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4 **DRAFT GUIDANCE DOCUMENT FOR THE DERIVATION OF AN**
5 **ACUTE REFERENCE DOSE**

6
7 The objective of the document is to provide stepwise approach for a harmonized guidance on
8 how to use optimally all available toxicological data, how to refine the exposure calculation
9 for the acute risk assessment and what to do if more data is needed for derivation of Acute
10 Reference Dose.

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

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

19
20 **A. Background**

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

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

33 Various guidance documents are available for setting Acute Reference Values (1, 2, 3, 4, 5,
34 and 7). The WHO panel of the Joint Meeting on Pesticide Residues (JMPR) adopted general

35 considerations in setting of ARfDs for pesticide chemicals (1, 2). Solecki et al. (4) described
36 in detail a step-wise process for establishing ARfDs, as well as specific considerations and
37 guidance regarding the identification of the most appropriate critical effects for selected
38 toxicological endpoints.

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

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

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

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

¹ http://www.who.int/ipcs/methods/harmonization/draft_document_for_comment.pdf

² <http://www.epa.gov/NCEA/bmds/index.html>; <http://epa.gov/osa/raf/publications/benchmark-dose-doc-draft.htm>

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

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

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

98 The results of the single exposure study should (i) clarify whether a substance poses an
99 unacceptable acute risk and (ii) allow the derivation of a refined ARfD for acute intake of
100 residues in food and drinking water.

101 At the 13th Meeting of the OECD Working Group on Pesticides in 2002, the JMPR presented
102 a proposal for an OECD Test Guideline animal study, designed to establish an ARfD for
103 dietary risk assessment for human health (8). The EC, Spain and Crop Life International
104 supported this proposal, suggested improvements and recommended that JMPR should
105 proceed by approaching the Working Group of National Co-ordinators to the Test Guidelines
106 Programme (WNT).

107 The 19th WNT meeting then agreed that an improved stepwise approach for a harmonised
108 guidance should be developed for the derivation of an ARfD. The WNT agreed to include the
109 project in the work plan for 2007 (9).

110

111 **B. Purpose**

112 The objective of this document is to provide a stepwise approach for a harmonized guidance
113 on how to set the ARfD based on all appropriate existing toxicological and exposure data. The
114 general considerations in setting of an ARfD in a an enhanced step-wise process, as well as
115 specific considerations and guidance regarding the identification of the most appropriate
116 critical effects for selected toxicological endpoints are described in detail. The general
117 biological background and the data available through standard toxicological testing for
118 regulatory purposes, interpretation of the data, conclusions and recommendations for future
119 improvements are described for these selected relevant endpoints. Special emphasis is placed
120 on evaluating whether toxic effects observed in the standard package of repeated dose toxicity
121 studies may also occur after single doses.

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

130 If the acute intake estimation exceeds such a potentially conservatively established ARfD in a
131 first step, reassessment of the risk assessment may be addressed as a second step in a
132 refinement of the exposure assessment and as a last resort in a third step a single exposure
133 study may be needed for the generation of toxicological data to establish and refine Acute
134 Reference Dose values.

135 This guidance document is intended to promote a harmonised scientific basis for the
136 derivation of ARfDs suitable for refined acute risk assessment in a range of acute human oral
137 exposure scenarios.

138 This proposed guidance document will:

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

146 This document presents specific guidance

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

152

153 **C. Basic Considerations**

154 The appropriateness of all available endpoints from subchronic and chronic studies to
155 establish ARfDs needs to be carefully considered in a *first step*. The pertinent biology of the
156 system affected should be considered to determine whether an acute exposure may
157 compromise the ability of the organ to compensate and maintain homeostasis. Particular
158 weight should be given to observations and investigations at the beginning of repeat dose
159 studies. Isolated findings, showing no specificity or clear pattern are not necessarily
160 indications of toxicity. In the absence of information to the contrary, all toxic effects seen in
161 repeat-dose studies should be evaluated for their relevance in establishing an ARfD.

