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Guidance document for setting an Acute Reference Dose in Dutch national pesticide evaluations

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Abstract

This report describes a proposal for the procedures for setting an Acute Reference Dose (ARfD) for pesticides evaluated in the Netherlands. This deals with both evaluations on the national level (on behalf of the Dutch Board for the Authorisation of Pesticides (CTB)) and evaluations at the European level (EU-monographs), either made within the scope of the Pesticide Act ("Bestrijdingsmiddelenwet" BMW) or the EU Directive 91/414.
Subjects covered by this report are: a definition of the concept of the ARfD, criteria for setting an ARfD, the relevance of effects for an ARfD, the use of assessment factors. In addition, a fututre outlook is presented on developments in the field of risk assessment for acute exposure to pesticides.

Contents

Samenvatting

Bij de toelating van bestrijdingsmiddelen op de (Nederlandse) markt worden de risico's voor zowel de chronische als de acute blootstelling aan (residuen van) pesticiden beoordeeld. Voor de chronische risicoschatting wordt de gemiddelde dagelijkse blootstelling vergeleken met de ADI ('Acceptable Daily Intake'). Voor acute blootstelling is het concept van de ARfD ('Acute Reference Dose) ontwikkeld. De ARfD is gedurende de laatste 5-10 jaar in gebruik genomen, speciaal om de risico's van bepaalde (groepen) bestrijdingsmiddelen in te schatten indien er een incidentele blootstelling is hoger dan de ADI. Hoewel de term ARfD reeds enige jaren in gebruik is binnen het veld van de bestrijdingsmiddelen, is het concept van de ARfD nog immer onderwerp van discussie.
In dit rapport wordt het concept van de ARfD verder uitgewerkt en worden er richtlijnen en beslisschema's gegeven die ertoe moeten leiden dat een waarde als de ARfD voor bestrijdingsmiddelen op een consistente manier wordt afgeleid, in ieder geval binnen Nederland.
Specifieke aandacht wordt besteed aan de volgende onderwerpen: strikte definitie van de ARfD, voor welke stoffen dient een ARfD te worden vastgesteld, welke toxicologische eindpunten zijn van belang voor een éénmalige orale blootstelling, hoe wordt voor deze eindpunten een No-Observed-Adverse-Effect-Level afgeleid, welke studies zijn wel of niet geschikt voor het afleiden van de ARfD, hoe dient men om te gaan met humane studies, welke extrapolatie- of veiligheidsfactoren worden gebruikt en welke condities verdienen speciale aandacht.

Summary

Within the procedures for the admission of pesticide on the (Dutch) market, the public health risks of both chronic and acute dietary exposure to (residues of) pesticides are evaluated. For chronic risk assessment, the mean daily exposure is compared to the ADI (Acceptable Daily Intake). For acute exposure, the concept of the ARfD (Acute Reference Dose) has been developed. The ARfD has been introduced over the last 5-10 years, specifically to assess the acute health risks of certain specific (groups of) pesticides, in case an occasional excursion above the ADI occurs. Although the ARfD is used for several years within the field of pesticide risk assessment, the concept of the ARfD is still subject to debate.
In the present report, the concept of the ARfD is further evaluated and guidance and decission schemes are presented in order to achieve a consistent way of allocating an ARfD, at least for the Dutch situation.
In this report, specific attention is paid to the following subjects: a further definition of the ARfD concept, when should an ARfD be allocated, which toxicological end points are relevant for acute oral exposure, how to set an No-Observed-Adverse-Effect-Level for these end points, which studies are appropriate for setting an ARfD, how are studies with humans used, which assessment factors are used, and which conditions require specific attention.

1 Introduction

The risk for health effects due to a lifetime dietary exposure to residues of pesticides is evaluated using the concept of the Acceptable Daily Intake (ADI). The ADI was defined by JECFA as 'an estimate of the amount of a substance, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk'. Occasional slight excursions above the ADI (e.g. due to a single meal on one day) may occur and are not necessarily of toxicological concern.
However, some pesticides or their residues may have specific acute toxicological properties or may induce long term effects after only a single dose at a level above the ADI. In this case, a short term excursion above the ADI may pose a health risk. The ADI is not an appropriate toxicological limit for estimating the risk of adverse health effects for such a short-term exposure to (residues of) pesticides at a level above the ADI. For the risk assessment of such short-term exposures, the concept of the Acute Reference Dose (ARfD) has been developed [1] triggered by concern for the acute toxicological risks of some types of pesticides (e.g. organophosphates). The ARfD is used primarily in the regulatory process of pesticides in order to evaluate the possible acute health risks of exposure to a pesticide through dietary intake. In addition, the ARfD may be used also in the actual risk assessment of the acute health risks of food products with high pesticide residues above the MRL.
Guidance for setting an ARfD has been prepared by JMPR [1] while recently a concept EU-guideline for setting an ARfD has been proposed by Germany [2]. However, the guidance published by JMPR is prepared in general terms describing only the principal starting points. The concept EU-guidance document is more detailed but provides little actual guidance in setting the ARfD. Since ARfD's have to be set in current pesticide evaluations while adequate international guidance is not (yet) available; this guidance document describes the procedures for setting an ARfD in Dutch national pesticide evaluations.

2 Concept of the ARfD

The ARfD has been developed to assess the risk for adverse health effects after a single or a short-term oral exposure. The ARfD has been defined by JMPR [1] as:

> ...*"an estimate of the amount of a substance in food or drinking water, expressed on a body weight basis, that can be ingested over a short term 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 evaluation. It is usually expressed in milligrams per kilogram of body weight."*

The definition of the ARfD as stated above allows variable interpretation of the ARfD concept since the definition states 'a short period of time'. Despite the addition of 'usually during one meal or one day', it can be argued which time intervals will be or will be not covered by the concept of the ARfD [3]. Confusion exists which exposure duration should indeed be covered, acute or short-term exposures [4]. Within the scope of the ARfD, acute and short-term exposures have been often intermingled also in policy documents [2,3] but from a toxicological point of view this is unacceptable. In fact, the ARfD is sometimes also used for the risk assessment of short-term excursions above the ADI for several subsequent days. In most cases, this does not provide a problem but for some pesticides the severity of toxicological effects may be substantially different after a single or repeated dosages (even for a few days). This type of confusion possibly also influences the process of setting the ARfD because importance is attached also to elements of repeated toxicity and recovery. Therefore, the concept of the ARfD requires a strict definition.

When considering exposure to a specific pesticide or its residues through the diet within the context of the ARfD, basically three conceptually different exposure scenarios can be expected. These scenarios are described below in a general and more or less simplified way and are intended to serve as examples only. In these examples, the exposure of a given individual to a given pesticide is depicted.

2.1 Exposure scenario 1.

This scenario refers to a condition called 'the hot apple'. Because of variability in pesticide residues and the possible mixing of food commodities from different origin on the market (5,6), the consumption of only a single food commodity or a single meal with a 'high' pesticide residue may result in a single exposure to a specific pesticide at a level above the ADI (see figure 1). This is basically the type of exposure that has led to the development of the ARfD (6). In this scenario, the ADI is exceeded only for a single day. It is considered that the possibility of a repeated exposure to a 'hot apple' within a short period of time is negligible.

