all available data.

INITIAL CONSIDERATIONS

In 2002 an analysis of the ARfD values set by several regulatory bodies was performed (1). There were large differences in the ARfD values between the analysed regulatory bodies (up to 2500-fold for some individual pesticides). In result of this analysis it was concluded “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.” In the mean time the regulatory authorities made more comprehensive experiences with the derivation of ARfD values and notifiers and authorities made also the first experiences with the design of additional acute or short term studies for the derivation of ARfDs. Therefore, it was considered necessary to perform a new analysis in order to identify the toxicological studies on which the ARfD values are based in 2008. This analysis was recommended as a basis for a harmonized guidance on how to use available data on ARfD derivation and also for the development of an ARfD study design. The current analysis of the ARfD values was based on the last revision of this annotated list of active pesticide substances which is published by the European Food Safety Authority (EFSA) on the EFSA website with the specification SANCO 3010, rev 10/11/2008. The data basis for the ARfD derivation of 198 substances was analysed. The portion of special ARfD studies is very low. Only 4% of the ARfDs are based on such studies. In some cases such submitted ARfD studies have not been accepted by the authorities because of quality deficiencies as a result of a missing guidance paper. Therefore, in the EU peer review process some of the submitted special ARfD studies have not been used for the ARfD derivation. The results confirm once more that the development of an acute study design that produces more comprehensive toxicological data for setting ARfDs would be of high value.

This in-vivo single exposure study is not intended to become a routine data requirement. As discussed in the guidance document, the single exposure study should refine the Acute Reference Doses and only be considered after the available toxicology and exposure information a compound has been appropriately evaluated. The relevant species and
toxicological endpoints should already be documented and reasonably well understood because this study is only designed to refined endpoints and dose of concern in the existing repeated dose studies. Observations on the experimental animals are based on those listed in the revised OECD Test Guideline 407. Therefore, additional validation of these test parameters in this study is not considered necessary.

PRINCIPLE OF THE TEST

An important principle in the design of the single exposure study is to consider all available information on the substance (e.g., physico-chemical, toxicokinetic and toxicodynamic properties of the test substance, available relevant information on structural analogues of the substance, results of previously conducted toxicity studies of the test substance) so that this study is conducted in the most appropriate way.

Some information on ADME may be able to be derived from chemical structure and physico-chemical data and results from toxicity studies (e.g. on NOAEL, indications of induction of metabolism).

The collection of all available information is important for a decision on the route of administration, the choice of the vehicle, the selection of animal species, and the selection of dose levels and possibly for modifications of the dosing schedule.

The test substance is administered orally as a single exposure in graduated dose levels to several groups of experimental animals, one dose being used per group. A vehicle control group is also included. Most toxicity should be manifested within 24 hours. Thus, animals are terminated at 24 hours. A later time point should be included, between 48-120 hours after treatment if it is anticipated that the toxicities of interest will not be adequately evaluated by 24 hours. Appropriate justification should be submitted to explain the inclusion or exclusion of a second time point.

For animal welfare reasons, the single exposure study protocol is not intended to examine reversibility of acute effects. Although, reversibility can be one of the key criteria in arriving at a judgment on the adversity of an effect and the inclusion of recovery periods may be also helpful for the assessment of risk from intermittent exposures, this information should only be considered on the available data from repeated dose studies, since specific testing of reversibility would require more animals and this should be avoided.

The objective of the single exposure study is NOT:

- to identify lethal doses or provide data on mortality after acute exposure to a chemical,
to investigate the reversibility of acute effects, or

to investigate developmental effects or corrosive/irritation properties.

DESCRIPTION OF THE METHOD

This protocol covers investigations of a comprehensive range of relevant endpoints which may arise after a single exposure, or during one day of dietary exposure to a test substance. In particular, it is tailored to determine the most appropriate NOAEL to derive a refined Acute Reference Value. Special emphasis is placed on evaluating whether toxic effects observed in the standard package of repeated dose toxicity studies may also occur after single doses. It can also address additional parameters not usually examined in repeated dose studies, as well as provide further information on the dose-response curve and time to peak of acute toxic effects after a single exposure. The introduction of a new animal demanding test for acute toxicity; especially as such as Test Guideline 401, is definitely not the goal of this project.