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

164 After reviewing the available toxicological database, the possible exposure scenarios should
165 be considered in a *second step*, based on the guidance in Annex 2. A tiered human health risk
166 assessment should be conducted that includes a comparison of the Acute Reference Value
167 with the potential acute oral exposure (or internal body burden), based on a worst- case
168 assumption. If this worst-case assessment does not indicate unacceptable health risks, no

169 further refinement of the acute risk assessment may be warranted. But, if this risk assessment
170 indicates a borderline or a clear concern, then the next tier should focus on a further
171 refinement of the exposure assessment (from refined acute intake estimation). If the health
172 risks are now acceptable, no further refinement is warranted.

173 If the refined exposure assessment still shows unacceptable health risks and if conservative
174 assumptions were used in setting the ARfD, then in a *third step* it should be considered, if a
175 single exposure study may be warranted to establish a refined Acute Reference Dose and how
176 to perform and tailor this single exposure test, based on the guidance in Appendix to Annex 2.
177 This may only be necessary for a very limited number of substances.

178

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

180

181 **STEP ONE**

182

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

185

186 2. *Consider the principles for not setting an ARfD*

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

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

196

197 3. *Selection of appropriate endpoints for setting an ARfD*

- 198 - Select the toxicological endpoints most relevant for a single (day) exposure in the most
199 relevant species.
- 200 - Select the most relevant or adequate study in which these endpoints have been
201 adequately determined.
- 202 - Identify the NOAELs for these endpoints.
- 203 - Select the most relevant endpoint providing the lowest NOAEL.

204 An endpoint from a repeat-dose toxicity study should be used if the critical effect of the
205 compound has not been adequately evaluated in a single-dose study. This is likely to be a
206 more conservative approach and should be stated.

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

209

210 4. Selection of appropriate safety factors for setting an ARfD

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

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

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

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

222 - If a specific factor cannot be derived, consider if there is any information to
223 indicate reduced or increased uncertainty. A combined SF may be based (16) on
224 (AKAF or AKUF) × (ADAF or ADUF) × (HKAF or HKUF) × (HDAF or HDUF)

225 ○ where AK represents inter-species toxicokinetic variability

226 ○ AD represents inter-species toxicodynamic variability

227 ○ HK represents human interindividual toxicokinetic variability

228 ○ HD represents human interindividual toxicodynamic variability

229 ○ AF represents a chemical-specific adjustment factor

230 ○ UF represents a default uncertainty sub-factor

231

232 - If not, the 100-fold (or 10-fold) default should be used. When using data obtained
233 from experimental animals, the default safety factor is 100. This comprises a factor
234 of 10 to allow for inter-species differences and a factor of 10 for intra-species
235 (human inter-individual) differences. The overall safety factor is the product of
236 these two factors, i.e. 10 × 10.

237 Whenever a safety factor other than a default is used, a clear explanation of the derivation of
238 the factor must be provided.

239
240 **STEP TWO (Annex 1)**

241
242 *5. Application of the ARfD for the acute risk assessment*

- 243 - Determine whether the acute exposure estimate is exceeding the ARfD.
244 - If the acute intake estimation does not exceed the ARfD, no further refinement is
245 necessary.

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

248

249 *6. Refinement of the exposure calculation for the acute risk assessment*

- 250 - In determining a refined exposure calculation, a stepwise approach is proposed.

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

253

254 **STEP THREE (Annex 2)**

255

256 *7. Experimental refinement of the ARfD derivation*

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

260

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

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

- 267 • *Haematotoxicity*: The induction of methaemoglobinaemia is considered to be a critical
268 effect in consideration of acute responses to chemical exposure. For acute exposure to
269 methaemoglobin-inducing xenobiotics, a level of 4% methaemoglobin (or higher) above
270 background in dogs or a statistically-significant increase in rodents cf. background is
271 considered to be relevant to set an ARfD. Haemolytic anaemias induced by mechanical
272 damage, immune mediated anaemia, oxidative injury to RBCs and non-oxidative damage

273 are considered to be less relevant for ARfD derivation since the severity of such effects
274 appear to generally depend on prolonged exposure. If changes in haematological
275 parameters are observed early in a repeated-dose study and do not appear to progress
276 during the course of the study, then such effects can be considered as relating to acute
277 exposure to the substance. In assessing whether effects observed in repeated-dose studies
278 should be used for setting an ARfD, one has to evaluate the mechanism of action. If
279 known, this could provide arguments for selecting or not selecting the endpoint for setting
280 an ARfD.