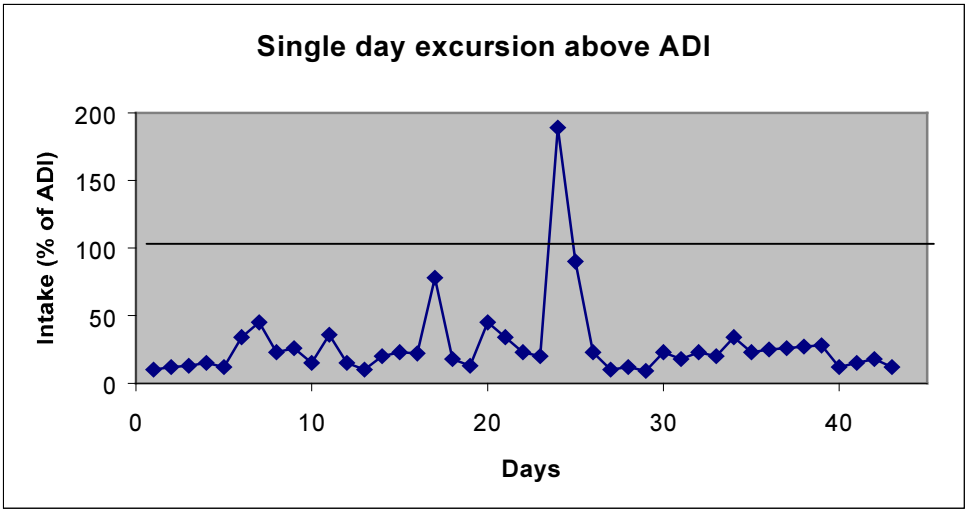

Figure 1. Example of an exposure involving a single day excursion above the ADI.

2.2 Exposure scenario 2.

In this scenario, the exposure of people is above the ADI for several subsequent days. This situation occurs when people may ingest a specific food commodity with a high residue on several consecutive days. For example, eating some grapes every day from the same bunch of grapes with high residues.

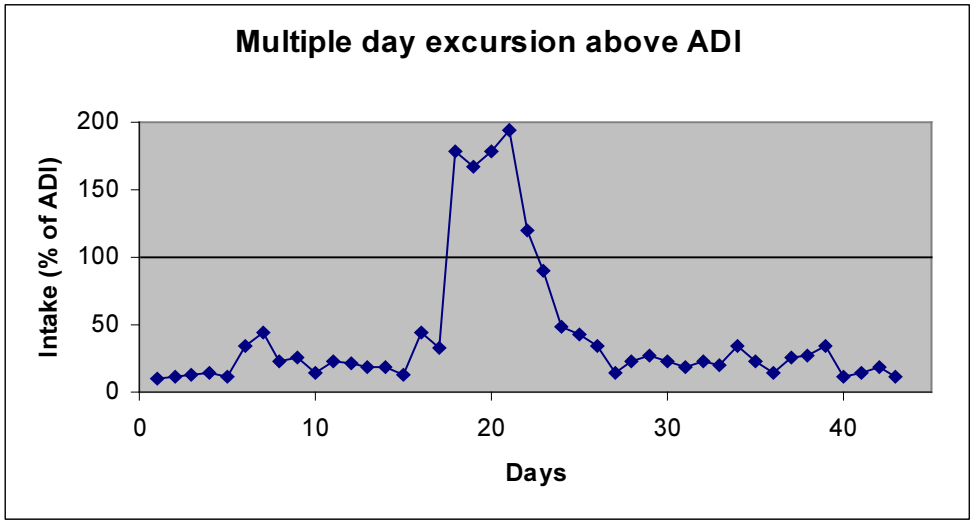

Figure 2. Example of a multi-day excursion above the ADI.

2.3 Exposure scenario 3.

Within the procedures for admission of a pesticide on the market, a risk assessment for chronic exposure to pesticide residues is based on the use of daily mean consumption figures of food commodities. The mean consumption figures are calculated taking into account also non-consumers. Therefore, the actual consumption figure for a certain product (e.g. the amount of spinach consumed during a single meal) may be higher than

the mean consumption figure used in the calculations, especially for products which are consumed with a low frequency (e.g. some exotic fruits).

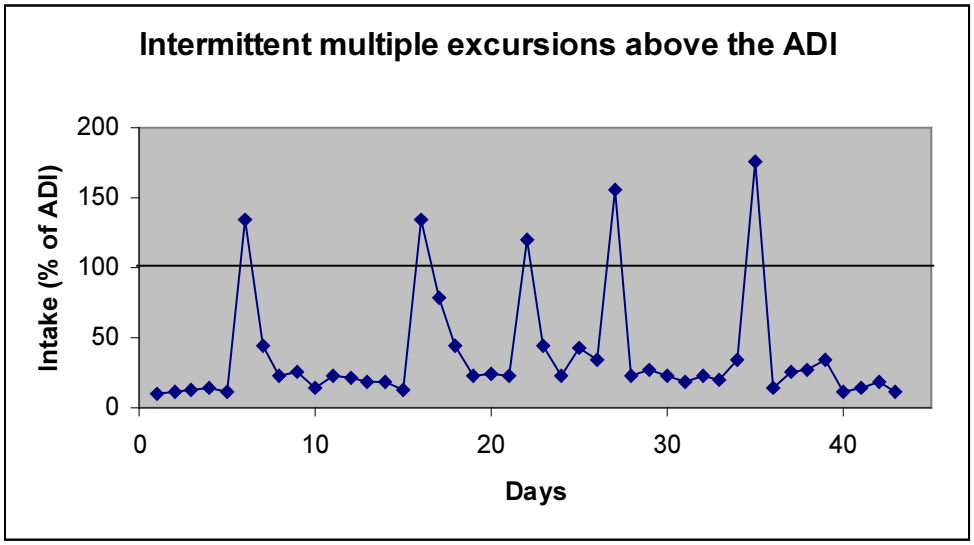

Figure 3. Example of repeated intermittent excursions above the ADI.

Theoretically, consumption of such products may in some cases lead to an exposure scenario as depicted in figure 3 in which the ADI may be exceeded on repeated occasions within a limited period of time. Whether in daily practice such conditions occur frequently or not is presently unknown.

In principal, these three basic exposure scenarios cannot be covered by a single toxicological limit value since for each of these scenarios different aspects of toxicology should be taken into account [4].
For example, for scenario 2 one should consider the effects of repeated dosing into setting of a limit value. However, each chemical has its own characteristics with respect to repeated exposure and, moreover, it should be defined which time frame should be covered: 3 days, 5 days, 1 week, 2 weeks, or 1 month? A risk assessment for these types of exposures should use some type of 'short-term Reference Dose'.
In the case of scenario 3, the repeated excursions above the ADI may or may not result in acute health risks (see introduction). However, when the acute risks of this exposure pattern are evaluated, the kinetics and the reversibility of effects are of pivotal importance and should be taken into consideration when setting a limit value. Apart from deriving a type of reference dose for such a single peak exposure one should also account for a 'safe lag time' between the peak exposures depending on the kinetics and reversibility of the effects induced. Again, such characteristics are substance specific and the question arises which lag time should be covered. A risk assessment for these types of exposures should use some type of 'Intermittent Reference Dose' or use some type of time-weighed average dose.
Thus, when done properly, the health risks of each of the above exposure scenarios should be assessed using its own limit values derived specifically for that purpose.