The ILSI Health and Environmental Sciences Institute (HESI), through its Agricultural Chemical Safety Assessment (ACSA) Committee designed an animal single-dose study to provide data relevant to 1-Day human exposure is proposed with full evaluation at 24 hours and 7 days, with histology, clinical chemistry, haematology and other specialized investigations that may be indicated by structure activity or information from other studies as a first step of the proposed tiered approach (11). The ILSI HESI approach outlines also a draft protocol for an single dose test in dogs or rodents as step 5, which should contribute to moving away from paradigms that involve extensive animal testing for ‘every possible adverse outcome’ to a more science-based tiered approach and to reduce dog (e.g. one year study) and other testing requirements.

This single exposure study should be performed only after determining the most likely exposure route for humans so that the study can be designed for this route (oral, dermal, or inhalation) and cover relevant levels of exposure. In general, oral administration would be the route most often considered. If the appropriate pharmacokinetic and port of entry effects data are available route-to-route extrapolations might be performed. Additional guidance for dermal and inhalation methods can be found in appropriate OECD test guidelines.

Selection of Animal Species

The selection of animal species should be based on the results of the repeated dose studies, which usually restricts the choice to the rat or the dog. It should not be required to perform the study in both species.
Occasionally, mice may be more sensitive than rats or a better model for humans. If the mouse is the preferred rodent species, the principles described for the rat should be adapted accordingly. There are for example differences in the activity of enzymes in the tyrosine catabolic pathway between rats and humans. Toxic effects of some active substances in rats are largely attributable to increased plasma tyrosine levels following HPPD inhibition. Therefore, in these cases the mouse is more predictive of the exposure in humans. Rabbits are not relevant for such single dose studies, since if the rabbit is the most sensitive species for the derivation of an ARfD in a developmental study, no further ARfD refinement is justified. A justification should be given for the selection of the species. It should be demonstrated that the animals selected will respond to the relevant parameters with a higher sensitivity than other species and to be more relevant to human health risk assessment. For example we don't want someone doing a study in dogs with a phenoxy acid such as MCPA, because of the dogs are the most sensitive species for MCPA, but the rat is considered more relevant to human health exposure. Preferably, the animals used in this study should be from the same strain and source as the animals used in the key studies of the existing toxicological database for the test substance.

**Rats:** At the commencement of the study the weight variation of the animals used should not exceed $\pm 20\%$ of the mean weight. The test compound should be administered when the animals are between 8 and 10 weeks old. However, if there is evidence that an early postnatal stage may be more sensitive to the effects of the compound, it might be appropriate to conduct a special study which uses younger animals (weanlings) to evaluate the toxicity of interest (e.g., cholinesterase inhibition at postnatal days 11-21).

**Dogs:** Young adult animals should be used. The test compound should be administered to dogs 4-6 months of age and not older than 9 months of age.

**Housing and Feeding Conditions**

The feed should be analysed for contaminants. A sample of the diet should be retained until finalisation of the report.

**Rats:** The temperature in the experimental animal room should be 22 °C ($\pm 3$ °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. Animals 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:** For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark.

**Preparation of Animals**

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.

**Preparation of Doses**

This study should have a minimum three dose groups plus vehicle control group for deriving a NOAEL/LOAEL. If a benchmark approach is intended, more than three dose groups should be considered. In this case, the number of animals per group could be reduced, as long as the necessary statistical requirements are fulfilled. Where necessary, the test substance is dissolved or suspended in a suitable vehicle. The toxic characteristics of vehicles other than water must be known. The homogeneity of the test substance in the vehicle should be assured.