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

286 • *Neurotoxicity*: The nervous system has limited capacity for repair and regeneration.
287 Therefore, any neurotoxicity seen in repeat-dose studies could be the result of a single
288 exposure that is not repairable i.e. any evidence of neurotoxicity should be considered
289 relevant to an ARfD assessment unless it can be demonstrated that the effects are produced
290 only after repeated exposures. In addition to long-term or irreversible effects associated
291 with acute exposure, attention should be paid to transient effects, as these could be
292 considered as adverse under some circumstances.

293 Delayed neurotoxicity following single chemical exposures can occur and thus any acute
294 exposure study should have an adequate period of investigation.
295 In functional observation batteries (FOB) a large amount of data is produced; interpretation
296 of such studies should include a consideration not only of the statistical significance of
297 results but the nature, severity, persistence, dose-relationship and pattern of the effects.
298 Isolated findings showing no specificity or clear pattern do not necessarily indicate
299 neurotoxicity.

300 The most common neurotoxic end-point used to date in the derivation of ARfDs for
301 pesticides is inhibition of acetylcholinesterase. The JMPR has previously defined criteria
302 for the assessment of cholinesterase inhibition; these apply equally to the setting of ADIs
303 and ARfDs. For inhibition of acetylcholinesterase a specific cut off (20%) is used routinely
304 to differentiate between adverse and non-adverse effects.

305 • *Kidney and liver effects*: If effects on these organs cannot be discounted as being either
306 adaptive or as the result of prolonged exposure, an ARfD can be derived on the basis of

307 these effects. Such an ARfD is likely to be conservative and it may be possible to
308 subsequently refine it using an appropriately designed single-dose study. When
309 interpreting data on liver and kidney toxicity in repeat-dose studies, one has to consider
310 two important aspects, firstly, the type of effect observed and secondly, any information on
311 correlations between exposure duration and effect.

312 For liver toxicity it is considered that findings of increased serum cholesterol, cirrhosis,
313 induced activity of metabolising enzymes, regenerative hyperplasia, hepatocyte
314 hypertrophy, fibrosis, or sclerosis in repeat-dose studies are, in isolation, either adaptive or
315 the result of prolonged exposure and therefore are not applicable for deriving an ARfD.
316 For kidney toxicity it is considered that the following findings of kidney toxicity in repeat
317 dose studies are, in isolation, the result of prolonged exposure and are not applicable for
318 deriving an ARfD: increased organ weight; regenerative hyperplasia; altered serum calcium
319 and phosphate.

320 All other findings of liver and kidney toxicity should be considered as potentially relevant
321 to the derivation of an ARfD.

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

325 • *Developmental effects:* Any treatment-related adverse effect on fetuses or offspring which
326 has resulted from exposure during any phase of development should be considered as
327 potentially appropriate to use in acute dietary risk assessment, despite the fact that the
328 treatment period typically consists of repeated dosing. ARfDs based on reductions in fetal
329 bodyweight gain may be conservative and should be evaluated in the context of all
330 pertinent data, including other developmental effects. Consideration should be given to the
331 degree of maternal toxicity when considering whether fetal effects may be occurring as a
332 direct effect of the chemical; severe maternal toxicity means a direct effect is less likely.

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

336 When gastro-intestinal effects occur, they are most commonly observed only after a bolus
337 administration of a compound (by gavage or capsule) in fasted animals and administration
338 of similar doses in food does not cause the same effects. In this case, the gastro-intestinal
339 effects are most likely due to a local irritant effect of large amounts of the compound in the
340 gastrointestinal tract. Since the ARfD applies to ingestion of a compound in food or

341 drinking water, local gastro-intestinal effects exerted by bolus administration are not
342 considered to be relevant for setting an ARfD.

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

354 Thirdly, chemicals may exert an effect on the gastro-intestinal tract through a systemic
355 action. For instance, it is known that the dopamine agonist apomorphine causes vomiting in
356 humans and dogs (not in rodents), through a direct stimulation of the chemoceptor trigger
357 zone for emesis in the area postrema of the medulla oblongata of the CNS. Such an indirect
358 effect on the GI tract is considered to be relevant for setting an ARfD. For such indirect
359 gastro-intestinal effects, inter- and intraspecies differences in the toxicokinetics as well as
360 the toxicodynamics of the substance should be taken into account. If it has been determined
361 that the indirect effect on the gastro-intestinal tract is the result of a pharmacological
362 (receptor-mediated) action of a compound, a reduction of the default 10 x 10 assessment
363 factors may be appropriate (16).