2.4 The ARfD concept further defined

At present, all 3 exposure scenario described above are (sometimes) linked to the concept of the ARfD and there is a lack of international consensus about these issues. However, for the purpose of (inter)national harmonisation, the ARfD concept should be based on only one specific exposure pattern. In the present guidance document, we propose that the concept of the ARfD in the present guidance document is limited to scenario number 1; i.e. a single excursion above the ADI for one single day. This is the exposure scenario which has originally triggered the discussion on the acute health risks of pesticides.

Although in principle toxicological differences may occur between exposure as a consequence of a single sitting (one single meal) or due to spread intakes over a single day (several meals), this guidance document will not discriminate between these two exposures. The ARfD will be expressed as the maximal intake within one day. Exposure through a single sitting (or by gavage in animal studies) may than be considered as 'the worst case' [6].

For the Dutch situation, the ARfD is defined as:

> ...*"an estimate of the amount of a substance in food or drinking water, expressed on a body weight basis, that can be ingested 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 evaluation. It is expressed in milligrams per kilogram of body weight on a single day of exposure."*

Along with this concept the possibility of being exposed to a second peak exposure above the ADI within a limited period of time is considered negligible [7]. This indicates that – by default – characteristics of repeated exposure and recovery of effects are not primary relevant for setting an acute reference dose because one does not account for a second exposure.

3 When should an ARfD be allocated ?

An ARfD is, similar to an ADI (RfD) or an AOEL, explicitly a toxicological limit value. The ARfD is therefore based solely on the toxicological properties of a chemical substance. From this it follows that an ARfD should principally be allocated for all substances [8].
However, for some substances, evaluation of the toxicological database will show that the substance has no particular acute toxicological hazards. Therefore, allocating an ARfD may be considered unnecessary or irrelevant [6]. Whether an ARfD needs to be allocated or not is defined using the following criteria. An ARfD needs not to be allocated when each of the following criteria are met:

a) The test substance does not induce any effects (including behavioural, clinical signs, and gross pathology) in an acute oral toxicity study at a dose 2000 mg/kg bw. Hence, the substance needs no classification for acute oral toxicity according to EU-guidelines.
and b) The test substance is not acutely neurotoxic (or expected to be) based on the available set of toxicological information.
and c) There are no indications or triggers from repeated dose studies indicating the occurrence of toxic effects after an acute exposure.
and d) No embryo- or foetotoxicity or developmental effects are present at levels which do not induce maternal toxicity.

In every other case, an ARfD should be derived. This is illustrated in the figure 4.

When it is concluded not to allocate an ARfD using the criteria above, a transparant argumentation on how this conclusion is reached should be included in the review report of the pesticide. It should be noted that argumentation for not allocating an ARfD is equally important as argumentation for allocating an ARfD on a specific effect.
In addition, deviation from the criteria above may be acceptable occasionally as long as a solid scientific argumentation is included.

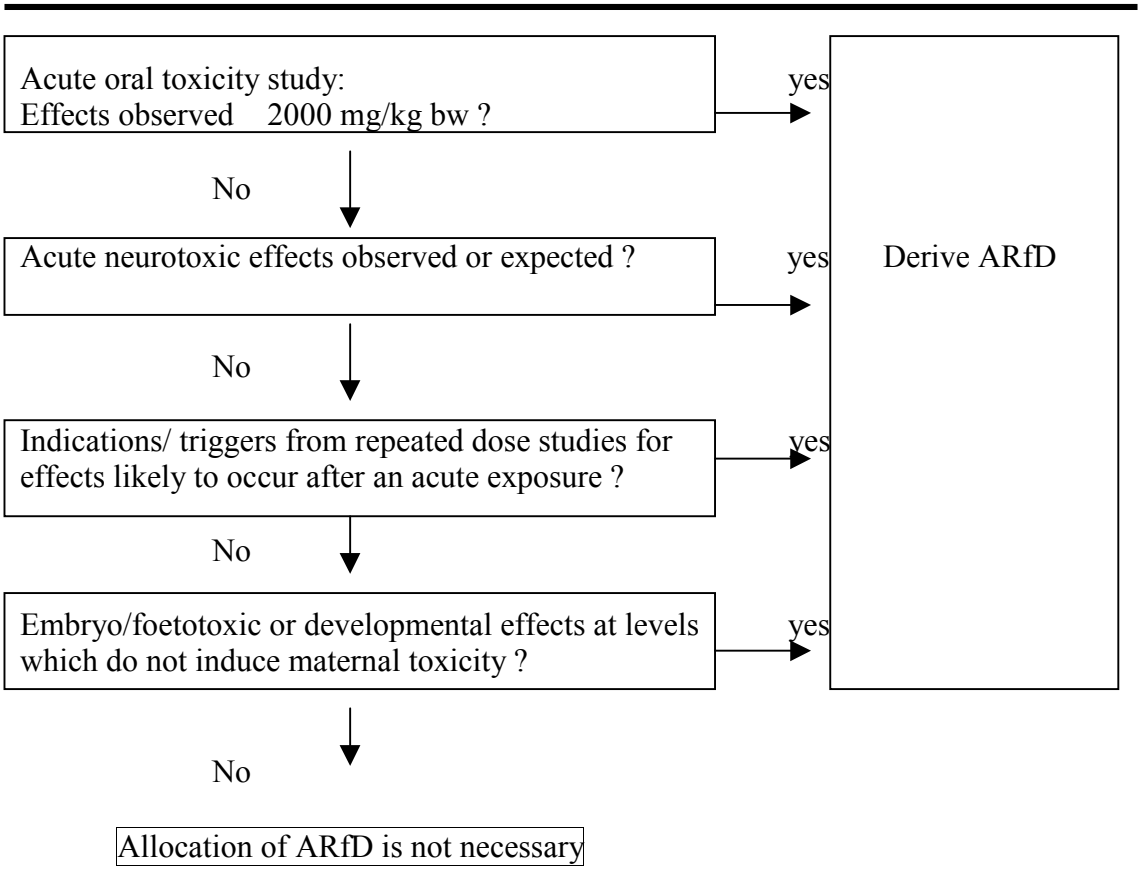

Figure 4. Schematic decision tree whether or not to allocate an ARfD (after ref. 6).

4 How to set the ARfD.

The procedure for derivation of an ARfD involves 4 steps which will be discussed in separate paragraphs below.

Selection of toxicological endpoints relevant for acute exposure.
Selection of the most appropriate study
Determination of the NOAEL and LOAEL for the selected endpoints
Establishment of the assessment factors to be used.