Based on the definition of the Acute Reference Dose, the acute intake is generally assessed on a per day basis. A worst-case exposure scenario would be to assume that daily intake occurs in a single meal. Therefore, the most appropriate animal dosing would be by gavage in rodents and by capsule in dogs. This dosing regimen would be particularly relevant when effects are C\text{max}-dependent and rapidly reversible (e.g. inhibition of acetylcholinesterase by carbamates). However, other means of dosing may also be appropriate. If exposure is in the food, the dogs should consume their daily ration completely within one hour. Data on the palatability of the intended dose levels in diet must be available.

**PROCEDURE**

**Number and Sex of Animals**

**Numbers of Animals:** The numbers of experimental animals used should be based on statistical power calculations and the variability of the specific end-points noted in the repeated dose studies as being especially relevant. For reasons of animal welfare as few
animals as possible should be used. For each dose, equal numbers of animals should be
sacrificed at the 24 hour termination time point, and if included, the later (second) time point.
If a later time point is included, additional subgroups of the same size should be used.
At least 10 rats (5 rats per sex and per group) should be used at each dose level, including the
vehicle control group. If a vehicle is used, a negative control is not required in addition to a
vehicle control. If only one sex is evaluated, then the number of animals could be increased, if
necessary, to provide more power to detect the toxicity of interest or more dose-groups could
be included to provide data for benchmark modelling.
A minimum of four dogs per sex and per dose group should be used for the 24-hour
evaluation. Only one sex should be evaluated for the toxicity of interest unless the
preliminary data suggest both sexes should be evaluated.
If identification of the toxic effect(s) of interest is possible in live animals at the 24 hour time
point, an additional subgroup may not be necessary and it may be sufficient to use the same
group of animals for the sacrifice at the later time point for pathomorphological examinations.
Sex: Both males and females could be used if necessary. Females should be nulliparous and
nonpregnant. Because existing information should be used to tailor and appropriately focus
this study, if existing data on the chemical show that one sex is clearly and consistently much
more sensitive than the other for the endpoint(s) identified as being relevant for acute toxicity,
then the study design should be modified to include only the more sensitive sex.

Dose Selection
At least three dose levels and a concurrent vehicle control should be used. Dose levels should
be selected taking into account any existing toxicity and ADME data available for the test
compound. The data should be sufficient to produce a dose-effect curve. Thus, dose levels
should be spaced to produce a gradation of toxic effects, ranging from recognisable toxicity
but not death or severe suffering at the highest dose to no or only very slight effects at the low
dose. If it is intended to establish a benchmark dose level rather than a NOAEL/LOAEL, it
may be sensible to increase the number of dose groups. A reduced spacing of dose levels may
allow the study to be conducted with fewer animals per subgroup, depending on the statistical
requirements for this approach.
Possible starting points for setting dose levels are known LD$_{50}$ values in animals and expected
exposure levels in humans. Furthermore, also cytotoxicity data according to NIH Publications
No: 01-4500 (1) can be used.
In addition, the highest/overall NOAEL from the repeated dose studies using the same animal species could be selected as the low dose and together with one or two of the effect doses from the repeated dose studies. The high dose may be limited to 1000 mg/kg bw/d, unless expected human exposure indicates the need for a higher dose level to be used.

Special consideration should be given if the NOAEL from the repeated dose studies is representative for provoking acute effects, e.g. clinical effects observed at the begin of a repeated dose study.

If the test substance is a pesticide and the results of the study will be used for the derivation of an ARfD related to acute intake estimations, the high dose need not be greater than 500 mg/kg bw/d.

Administration of Doses

The most appropriate dosing would be by gavage in rodents and by capsule in dogs. Gavage should be done in a single dose to fasted 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/100 g body weight, except for aqueous solutions, where 2 mL/100 g bw may be used. With the exception of irritating or corrosive substances, which are likely to cause exacerbated effects with higher concentrations, variability in volume should be minimised by adjusting the concentration to ensure a constant dosing volume at all dose levels.