364 The reasons for establishing (or not establishing) an ARfD on the basis of gastro-intestinal
365 effects observed after single or short-term dosing, and the assessment factors applied
366 should always be justified by appropriate explanatory text.

367

- 368 • *Other findings indicative of adverse effects elicited by an acute exposure:*
- 369 ○ Clinical signs observed in acute (LD50) Toxicity studies
- 370 ○ Clinical signs and mortality in developmental neurotoxicity studies
- 371 ○ Behavioural abnormalities and clinical signs in the first days of repeated dose
- 372 studies, which are not indicative of neurotoxicity
- 373 ○ Decreased body weight gain, reduced food and /or water intake signs in the
- 374 first days of repeated dose studies, which are indicative of general toxicity and
- 375 not based on palatability of the feed.

376

377

378 **F. Animal Welfare Consideration**

379 For reasons of animal welfare, the request for additional experimental animal data should

380 always be a last resort in the risk assessment process; additional single exposure study should

381 NOT be performed:

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

394 In the single exposure study, a minimum but sufficient number of animals of the most

395 appropriate species should be utilised to produce the required additional data. Dogs should be

396 used only when it has been demonstrated that they are the most sensitive species to the test

397 substance if a single exposure study needs to be conducted.

398 If the rabbit is the most sensitive species, i.e. in a developmental study, no additional

399 experimental animal data should be submitted from a single exposure study in rabbits.

400

401 **G. Consideration of the Route of Exposure**

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

405 General principles and concepts which can be applied to dermal and inhalation exposure
406 routes should be addressed in separate OECD guidance documents. If for example the most
407 likely exposure route of a chemical for humans is inhalation, studies with inhalatory exposure
408 would be the preferred route of administration. This would eliminate the need for a route-to-
409 route extrapolation, with all its uncertainties, assumptions and limitations. Additional
410 guidance for dermal and inhalation studies can be found in appropriate OECD test guidelines.
411 However, if appropriate pharmacokinetic studies are available and/or part of entry effects data
412 are available route-to-route extrapolations might be performed to avoid additional route
413 specific animal testing.

414 Therefore, an ARfD may be transformed into an internal value considering the extent of
415 absorption of the substance along the respective route of application, if appropriate data are
416 available. This acute internal value can be compared to the different routes of exposure
417 without additional animal testing. This approach is equivalent to the acute systemic AEL
418 applied in different regulatory frameworks, e.g. for biocides (5).

419

420 **H. Consideration of Human Data**

421 Human data from accidental or deliberate poisonings, biomarker monitoring studies,
422 epidemiology studies, volunteer studies, and clinical trials on the same or structurally-similar
423 compounds can provide useful data to help establish ARfDs. The use of human volunteer data
424 in chemical risk assessment is a controversial issue, with a range of views held by different
425 countries and individuals. Therefore, the portion of ARfD values derived from human studies
426 varies in a wide range between different authorities. In a retrospective analysis of EU ARfDs
427 only 0.5% of the values were derived from human studies. In an older retrospective analysis
428 not restricted to Europe approximately 10% of the ARfD values were derived from human
429 studies (1). However it is recognised that the use of such data can reduce the level of
430 uncertainty inherent in extrapolating from animal models, if such data exist from the past or
431 from studies in nutritional physiology and medicine. For some substances like copper which is
432 used as a pesticide but which is also an essential nutritional compound the results from human
433 studies may be indispensable. There needs to be adequate consideration of both scientific and
434 ethical issues. The JMPR has considered human data at many of its meetings. The JMPR

435 reaffirmed the principle that endpoints from existing human volunteer studies could be used
436 for setting health intake standards if they had been conducted in accordance with relevant
437 ethical and scientific guidelines (2).

438 The PPR Panel has also published the opinion (12) that human data on a pesticide, whether
439 from volunteer studies or from other investigations of human exposures in the workplace or
440 environment, can be extremely valuable in placing the animal data in context and, when
441 available, should always be evaluated even when they are not used to derive a reference value.
442 Due to the ethical implications of studies in humans, they must be conducted in accordance
443 with principles such as those expressed in the Declaration of Helsinki (13) or equivalent
444 statements prepared for use by national and/or multinational authorities (4).