4.1 General considerations

In general, setting of the ARfD takes into account the complete toxicological database, i.e. all available toxicological information (including kinetics and metabolism) at the time of evaluation. It is not possible to pre-define the most appropriate study or effect that should be used for allocating an ARfD. As a general concept, the ARfD should be based on the most sensitive acute toxicological endpoint(s) of relevance to humans, derived from the most appropriate study in the most appropriate species, allocated on a case-by-case basis. Using this approach, the procedure for setting an ARfD is focussed primarily at selecting the most relevant endpoint(s) for acute exposure rather than selecting the most relevant study. Selecting the study from which to derive the ARfD should be the second step in the process, but not secondary in importance to selecting endpoints.

It should be emphasised that the effects used to derive an ARfD must be relevant for acute exposure to the substance under consideration, i.e. it will be likely that these effects may be induced by a single day exposure. Although in many cases it cannot be concluded that specific effects are indeed likely to occur after acute exposure, their occurrence after acute exposure can certainly not be excluded. This holds true especially for specific embryo- or foetotoxic effects. In such cases, the effects are considered relevant for acute exposure and may be used for setting an ARfD.
The toxicological endpoints selected for deriving an ARfD may be quite different from the endpoint(s) used for setting the ADI or AOEL. Setting the ARfD is therefore a process that is independent from setting the ADI or the AOEL. However, this does not exclude the possibility that the ARfD may eventually be similar to the ADI or AOEL since for some substances the acute toxicological characteristics remain the most pronounced effects even after repeated exposure (e.g. for reversible cholinesterase inhibitors). From the definition of the ADI (see introduction) it can be concluded that the ARfD cannot be set at a lower level than the ADI.

In the procedure for setting an ARfD, several choices will need to be made by the risk assessor (selecting relevant endpoint, selecting study, selecting assessment factors etc.). Because many of these choices are made on a case-by-case basis, this urges for a high level of transparency in reporting. Therefore, all choices made during the procedure for setting an ARfD should be clearly argued and reported.

4.2 Selecting relevant toxicological endpoints and studies

4.2.1 Relevant endpoints and mechanism of action

As stated above, selection of the most appropriate endpoint for setting the ARfD should be based on consideration of the complete toxicological database available. Knowledge of the mechanism of action of a specific substance is very helpful in identifying the most relevant endpoint for acute exposure. Sometimes this may lead to a straightforward identification of relevant endpoints. For example, organophosphates and carbamates inhibit AChE, pyrethroids affect voltage-gated sodium channels and hence nerve function, and aniline-derivates may induce methaemoglobin formation. However, other effects induced by these substances should not be automatically discounted (see below).

Kinetic data may also be of help in identifying the most relevant target sites or toxicological endpoints. For example, data on the distribution of a substance after a single oral administration can identify the tissues with exposure to the substance. In other cases, the elimination half-life may be of importance in setting an ARfD (see also section 8.3). Unfortunately, the quantity and quality of the kinetic data are not always sufficient for this purpose.

For other pesticides their mechanism of action is less clear and in these cases the most relevant effects for acute exposure can be identified only after examination of all toxicological effects induced by the substance taken into account their relevance for acute exposure. Sometimes, the effects considered most relevant for acute exposure are not critical in repeated dose studies, i.e. they may not occur at the lowest effect dose (see also section on NOAEL).

In Appendix A, a list of some relevant toxicological endpoints for acute exposure is given along with argumentation and some major points of attention. It should be emphasised that other toxicological effects not listed in this Appendix should not be automatically considered irrelevant and discounted. The relevance for effects after acute exposure which are not listed in Appendix A should be assessed on a case-by-case basis.

4.2.2 Problems in selecting endpoint or study

Because the database of substances currently under evaluation is not designed for allocating an ARfD, it may be difficult to select an appropriate endpoint or study for deriving the ARfD. Two situations may occur which need some attention.

1.

Based on all information available, the substance under evaluation is considered to be able to induce distinct acute toxicological effects (in line with the criteria formulated above). However, the most appropriate information or study for deriving an ARfD may not be available (e.g. ChE inhibition measurements are performed only in an 28-day study but not at the time of peak inhibition after a single exposure; structure analogy reveals that the substance is a carbamate or pyrethroid but no relevant neurotoxicity measurements are available). In such cases, the ARfD is derived from other (repeated dose) studies in which the selected relevant endpoint has been investigated. Such an ARfD does not necessarily represents a ‘conservative’ value since some effects (e.g. MetHb-formation, AChE inhibition) may actually adapt to some extent or the effect was not measured at the time of peak effect. This may sometimes lead to an underestimating of the actual risk after acute exposure. If necessary, an additional assessment factor for

inadequacy of the database may be used in deriving the ARfD (of which the choice should be transparently argued within the evaluation document).

2.
For some substances, the criteria formulated above for not setting an ARfD are not met but the available toxicological database does not provide clear indications to identify a specific relevant toxic endpoint(s) for acute exposure. This may partly be due to the absence of adequate acute oral toxicity studies. In the absence of relevant triggers in repeated dose studies for effects upon acute exposure, the ARfD is derived from general endpoints (most likely more relevant to repeated exposure) observed in the most appropriate study. By default, the study with the shortest exposure duration is used for this purpose unless other information indicates otherwise. In this case, the ARfD is a rather conservative value, most likely overestimating the actual risk upon acute exposure.

4.3 Allocating a NOAEL for the acute toxicological endpoints

For setting an ARfD, a No-Observed-Adverse-Effect-Level (NOAEL) is used. This NOAEL is derived specifically for the selected toxicological endpoint(s) considered to be relevant for setting the ARfD. Therefore, this 'acute-NOAEL' may be different from the actual NOAEL derived in a particular study. In the following fictive and simplified example of a 28-day oral study, the selected endpoint for acute exposure (methemoglobin formation) is increased only at the highest dose level (table 1). Other treatment related effects (considered to be not relevant for a single exposure) occur at the mid-dose. The NOAEL in this study is 1 mg/kg bw/day but the 'acute-NOAEL' for MetHb-formation that may be used for setting an ARfD, is 10 mg/kg bw/day.

Table 1. Fictive and simplified example of an oral 28-day study illustrating the selection of an 'acute NOAEL' (see text for explanation).

Effect	Control	1 mg/kg bw/day	10 mg/kg bw/day	80 mg/kg bw/day
Body weight (gain)			ds	ds
Food consumption				ds
Methaemoglobin (%)	0.4	0.3	0.6	3.5 *
Liver weight	0	+ 4%	+ 15%	+ 28%
Kidney weight	0	- 2 %	+ 13 %	+ 17 %
Histopathology Hypertrophic hepatocytes				is

ds = decreased significantly, is = increased significantly, * = statistically significant.

When selecting an 'acute-NOAEL' for a specific effect, all studies in which the selected effect is determined should be taken into account. Problems in setting acute exposure limits may arise when one focuses only on a single study. In fact, a study which appears to be the most appropriate study at first sight, may be less relevant when compared with other toxicological information (e.g. effect levels are quite low compared to any other effect level in the database due to species differences, inadequate analytical methodology, or dosing regimes and vehicles). Thus, when selecting an acute NOAEL for a selected endpoint, the complete toxicological package should be taken into account. In the following fictive and simplified example it is shown that the lowest

NOAEL for a selected acute endpoint is observed in a 14-day oral study (table 2). However, in studies of longer duration higher NOAELs and LOAELs for this effect were observed. In this case, the 14-day study was a gavage study in which a vehicle was used which markedly enhanced gastrointestinal uptake compared to dietary exposure. In this case one could conclude that the NOAEL of 2 mg/kg bw/day from the 28-day oral rat study is the most appropriate.