Apart for treatment with vehicle instead of the test substance, the animals in the control group should be handled in an identical manner to those in the test group. If a vehicle is used to administer the test substance, the control group should receive the vehicle in the same volume used as total application volume (vehicle + test compound) in the treated groups. If different volumes are administered to the different treatment groups, the control should receive the vehicle at the highest volume used.

If administration is via feed in the dog, the single dose should be consumed completely in one meal within approximately one hour; a confirmation of this consumption time should be provided in the study report.

Clinical Observations

Clinical observations should be made in all animals at least once before exposure to the test substance (to allow for within-subject comparisons) and at least 0.5, 1, 2, 4 and 24 hours after
dosing. The peak period of the anticipated effects should be considered when determining the time points for clinical observations.

If later time points are evaluated (e.g., 48-120-hour subgroups), further observations should be made at least twice daily after the first 24 hours.

Observations should be carefully recorded, preferably using scoring systems, explicitly defined/reported by the testing laboratory. Effort should be made to ensure that variations in the test conditions are minimal and that observer bias is excluded.

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, and unusual respiratory pattern).

Changes in gait, posture, response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g. excessive grooming, repetitive circling) or bizarre behaviour (e.g. self-mutilation, walking backwards) should also be recorded.

**Body Weight and Food/Water Consumption**

All animals should be weighed on the day of treatment and prior to sacrifice of the subgroup. In addition, the animals of the 48-120-hour subgroups (if present) should also be weighed every 24 hours after treatment.

Measurements of food consumption and drinking water intake should be made daily.

**Toxicokinetics**

Information on toxicokinetics should be obtained before commencing this single exposure study. However, frequently toxicokinetic data will only be available for the rat. If the dog is used as the more appropriate species in the single exposure study, additional information on toxicokinetics may be necessary. Collection of samples for substance plasma levels at different time points can be incorporated into the design of the study if it does not interfere with other investigations. Blood samples should be taken at least at subgroup termination time points.

**Functional Observations**

If existing data indicate that the critical effect of the compound is neurotoxicity, then the acute neurotoxicity test guideline should be considered (see OECD 424 and OPPTS 870.6200). Alternatively, the elements described in this guideline may be combined with the design of an acute neurotoxicity battery study, as long as none of the requirements of both guidelines are
violated by the combination. The parameters included may be tailored based on the extent of 
existing knowledge.

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

This evaluation should be conducted in the peak period of the anticipated effect, e.g. 1, 2 or 4 hours, as well as just before sacrifice of the subgroups. If the peak effect is expected to be close to 24 hours then the 24 hour observation is sufficient.

**Haematology**

The haematologic examination is only required if data from repeated dose studies indicate that the blood cells and/or the haematopoietic system are target sites. The following haematological examinations should be made just prior to or as part of the procedure for killing the animals at the end of the test period: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, and blood clotting time/potential. Justification should be given, if these parameters are not investigated.

Additional guidance for haematological and clinical biochemistry parameters can be found in OECD test guideline 412.

**Clinical Biochemistry**

The clinical biochemistry examination is only required if data from repeated dose studies indicate that these parameters are of concern. The parameters evaluated may depend on the species selected (typically rat or dog) and on the results of the repeated dose studies. Clinical biochemistry determinations should be performed on blood samples of all animals taken just prior to or as part of the procedure for killing the animals in each subgroup at the end of the test period. In general, the following investigations of plasma or serum should be included: glucose, total cholesterol, urea, creatinine, total protein, 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 and bile acids may provide useful information under certain circumstances.

In addition, the investigation of serum markers of acute tissue damage should be considered. These need to be identified for chemicals in certain classes or on a case-by-case basis.
If a specific, potentially acute effect of the test substance has been observed using special
techniques in repeated dose studies, then these techniques should also be used in this study.