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

453

454 **I. Consideration of Different Subpopulations**

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

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

459 However, it is also important to ensure that the ARfD is adequate to protect the embryo/foetus
460 from possible *in utero* effects. Therefore, use of data from developmental studies for the
461 derivation of Acute Reference Values is considered, as a more conservative approach.
462 Because of critical windows of sensitivity for developmental effects, it should be assumed that
463 most developmental endpoints from repeated dosing studies are relevant for setting acute
464 dietary doses, unless there is evidence to the contrary (1, 2). There are several OECD test
465 guidelines that serve to evaluate potential developmental toxicants following prenatal and
466 postnatal exposures, including prenatal toxicity (OECD 414), reproductive (e.g. OECD 416)
467 and developmental neurotoxicity (OECD 424) studies.

468 While an Acute Reference Dose based on developmental (embryo/foetal) effects would be
469 appropriate for women of child-bearing age, it is recognised that the same value may be overly
470 conservative with respect to other subgroups in the population. For example, children aged 1
471 to 6 years; the use of a refined Acute Reference Dose based on *in utero* effects could be
472 inappropriate as they unlikely to be at risk for the developmental toxicity observed. In this
473 situation, separate modelling with respect to acute dietary intake of residues can be performed
474 taking into account age-specific acute consumption data. Alternatively, it might be necessary
475 to address higher sensitivity of children to other forms of acute toxicity by testing during early
476 life-stages.

477 Therefore, in some situations it may be necessary to set an Acute Reference Dose for the
478 general population and another value for other populations of concern.

479

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- 540

541

542 **Annex 1**

543

544 **REFINEMENT OF THE EXPOSURE CALCULATION FOR THE ACUTE RISK**
545 **ASSESSMENT**

546

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

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

570

571 Further approaches are under discussion but are not yet implemented.

572

573 Calculations of intake recognize four different cases (1, 2a, 2b and 3). Case 1 is the simple
574 case where the residue in a composite sample reflects the residue level in a meal-sized portion

575 of the commodity. Case 2 is the situation where the meal-sized portion as a single fruit or
576 vegetable unit might have a higher residue than the composite. Case 2 is further divided into
577 case 2a and case 2b where the unit size is less than or greater than the large portion size
578 respectively. Case 3 allows for the likely bulking and blending of processed commodities such
579 as flour, vegetable oils and fruit juices.

580

581 The following abbreviations are used in the equations:

582

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

583

584 *Case 1*

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

587

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

588

589 *Case 2:*

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

592

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

594

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

595

596 The Case 2a formula is based on the assumption that the first unit contains residues at the
597 $[\text{HR} \times v]$ level and the next ones contain residues at the HR level, which represents the residue
598 in the composite from the same lot as the first one.

599

600 *Case 2b:* Unit edible weight of raw commodity exceeds large portion weight.

601

$$\text{IESTI} = \frac{\text{LP} \times (\text{HR}) \times v}{\text{bw}}$$

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

604

605 *Case 3*

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

608

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

609

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

616

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

619

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

628

629 Another refinement option is the more detailed analysis of consumption data and the
630 refinement of LP. In many consumption surveys individual intakes of commodities arising
631 from various food items are aggregated over the day based on the RAC. Due to this
632 combination, information about the processing state of the food is lost: e.g. the intake of raw
633 apples, apple juice and apple pie are combined to a total figure for apples based on the RAC.

634 This aggregation normally results in an overestimation of the exposure and should be taken
635 into account, if further information is available.

636

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

641

642 A further refinement option is the replacement of the default variability factor ν by
643 experimental data. Though on FAO/WHO level a default factor of 3 is already used, which
644 can not be reduced much further by using experimental data, EU Member States on the other
645 hand still use factors of 5, 7 and 10, depending on the commodity. In those cases it might be
646 appropriate to conduct a supervised residue study to determine the unit to unit variability.
647 Data should be representative for different fruit sizes and fruit exposure situations. For
648 statistical reasons, at least a total of 120 single units should be analyzed. According to
649 Hamilton et al. (21) at least 119 samples are needed to estimate the 97.5 percentile with a
650 95 % confidence interval.