Table 2. Fictive and simplified example of NOAEL and LOAEL values for a selected relevant endpoint.

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
14-day oral gavage study rat	0.05	0.5
28-day oral diet study rat	2	10
90-day oral diet study dog	1	10

When an acute NOAEL cannot be established for the selected toxicological endpoint because only a LOAEL is available, various approaches can be used:
a) the use of additional assessment factors
b) dose-response modelling (e.g. Bench-mark approaches, PROAST-modelling)
c) requesting additional data.

ad a)
The use of additional assessment factors is discussed in the separate section on assessment factors.

ad b)
Most standard studies presently incorporated in pesticide dossiers are not designed for Benchmark-like approaches. However, dose-response modelling may be very helpful in establishing a dose without an adverse effect. Especially when the effect shows a clear dose-response relationship, dose-response modelling is a fruitful tool even though the procedure can be somewhat laborious. In this context it should be mentioned that the PROAST-procedure developed by RIVM is able to perform curve-fittings using only the mean and standard deviations as reported in most studies [9]. Dose-response modelling has a clear-cut advantage over the use of additional assessment factors: the procedure uses substance-specific data as much as possible providing a more scientifically based ARfD.

ad c)
Additional data may sometimes be necessary for appropriate setting of an ARfD. However, at present the national as well as the EU data requirements do not include specific needs for a specific study for setting an ARfD, although setting an ARfD has been incorporated in the guidelines for evaluation of pesticides in the EU [10]. Nevertheless, Dutch legislation provides a basis for requesting additional data when a pesticide or its residues is suspected to pose an acute health risk for consumers. Recently, the Dutch Board for the authorization of pesticides (CTB) has published its intention of incorporating the acute dietary risk assessment into their regulatory procedures [11] as has also been recommended at EU-level by the SCP [12]. In a special section at the end of the document it is discussed in which cases additional data should be requested.

4.4 Assessment factors and sensitive subgroups

4.4.1 Default assessment factors

An ARfD is derived from an oral NOAEL for specific acute effects by making use of assessment factors (see for a review on assessment factors ref. 13). Similar to the situation for setting chronic limit values (ADI, TDI or RfD), the inter- and intraspecies variation has to be taken into account. For each factor, an internationally accepted default value of 10 is mostly used. Therefore, the total default assessment factor for setting an ARfD is 100.
When the intraspecies and interspecies factors are divided into a factor for toxicokinetic and toxicodynamic differences, substance specific information on kinetics may be used to substitute the default values (see for a discussion on this subject ref. 14).
When human data are used for setting the ARfD, only the intraspecies factor of 10 should be used (total default assessment factor is 10).

4.4.2 Sensitive groups

In general terms, the default assessment factor of 10 for intraspecies variation is in principle considered to be sufficient to account for the total variability in sensitivity within the human population, i.e. to extrapolate from the mean human to the sensitive human. This includes all 'YOPIG'-groups (Young, Old, Pregnant, Ill, and the Genetically susceptible people). Hence, no additional assessment factors will be applied for e.g. children. Only when substantial information is available indicating that a factor of 10 for intraspecies variation would not be sufficient, a factor of more than 10 may be used. In this case, it should be clearly stated within the report on which grounds this assessment factor is allocated.

4.4.3 Direct effects

For some effects an assessment factor smaller than the default of 100 may be appropriate. For example, irritation of the GI-tract or the induction of vomiting (sometimes observed in dogs (rodents do not vomit)) are much more related to the quantity of the chemical locally present, i.e. the concentration at the site of action, than to the dose expressed on a body weight basis. In addition, some other (pharmacological) effects (e.g. direct interaction with ion channels) are related to C_{max} rather than the AUC of a substance [14]. For such cases, it has been argued to reduce the total assessment factor by a factor of 2 (e.g. the default assessment factor of 100 is reduced to 50) [14].

4.4.4 Correction for Exposure Duration

In the setting of the ADI or AOEL, frequently NOAELs from studies with a short exposure duration have to be extrapolated to a longer exposure period for humans. For example, when a NOAEL from a 90-day oral rat study is used for setting the ADI, an additional assessment factor is used for extrapolation of semi-chronic to chronic exposure.
In setting an ARfD, such assessment factors are usually not necessary since the concept involves only a single exposure and the studies available in a standard dossier involve either a single treatment or exposure periods of longer duration. This would rather suggest to include a correction for repeated exposure by using an assessment factor of less than 1 when the ARfD is derived from a subacute or semichronic study. This will

reduce the overall assessment factor [4]. However, this is generally not applied since no data will be available on which the choice of such a factor may be based on. Hence, an ARfD derived from a study with repeated dosing may in a number of cases (depending on the type of effect induced) result in a rather conservative value for the ARfD overestimating the actual risk during acute exposure.

4.4.5 Extrapolation of a LOAEL to a NOAEL

As indicated above, sometimes a NOAEL cannot be established for the selected endpoint and only a LOAEL is available. To extrapolate a LOAEL to a NOAEL an additional assessment factor may be used ranging from 2 to 10 (default is 10). This assessment factor is chosen taken into account the following aspects: the dose-response relation for the specified effect (steepness of dose-response curve and extent of effect at LOAEL), the experimental set-up of dosages (i.e. the increment between the dosages used in the study), and the overall quality of the study (group size, sensitivity of measurements), and the type of dosing (gavage versus diet).

4.4.6 Additional modifying assessment factors

Some regulatory bodies apply additional assessment factors for the nature and/or severity of effects (in case of carcinogenicity or teratogenicity) and the inadequacy of the database. These factors are termed modifying factors in U.S. evaluations. A review on the use of such assessment factors by Renwick [15] revealed that a) these factors are frequently inadequately applied, b) factors for nature and severity of the effect and inadequacy of the database are often intermingled, and c) the use of these factors shows a large amount of inconsistency between evaluations of a particular organisation as well as between different organisations. In addition, it cannot be excluded that a substantial amount of emotional, subjective or societal arguments are involved in the application of these types of factors [6,15]. A further discussion on this aspect is considered beyond the scope of this document.
As a starting point, no additional or modifying factors should be used for setting an ARfD. A modifying factor may be used only if the data available are inadequate while reasonable suspicion exists to indicate that the substance under evaluation might pose an acute health risk. In that case, the additional modifying assessment factor may be a tool for triggering additional information.

4.5 How to use data/studies in current submissions

The set of information currently required for regulatory purposes is not directed towards the derivation of an ARfD. However, it is currently necessary to derive ARfD's from the information provided in standard submissions. What to do with the various types of studies in current dossiers in the scope of setting an ARfD has been the subject of several documents and publications [1,2,3,6,16]. In Appendix B, the oral studies currently available in standard submissions are discussed in terms of their use for setting an ARfD.