- Cholinesterase inhibition in plasma, red blood cells, brain and peripheral nervous
tissue should be measured for compounds known to inhibit these enzymes.
- Blood methaemoglobin should be measured for compounds known to increase
methaemoglobin formation. In this case it is advisable that blood samples are obtained
at the time of peak effect if it does not interfere with other investigations since Met-Hb
formation is an acute effect and Met-Hb is rapidly degraded.
- For endocrine modulators, specific hormones, which could be affected after single
exposure, should be measured.

**Urinalysis**

Urinalysis determinations are optional and only necessary if data from repeated dose studies
indicate that this is a critical parameter to be evaluated. Urinalysis determinations should be
performed just prior to termination. The following parameters should be evaluated:
appearance, volume, osmolality or specific gravity, pH, protein, glucose, blood and blood
cells, cell debris.

**Pathology**

Methods for humane killing according to OECD series on testing and assessment No. 19 have
to be considered. The pathological and organ weight evaluations should focus on
tissues/endpoints that are found to be targets in the repeated dosing studies.

**Gross necropsy**

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, the cranial, thoracic and
abdominal cavities and their contents.

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),
gonads, accessory sex organs (e.g. uterus, prostate), 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, and a section of bone marrow (or, alternatively, a freshly mounted bone marrow aspirate). Specific attention should be paid to likely target organs based on the known properties of the test substance. If an inhalation study is performed, the respiratory tissues preserved should be those mentioned in OECD TG 412. Skin should be preserved when a dermal study is performed.

**Organ weight**

Unless existing data from repeated dose studies with the test substance indicate that an organ is not a target site, the following organs should be trimmed of any adherent tissue, as appropriate, and their wet weight should be measured as soon as possible after dissection to avoid drying: liver, kidneys, adrenals, testes, epididymides, thymus, and spleen.

In addition, if relevant as target organ for acute effects of the test substance, the wet weight should be determined for the following organs as soon as possible after dissection to avoid drying: paired ovaries, uterus, seminal vesicles (including coagulating glands), and prostate (dorsolateral and ventral part combined). Alternatively, seminal vesicles and prostate may be trimmed after fixation. Clamp or ligature should be present during fixation as leakage of fluid provokes damage to fine structures in seminal vesicles.

The following organs should be weighed after fixation: thyroid (trimming should also be performed after fixation in order to avoid tissue damage) and dorsolateral and ventral parts of the prostate separately after separation.

**Histopathology**

Full histopathology should be carried out on the preserved organs and tissues of all animals in the control and high dose groups unless existing data from repeated dose studies indicate that an organ is not a target site. These examinations should be extended to animals of all other dose groups, if treatment-related changes are observed in the high dose group.

All gross lesions shall be examined.

**DATA AND REPORTING**

Individual animal data should be provided. Additionally, all data should be summarised 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 sacrificed for humane reasons and their respective cause of death, the number showing signs of toxicity, a description of the signs of toxicity observed, including time of onset, duration, and severity, the number of animals showing lesions, the type of lesions and the percentage of animals displaying each type of lesion.
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.

Test Report

The test report must include the following information:

Aim of the study:
- Justification for conducting such a single exposure study
- Rationale for the specific design (e.g. choice of species and sex, dose selection, endpoint selection)

Guidelines and Quality Assurance:
- Test type (Guideline)
- GLP

Test substance:
- physical nature, purity and physicochemical properties
- identification data

Test animals:
- species and strain used
- number, age and sex of animals
- source, housing conditions, diet etc.
- individual weight of animals at the start of the test

Test conditions:
- rationale for dose level selection
- details of test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation
- details of the administration of the test substance
- conversion from diet test substance concentration (ppm) to the actual dose (mg/kg bw/d), if the test substance was administered via the diet
- details of food and water quality