651

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

662

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

- 666 • Uncertainty and variability of the parameters used in the estimation;

- 667
- 668
- 669
- 670
- 671
- 672
- 673
- 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;

674 **Annex 2**

675

676 **GUIDANCE FOR CONDUCTING A SINGLE EXPOSURE TOXICITY STUDY**

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

679

680 **INITIAL CONSIDERATIONS**

681 In 2002 an analysis of the ARfD values set by several regulatory bodies was performed (1).
682 There were large differences in the ARfD values between the analysed regulatory bodies (up
683 to 2500-fold for some individual pesticides). In result of this analysis it was concluded “that
684 the current database of toxicological studies is not optimal for the derivation of the ARfD.
685 More specific information on the acute toxicity other than lethality is often needed for setting
686 an adequate ARfD.” In the mean time the regulatory authorities made more comprehensive
687 experiences with the derivation of ARfD values and notifiers and authorities made also the
688 first experiences with the design of additional acute or short term studies for the derivation of
689 ARfDs. Therefore, it was considered necessary to perform a new analysis in order to identify
690 the toxicological studies on which the ARfD values are based in 2008. This analysis was
691 recommended as a basis for a harmonized guidance on how to use available data on ARfD
692 derivation and also for the development of an ARfD study design. The current analysis of the
693 ARfD values was based on the last revision of this annotated list of active pesticide
694 substances which is published by the European Food Safety Authority (EFSA) on the EFSA
695 website with the specification SANCO 3010, rev 10/11/2008. The data basis for the ARfD
696 derivation of 198 substances was analysed. The portion of special ARfD studies is very low.
697 Only 4% of the ARfDs are based on such studies. In some cases such submitted ARfD studies
698 have not been accepted by the authorities because of quality deficiencies as a result of a
699 missing guidance paper. Therefore, in the EU peer review process some of the submitted
700 special ARfD studies have not been used for the ARfD derivation.

701 The results confirm once more that the development of an acute study design that produces
702 more comprehensive toxicological data for setting ARfDs would be of high value.

703

704 This *in-vivo* single exposure study is not intended to become a routine data requirement. As
705 discussed in the guidance document, the single exposure study should refine the Acute
706 Reference Doses and only be considered after the available toxicology and exposure
707 information a compound has been appropriately evaluated. The relevant species and

708 toxicological endpoints should already be documented and reasonably well understood
709 because this study is only designed to refined endpoints and dose of concern in the existing
710 repeated dose studies. Observations on the experimental animals are based on those listed in
711 the revised OECD Test Guideline 407. Therefore, additional validation of these test
712 parameters in this study is not considered necessary.

713

714 **PRINCIPLE OF THE TEST**

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

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

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

726 The test substance is administered orally as a single exposure in graduated dose levels to
727 several groups of experimental animals, one dose being used per group. A vehicle control
728 group is also included. Most toxicity should be manifested within 24 hours. Thus, animals
729 are terminated at 24 hours. A later time point should be included, between 48-120 hours after
730 treatment if it is anticipated that the toxicities of interest will not be adequately evaluated by
731 24 hours. Appropriate justification should be submitted to explain the inclusion or exclusion
732 of a second time point.

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

739

740 **The objective of the single exposure study is NOT:**

741 ➤ to identify lethal doses or provide data on mortality after acute exposure to a chemical,

- 742 ➤ to investigate the reversibility of acute effects, or
743 ➤ to investigate developmental effects or corrosive/irritation properties.

744

745 **DESCRIPTION OF THE METHOD**

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

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

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

771

772 **Selection of Animal Species**

773 The selection of animal species should be based on the results of the repeated dose studies,
774 which usually restricts the choice to the rat or the dog. It should not be required to perform the
775 study in both species.

776 Occasionally, mice may be more sensitive than rats or a better model for humans. If the mouse
777 is the preferred rodent species, the principles described for the rat should be adapted
778 accordingly. There are for example differences in the activity of enzymes in the tyrosine
779 catabolic pathway between rats and humans. Toxic effects of some active substances in rats
780 are largely attributable to increased plasma tyrosine levels following HPPD inhibition.
781 Therefore, in these cases the mouse is more predictive of the exposure in humans. Rabbits are
782 not relevant for such single dose studies, since if the rabbit is the most sensitive species for the
783 derivation of an ARfD in a developmental study, no further ARfD refinement is justified.