5 Human data

5.1 Human volunteer studies

At present there is no requirement for the performance of such studies for pesticide regulatory purposes. In fact, the performance of human volunteer studies is not encouraged within EU-regulatory procedures and is strictly bound to various regulations primarily with respect to ethical aspects [6].
Nevertheless, for groups of pesticides with a known mechanism of action (e.g. OP-esters and carbamates) such studies are frequently available. In the case of AChE inhibiting compounds, one can easily measure the critical endpoint (AChE inhibition in blood) at various time points in human volunteers. Often a relevant exposure period of 1 to 14 days is used. Because the use of human volunteer studies remove the uncertainty of interspecies extrapolation, such data are extremely useful for setting an ARfD. However, in these studies often only one dose level is used and sometimes the study is performed with a low number of subjects. Moreover, some volunteer studies (sometimes rather dated) may be of questionable significance. These aspects should be taken into account when using these data for setting an ARfD.

5.2 Human poisoning / accidents

Case reports of human poisoning or accidental exposure can normally not be used for setting an ARfD since the extent of exposure is unknown or unreliable.

6 Requesting additional data

The most ideal situation will be that submitted dossiers for pesticides will include data specifically designated for establishing an ARfD. However, as stated before, current data requirements for pesticides do not (yet) include the need for submission of these specific data. Therefore, the ARfD is allocated on the basis of the information available in the toxicological dossier. In some cases, the available data are insufficient for an estimation of the ARfD and one might decide not to allocate an ARfD unless new data becomes available.
In several other cases (see for example the numbered paragraphs conditions 1 and 2 in the section on 'problems in selecting relevant endpoints'), the ARfD is derived from repeated dose studies or even from general endpoints which are more relevant for repeated exposure than for acute exposure. In each of these cases, the reliability of or the confidence in the allocated ARfD should be evaluated (despite the possible use of additional assessment factors). New data may be needed for a better estimation of the ARfD. Upon deciding of requesting additional toxicity data, aspects of exposure estimates (through dietary intake calculations) and risk assessment (comparison of intake with the allocated ARfD) should be included also.
This proces may be illustrated by the following two examples.

Example 1) An ARfD for a certain pesticide is derived from a repeated dose study. The availability of a new acute oral toxicity study in which the relevant endpoint(s) have been determined is expected to possibly lower the ARfD by a factor of 2 – 3. However, when dietary intake calculations reveal that exposure is only about 1% of the derived ARfD, it may be considered unnecessary to request additional toxicity data.

Example 2) An ARfD for a certain pesticide is derived from general endpoints from a repeated dose study. This ARfD is conisdered to be rather conservative and new data are expected to increase the ARfD by a factor of 3 – 5. When dietary intake calculations reveal that the exposure exceeds the derived ARfD by a factor of 2, requesting additional toxicity data may be appropriate for setting of more reliable and relevant ARfD. Alternatively, a refinement of the intake calculations may be performed also.

To summarise, when requesting additional acute oral toxicity data one should 1) consider the reliability of and the confidence in the derived ARfD and the extent by which additional data may change this value and, 2) take into account the potential health risks using dietary intake calculations and the derived ARfD.

When additional data are requested, the submitted data should be of sufficient type and quality to fill the gaps of knowledge for the substance under evaluation. At present, several initiatives have been launched to prepare guidelines for toxicity testing relevant for the establishment of ARfD's (e.g. proposals from ECPA and JMPR) [17,18]. The JMPR guideline [18] is presently submitted to OECD for consideration. Additional studies should be performed preferably according to the JMPR test guideline [18].

7 Points of specific consideration

Within the process of developing the concept of the ARfD, the allocation of ARfD values for various substances, and the use of ARfD's, some specific points of consideration have been identified which will be discussed briefly below.

7.1 Subpopulations

In dietary risk assessment, a separate risk assessment is performed for children of age 1-6 because this group has a different consumption pattern (especially for fruits) compared to adults and has a higher dietary intake based on body weight. This is performed for both acute and chronic exposure. For the risk assessment of acute dietary intake, the ARfD is used.
A specific point of consideration arises when the ARfD used in the risk assessment is derived from developmental effects (effects on the foetus observed specifically in a developmental study). Such an ARfD is highly relevant for pregnant women but possibly irrelevant for other individuals within the population and has little relevance for the acute risk assessment of children. It presnetly unclear what to conclude when the calculated dietary intakes of children are above such an ARfD.
The situation as depicted above have led to the idea that separate ARfD's should be established for separate subgroups in the population [1,6,7]. However, in most cases there is no information on the differences in sensitivity between subpopulations (e.g. children vs adults) for a specific effect and hence there is no information to identify specific ARfDs for these subpopulations separately.
Furthermore, we propose that the ARfD is considered to be a toxicological limit value and is therefore intended to protect the most sensitive individuals within the population analogous to the ADI [1]. As a starting point in this guidance document, therefore, only a single ARfD will be allocated per substance and hence no separate ARfD's will be established for different subpopulations.

7.2 Gavage versus Diet

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.
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 (especially when the substance has a very short half life). In these cases, gavage dosing can possibly not be considered as a 'worst case' condition (see also JMPR guidelines for acute toxicity testing [18]). The possible difference in the ARfD value based on differences in gavage vs. dietary exposure are considered to be relatively small compared to other uncertainties such as the accuracy of the NOAEL (dependent on study protocol) and the use of assessment factors. Therefore, gavage dosing is

[1] The concept of the ADI is aimed at protecting all individuals over a lifetime exposure. This means that the ADI is principally based on the most sensitive effect extrapolated to the most sensitive human being.

considered a relevant treatment for setting an ARfD, also for organophosphate pesticides unless the available information indicates otherwise (e.g. kinetics or the use of irrelevant/peculiar vehicles, see also example in table 2).

7.3 Substances with a substantial half-life

For some pesticides, the kinetic information may show that the elimination half-life of the substance is substantial (e.g. >> 24h). For these subtances, there is a risk for accumulation of the substance in the body. When the oral exposure to such a particular pesticide is above the ADI, the risk of accumulation or an increased systemic exposure for more than one day should be evaluated.
If an ARfD is allocated for a substance with a substantial half-life, the risk of accumulation of the substance should be also taken into account when deriving a value for the ARfD. This can only be done on a case-by-case basis.

7.4 Short-term RfD.

In some documents (e.g. the UK-PSD policy document on setting an ARfD [3]), it is proposed that when no adequate data are available for setting an adequate ARfD, one should establish a ‘short-term’ RfD based on toxicity endpoints from subacute or semichronic studies. Such a terminology has been used also in the EU-concept guidance document on the ARfD [2]. This ‘short-term’ RfD refers essentially to condition 2 in the section on ‘Problems in selecting endpoint or study’ in paragraph 4.2.
In our opinion the terminology and establishment of a ‘short-term’ RfD should not be performed because it suggests to be a basically different type of limit value compared to the ARfD. This will result in confusion about the concept, setting, and use of the ARfD [4].