Results:
- body weight/body weight changes
- food consumption, and water consumption, if applicable
- toxic response data by sex and dose level, including signs of toxicity
- nature, severity and duration of clinical signs
- functional observations (e.g., sensory reactivity, grip strength, motor activity assessments)
- haematological tests with relevant base-line values
- clinical biochemistry tests with relevant base-line values
- body weight at sacrifice and organ weight data
- gross necropsy findings
- a detailed description and tabulation of all histopathological findings
- statistical treatment of results
Summary and discussion of results
Conclusions, Critical effects, NO(A)EL, LO(A)EL (or benchmark dose, if applicable)
Appendix to Annex 2

A retrospective analysis of ARfD values of pesticides in the European Union

Introduction

In 2002 an analysis of the ARfD values set by several regulatory bodies was performed (1, 2). There were large differences in the ARfD values between the analysed regulatory bodies (up to 2500-fold for some individual pesticides). In result of this analysis it was concluded “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.”

In 2002 the ARfD value was still relatively new and no harmonised guidance for the derivation of an ARfD was available. In the mean time the regulatory authorities made more comprehensive experiences with the derivation of ARfD values for all pesticides, which were evaluated in the last six years. Notifiers and authorities made also the first practical experiences with the application of specific additional studies which were designed and performed for the derivation of ARfDs. Therefore, a new retrospective analysis was considered necessary in order to identify the toxicological studies on which the ARfD values are based in 2008. This analysis was recommended as a supportive basis for a harmonized guidance on how to use all available data on ARfD derivation and also for the development of an ARfD study design. This analysis should also identify how often such a specific single exposure test was submitted, what were the tested parameters, that such a study can be performed in future according to a harmonised OECD test procedure.

The current retrospective analysis was based on the data of active pesticide substances which have been evaluated and peer-reviewed in Europe and included in Annex I of EU directive 91/414/EWG between 2000 and 2008. The reason to use this EU data base for the ARfD analysis was that in the EU all ARfD values are especially well intensively discussed in a long peer review process by the regulatory authorities of all member states of the European Union and the results of this discussion are published regularly.
Material and Methods

The European Commission maintains a tabular list with all existing and new active pesticide substances. This table contains information about the stage in which the active substance is evaluated, the Rapporteur Member State, the current status of each substance as well as further data such as ARfD and ADI including the source for the derivation of these threshold values and the year.

The current analysis of the ARfD values was based on the last revision of this annotated list of active pesticide substances which was published by the European Food Safety Authority (EFSA) on the EFSA website. Substances of this list have only been considered for the retrospective analysis if they have already been included in Annex I of EU directive 91/414/EWG. This means that the risk assessment process on basis of a draft assessment report and a peer review by the EU Member States has been finalised.

Furthermore, substances of the EFSA list have only been considered if a statement on the ARfD was given. Microbial pesticides and other not clearly chemically defined substances like plant extracts have been sorted out. Finally 198 active substances (existing and new pesticide substances) have been considered for the analysis.

The sources for the ARfD derivation according to SANCO 3010 have been sorted into 8 groups of studies: 1. Special ARfD studies (i.e. single dose studies which have been performed to derive a refined ARfD; these are additional studies to the usual data requirements which have not been performed according to OECD guidelines), 2. Acute Neurotoxicity studies, 3. Repeated dose studies, 4. Multigeneration studies, 5. Developmental toxicity studies, 6. Developmental neurotoxicity studies (DNT), 7. Human studies, 8. No ARfD derived (i.e. considered not necessary because of low acute toxicity according to the guidance on the principles for not setting an ARfD).

The portion of every group was calculated in per cent of all studies.

Results

The percentages of the 8 groups of studies in this analysis of the data basis for the ARfD derivation of 198 substances are summarised in Table 1. For 95 pesticides, approximately the half of all substances (i.e. 48 %), no ARfD was considered necessary because of low acute toxicity of these pesticides. For 103 of the analysed substances (i.e. 52 %) an ARfD was established. Most of these ARfD values have been derived from developmental toxicity...
studies in rats or rabbits (26.8%). 10.1% of the ARfD values are based on acute neurotoxicity studies. The portion of special ARfD studies applied for an ARfD derivation is very low. Only 4% of the ARfDs are based on such studies. The use of DNT and human studies is negligible in the European Union.