8 Future outlooks and recommendations

The concept of the ARfD has been and still is subject to extensive discussions and developments. Because of these developments there is (at least for certain aspects) at present no definitive consensus on the ARfD. Therefore, it is anticipated that changes in the concept, the setting and the use of the ARfD may also occur in the future.
Specific points of attention in the concept and the process of setting the ARfD are:

1) In order to reduce the differences and confusions about the setting and use of the ARfD, the concept of the ARfD should be further defined as has been intended in this guidance document.
2) The subject of subpopulations is presently subject to discussion in (inter)national groups and new insights from these discussion should be evaluated in the future. These discussions will need to address the issues of allocating separate ARfD's for several sub-populations; whether there is an actual need for separate risk assessments for sub-populations from a regulatory perspective; and whether setting of an ARfD for the most sensitive effect in the most sensitive human beings (sub-population) will be sufficient to cover the health risk of the possible acute exposure to (residues of) pesticides.
3) When more data becomes available, the use of assessment factors for specific effects or conditions might deviate from the current default values of 10 for intra- and 10 for interspecies variation.
4) Clear opinions and/or guidance should be prepared in which it is identified which type of effects observed especially in developmental studies are relevant for acute exposure. RIVM/CSR will address this specific issue in a new project in 2001-2003.
5) With respect to the acute dietary intake calculations and/or exposure estimates, which are equally important to acute health risk assessment, some points of attention should be taken into account which have direct consequences also for the toxicological derivation of the ARfD. Firstly, it has to be established whether the assumption that exposure to a second peak above the ADI within a limited period of time is negligible is valid (see exposure scenario 1 in this document). Secondly, it has to be investigated whether exposure scenario 3 actually occurs in practice and with what kind of frequency and, if so, what regulatory measures have to be taken to avoid such exposure.

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APPENDIX A: Relevant effects for setting the ARfD

A.1 Clinical signs

Clinical signs appearing after a single oral dose or directly after the start of dosing in repeated dose experiments provide evidence for adverse effects of the test substance due to acute exposure. However, not all types of clinical signs are relevant since often several non-specific clinical signs appear just as a consequence of the dosing procedure, especially in gavage or intubation studies (e.g. . general lethargy, subdued behaviour, inactivity, vomiting). Therefore, relevant clinical signs are those that can be identified as treatment related or likely to be so. Elements that should be taken into account for identifying relevant clinical signs are: consistency of the clinical signs over various studies, consistency of the clinical signs in various species, dose-related increases in incidence and/or severity of clinical signs. Examples of relevant clinical signs are tremor and salivation for organophosphates or carbamates due to inhibition of acetyl cholinesterase (AChE).

A.2 Inhibition of Acetylcholinesterase

Organophosphates and some carbamates are known to inhibit AChE. This inhibition is often a very direct effect although some substances need to be metabolised to induce AChE-inhibition. In that case one of the metabolites is responsible for AChE inhibition. When using AChE-inhibition as the most relevant end-point for the ARfD, careful evaluation of the dose-time-effect relationship should be taken into account because e.g. carbamates inhibit AChE only transiently and inhibition of AChE is known to adapt to some extent (hence AChE inhibition should be measured at the peak time point). These examples indicate that the study and hence the experimental protocol used should be carefully considered when the ARfD is based on AChE-inhibition. See for more information ref. [19].

A.3 Delayed Neuropathy

Some organophosphate pesticides are capable of inducing a specific type of delayed neuropathy often referred to as OrganoPhosphate Induced Delayed Neuropathy (OPIDNP) [20]. This neuropathy, which will only become apparent after several days, can be induced after a single exposure to organophosphate pesticides. This toxicological end-point can be used for setting an ARfD although in most cases other toxicological effects (such as AChE inhibition) will appear to be more critical. See for more information ref. [20].

A.4 Neurotoxicity

Various pesticides have specific neurotoxic properties, which may become apparent as specific behavioural changes (see also clinical signs) or specific neuropathological lesions. When such neurotoxic properties have been adequately investigated in acute neurotoxicity studies (including an FOB, histopathological analysis of the brain, and/or biochemical alterations (e.g. receptor densities or neurotransmitter concentrations)), these types of effects may be highly relevant for setting an ARfD (either behavioural-, biochemical-, or neuropathological end-points).

A.5 Methemoglobin formation

A number of agents are able to induce the formation of methemoglobin. This effect can be induced after a single or several doses and is therefore a relevant end-point for

setting an ARfD. This relatively acute effect tends to adapt in repeated dose experiments, at least in the rat [21]. Therefore, the time of measurement is an important factor when methemoglobin induction is used for setting an ARfD.

A.6 Disruption of oxygen transport or mitochondrial uncoupling

Some substances are able to disrupt the transport of oxygen by the haemoglobin / erythrocytes (e.g. due to acute haemolysis). Some others are capable of inhibiting oxidative phosphorylation in the mitochondria ('cyanide' containing substances) thereby reducing the efficiency of ATP-generation (uncoupling) resulting in e.g. heat production. When these types of end-points have been measured appropriately, they may be relevant for setting an ARfD.

A.7 Teratogenicity

When a substance is known to induce franc teratogenic effects (structural irreversible effects) when administered during organogenesis it must be assumed that such effects may be induced also by a single exposure. In fact, for true teratogenic effects it is generally accepted (and has sometimes been shown) that these effects are induced within a limited time-frame (window) of sensitivity (the critical moment of development of the organ or structure). Therefore, the dose level in developmental toxicity studies at which no teratogenic effects are induced should be considered as a relevant no-effect-level for acute exposure. Teratogenic effects are therefore relevant for setting an ARfD.

A.8 Developmental effects

Developmental effects can be observed in developmental toxicity studies (teratogenicity studies) and multi-generation reproduction studies.
In developmental studies the period of exposure is relatively short (10 –15 days depending on species). Any effects specifically observed on the embryo- or the foetus in these studies are, by default, considered relevant for setting an ARfD. The induction of embryonic or foetal deaths (resorptions) is especially relevant. However, all effects should be carefully evaluated for their relevance for acute exposure (taken maternal toxicity into account).
The only studies in which the period from birth to adulthood is covered, including the period of lactation, are multi-generation reproduction studies. Although many effects in these types of studies are mainly caused by repeated exposure, specific attention should be paid to effects observed exclusively in the period directly after birth or during lactation. Examples are effects on birth-, live birth-, viability-, or lactation index, and effects on body weight gain specifically during the period directly after birth or during lactation. Expert judgement is needed to determine whether these effects are caused by acute rather than repeated exposure.

A.9 Developmental Neurotoxicity

For reasons already formulated at "Neurotoxicity", "Teratogenicity", and "Developmental Effects", specific developmental neurotoxic effects may be relevant for setting an ARfD.

A.10 Direct effects on the GI-tract / Stomach

Some substances may cause direct effects on the stomach or the GI-tract. Irritation of the stomach and GI-tract after one or a few dosages or clinically related signs such as

vomiting (sometimes observed for dogs) may be considered relevant for setting an ARfD. Most of these studies are performed using gavage dosing. See for a discussion on gavage studies the separate section in the main document.

A.11 Pharmacological Effects

Some substances are known to affect specific physiological target sites, e.g. ion channels. The effects from such a specific interaction may vary from changes in nerve conductivity, blood pressure changes to even paralysis. Since such effects are mostly caused by a direct interaction of the substance with the target site, they may occur also directly after a single exposure. When data on such interactions are available (e.g. from mechanistic studies) and the effects are considered to be adverse, these data may provide highly relevant information for setting an ARfD. Nevertheless, it should be taken into account that mechanistic studies are often performed to demonstrate the irrelevance of effects for humans. Therefore, the toxicological relevance of effects observed in mechanistic studies for human risk assessment has to be taken into account.

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APPENDIX B: The use of oral studies in current data submissions

B.1 Acute toxicity studies

In general, studies on acute oral exposure include only the classical oral LD_{50}-studies (OECD 401). These studies are intended to give an estimate of the LD_{50}-value and a gross indication of toxicity and are primarily used for classification purposes. The observations and reporting of effects other than lethality is often not sufficient for the use of setting an ARfD. However, the incidence and severity of treatment related clinical signs may be a basis for deriving an ARfD when an adequate dose-response relation is present and a NOAEL for these effects can be identified. Often only a limit-test is performed using only one dosage of 2000 or 5000 mg/kg bw. Unfortunately, this approach does not provide any dose-response information.
Since the classical OECD 401 study is subject to criticism because of animal welfare aspects, new study protocols have been introduced (OECD guidelines 420, 423 and 425). However, these studies use a low number of animals, a single sex, and are focussed primarily on the estimation of an LD_{50} range. As a consequence, such new studies may provide even less relevant information for setting an ARfD.
In conclusion, the acute oral toxicity studies are the studies with appropriate exposure durations but generally lack any indications for toxic endpoints other than lethality. Only when dose- and treatment related clinical signs are observed such studies may be used for setting an ARfD albeit mostly for identifying the upper range of the ARfD.

B.2 Subacute and semichronic studies

Subacute oral studies up to exposure durations of 28 days are not always present in current dossiers. In fact, the Dutch data requirements for pesticides do not include the submission of subacute oral studies although it is requested to submit such studies when available. These repeated dose studies include mostly 3 dosages or more and a range of observations on haematology, clinical biochemistry, and (histo)pathological analyses. Semichronic studies include the 90-day oral studies for rodents and the 90-day or 1-year oral studies for dogs. Extensive observations and the use of 3 or more dosages mean that such studies are likely to cover the key endpoints. However, the effects may be induced by a combination of single and repeated exposures while effects of the first one or two doses may be corrected by feedback mechanisms at the observation time at the end of the study. In this respect, interim examinations in these studies may provide valuable additional information on the time-dependent development of toxic effects and hence their relevance for setting an ARfD. Clinical signs (e.g. vomiting, diarrhoea) and effects on food intake and body weight are monitored daily or regularly in the initial phase of the study. Effects on these parameters observed after one or a few days may be of value for setting an ARfD.
Range-finding or sighting studies with short exposure durations (a few or 14 days) may be highly relevant for identifying the main target sites for acute and short-term exposure and thus provide valuable information for setting an ARfD.
The use of these repeated dose studies may often result in relative conservative ARfD's.

B.3 Chronic toxicity and carcinogenicity studies

In most cases these studies cover all relevant toxicity endpoints but the exposure duration is inappropriate for setting an ARfD. The first observations in haematology or clinical biochemistry are made after 3 months which is not relevant for setting an ARfD.

However, similar to semichronic studies, effects on clinical signs, food intake and body weight observed during the initial phase of the study may provide relevant information for setting an ARfD.
Chronic and/or semichronic studies in dogs may be used for setting an ARfD if these types of studies show that the dog is the most sensitive species for a certain type of effect relevant for acute exposure since studies of shorter duration are normally not available for dogs.
In general, chronic studies should not be used for setting an ARfD (with a possible exception for reversible ChE inhibitors such as carbamates).

B.4 Reproduction studies

These studies are the only studies available in which new-born and young animals are investigated. Furthermore, reproduction is investigated. However, exposure involves several weeks before mating and continues throughout maturation of the progeny. Specific attention should be paid to specific disturbances in mating performance and/or the F_1 -generation directly after birth and during lactation. Although for effects on mating performance one cannot discriminate between acute and repeated exposure, any effects on these parameters should be carefully evaluated. If the cause of reduced mating performance can be identified from other studies (e.g. observed atrophy of the testes in repeated dosing studies) one can evaluate whether the effects on mating performance is relevant for acute exposure.
The period from birth to weaning should be carefully evaluated since any effects on the young specifically in this period may be relevant for setting an ARfD. However, one should evaluate this data thoughtfully since many effects may be caused by repeated exposure or due to indirect effects on the parent animals.

B.5 Developmental toxicity studies

Developmental or teratogenicity studies mostly use gavage dosing for 10 to 15 days during gestation, depending on the species used. Therefore these studies use an appropriate dosing duration although the last days before sacrifice dosing is generally stopped allowing some recovery of effects to occur. Special attention in these studies is paid to effects on the embryo or the foetus, taking into account the presence of maternal toxicity. Although the dosing period is rather short, it is often impossible to discriminate between effects of a single day or repeated exposure. Especially, true malformations (teratogenic effects) and embryonic or foetal deaths (resorptions) are thought to be induced by a single exposure within a certain (sensitive) time window. For other effects such as foetal body weights and skeletal retardation (variations) their relevance for acute exposure is less clear.
As a starting point, any effects observed on the foetus should be considered relevant for setting an ARfD unless information is available to indicate otherwise.

B.6 Genotoxicity studies

In-vivo genotoxicity studies use mostly single exposures although dosing involves gavage or even parenteral injection. Nevertheless, these studies include endpoints not investigated in other types of studies. Therefore, these studies should always be checked for possible relevant non-genotoxic effects for setting an ARfD.

B.7 Additional studies

Neurotoxicity studies

Two types of neurotoxicity studies according to international accepted guidelines exist: a single exposure study and a repeated exposure study. These studies are specifically designed for monitoring the effects on specific endpoints (e.g. AChE-inhibition), neurobehavioral effects, and neuropathology (e.g. nerve degeneration). Many insecticides are neurotoxic substances (OP-esters, carbamates, or pyrethroids) and the (acute) neurotoxicity studies are highly relevant for setting an ARfD since the key endpoints are investigated specifically in these studies.

Mechanistic studies

These types of studies may include both in-vitro or in-vivo studies. Both types of studies are useful in identifying the key endpoints for toxicity (both acute and repeated). Although in-vivo studies may use repeated dosing, the total dosing period is often limited (subacute) and various measurements are made during the exposure period allowing the understanding of the effects induced and selecting the most relevant effects for acute exposure.

Because such studies are sometimes performed to demonstrate the irrelevance of specific effects for humans or the inapplicability of a certain animal model, these studies should be carefully evaluated. Nevertheless, such studies may provide valuable information for setting an ARfD.

Although an ARfD cannot be based on in-vitro data, the latter may be useful in identifying the mechanism of action and hence the choice of relevant endpoints for acute exposure.

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50. SBD/Voorlichting & Public Relations
51. Bureau Rapportenregistratie
52. Bibliotheek RIVM
53-67. Bureau Rapportenbeheer
68-85. Reserve exemplaren