For 19 pesticides (< 10 %) the ARfD can be considered as conservative, since it was based on repeated dose toxicity or multigeneration studies.

### Table 1 ARfD derivation in the EU pesticide evaluation program (198 active substances)

<table>
<thead>
<tr>
<th>Studies used for ARfD derivation</th>
<th>Number of derived ARfD values</th>
<th>%</th>
<th>Symbol in Figure 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Special ARfD study / mechanistic study (single dose or few repeated doses)</td>
<td>8</td>
<td>4.0</td>
<td>A</td>
</tr>
<tr>
<td>Acute neurotoxicity study</td>
<td>20</td>
<td>10.1</td>
<td>B</td>
</tr>
<tr>
<td>Repeated dose toxicity study</td>
<td>16</td>
<td>8.1</td>
<td>C</td>
</tr>
<tr>
<td>Multigeneration study</td>
<td>3</td>
<td>1.5</td>
<td>D</td>
</tr>
<tr>
<td>Developmental toxicity</td>
<td>53</td>
<td>26.8</td>
<td>E</td>
</tr>
<tr>
<td>Developmental neurotoxicity</td>
<td>2</td>
<td>1.0</td>
<td>F</td>
</tr>
<tr>
<td>Human study</td>
<td>1</td>
<td>0.5</td>
<td>G</td>
</tr>
<tr>
<td>No ARfD derived (not necessary)</td>
<td>95</td>
<td>48.0</td>
<td>H</td>
</tr>
</tbody>
</table>

**Figure 1 ARfD derivation in the EU pesticide evaluation program**

### Discussion and Conclusion

The results of the retrospective analysis of the ARfD values in the EU in 2008 are approximately in the same range of the ARfD values set by several regulatory bodies in 2002. Solecki et al. (1) concluded that 23% of the ARfD values were based on single dose studies. The majority of these acute studies were acute neurotoxicity studies in rats. 39% of the analysed ARfD values were based on maternal and/or developmental effects in

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3 (www.efsa.europa.eu) with the specification SANCO 3010, rev 10/11/2008
developmental toxicity studies in rats or rabbits. The conclusion that an ARfD is unnecessary varied between 14% and 54% of the analysed substances in 2002. The conclusion of Solecki et al. (1) “that the current database of toxicological studies is not optimal for the derivation of the ARfD” is not so applicable to the analysis of the EU ARfD values in 2008. The majority of ARfD values were based on studies in which specific acute alerts (i.e. developmental toxicity and neurotoxicity) was investigated. For these 75 substances no refinement of the ARfD with a special study is necessary. Only for the 19 pesticides in which the ARfD was based on repeated dose toxicity or multigeneration studies a refinement with a specific study might be considered, if the ARfD is exceeded by the acute intake assessment.

For a small portion of pesticides (4 %) special acute studies were submitted for the ARfD derivation. These special acute ARfD studies do not belong to the basic requirements and guidance papers do not exist. The only available acute studies apart from the LD\(_{50}\)-studies are currently the acute neurotoxicity studies which are used to derive approximately 20% of the ARfD values, but if neurotoxicity is not the most relevant acute alert, no specific study design are available. Special studies to evaluate the acute toxicity as a basis for the ARfD derivation are mostly performed additionally to the basic data requirements in the process of discussion of the toxicological assessment between notifiers and authorities. These studies may be required if the evaluation of acute toxicity and the derivation of an ARfD is difficult on the basis of routinely required studies. However, in some cases such submitted studies are not acceptable by the authorities because of quality deficiencies as a result of a missing guidance paper. Therefore, in the EU peer review process some of the submitted special ARfD studies have not been used for the ARfD derivation. The results confirm once more that the development of an acute study design that produces more comprehensive toxicological data for setting ARfDs would be of high value. One the other hand this analysis has shown that such a special ARfD study was considered necessary only for very few pesticides.