784 A justification should be given for the selection of the species. It should be demonstrated that
785 the animals selected will respond to the relevant parameters with a higher sensitivity than
786 other species and to be more relevant to human health risk assessment. For example we don't
787 want someone doing a study in dogs with a phenoxy acid such as MCPA, because of the dogs
788 are the most sensitive species for MCPA, but the rat is considered more relevant to human
789 health exposure. Preferably, the animals used in this study should be from the same strain and
790 source as the animals used in the key studies of the existing toxicological database for the test
791 substance.

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

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

800

801 **Housing and Feeding Conditions**

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

804 **Rats:** The temperature in the experimental animal room should be $22\text{ }^{\circ}\text{C}$ ($\pm 3\text{ }^{\circ}\text{C}$). Although
805 relative humidity should be at least 30 % and preferably not to exceed 70 % other than during
806 room cleaning, the aim should be 50-60 %. Lighting should be artificial, the sequence being
807 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used, with an
808 unlimited supply of drinking water. Animals may be housed individually, or be caged in small

809 groups of the same sex. For group caging, no more than five animals should be housed per
810 cage.

811 **Dogs:** For feeding, conventional laboratory diets may be used with an unlimited supply of
812 drinking water. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark.

813

814 **Preparation of Animals**

815 Healthy young adult animals are randomly assigned to the control and treatment groups.
816 Cages should be arranged in such a way that possible effects due to cage placement are
817 minimised. The animals are identified uniquely and kept in their cages for at least 5 days prior
818 to the start of the study to allow for acclimatisation to the laboratory conditions.

819

820 **Preparation of Doses**

821 This study should have a minimum three dose groups plus vehicle control group for deriving a
822 NOAEL/LOAEL.

823 If a benchmark approach is intended, more than three dose groups should be considered. In
824 this case, the number of animals per group could be reduced, as long as the necessary
825 statistical requirements are fulfilled.

826 Where necessary, the test substance is dissolved or suspended in a suitable vehicle. The toxic
827 characteristics of vehicles other than water must be known. The homogeneity of the test
828 substance in the vehicle should be assured.

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

837

838 **PROCEDURE**

839 **Number and Sex of Animals**

840 **Numbers of Animals:** The numbers of experimental animals used should be based on
841 statistical power calculations and the variability of the specific end-points noted in the
842 repeated dose studies as being especially relevant. For reasons of animal welfare as few

843 animals as possible should be used. For each dose, equal numbers of animals should be
844 sacrificed at the 24 hour termination time point, and if included, the later (second) time point.
845 If a later time point is included, additional subgroups of the same size should be used.
846 At least 10 rats (5 rats per sex and per group) should be used at each dose level, including the
847 vehicle control group. If a vehicle is used, a negative control is not required in addition to a
848 vehicle control. If only one sex is evaluated, then the number of animals could be increased, if
849 necessary, to provide more power to detect the toxicity of interest or more dose-groups could
850 be included to provide data for benchmark modelling.
851 A minimum of four dogs per sex and per dose group should be used for the 24-hour
852 evaluation. Only one sex should be evaluated for the toxicity of interest unless the
853 preliminary data suggest both sexes should be evaluated.
854 If identification of the toxic effect(s) of interest is possible in live animals at the 24 hour time
855 point, an additional subgroup may not be necessary and it may be sufficient to use the same
856 group of animals for the sacrifice at the later time point for pathomorphological examinations.
857 **Sex:** Both males and females could be used if necessary. Females should be nulliparous and
858 nonpregnant. Because existing information should be used to tailor and appropriately focus
859 this study, if existing data on the chemical show that one sex is clearly and consistently much
860 more sensitive than the other for the endpoint(s) identified as being relevant for acute toxicity,
861 then the study design should be modified to include only the more sensitive sex.

862

863 **Dose Selection**

864 At least three dose levels and a concurrent vehicle control should be used. Dose levels should
865 be selected taking into account any existing toxicity and ADME data available for the test
866 compound. The data should be sufficient to produce a dose-effect curve. Thus, dose levels
867 should be spaced to produce a gradation of toxic effects, ranging from recognisable toxicity
868 but not death or severe suffering at the highest dose to no or only very slight effects at the low
869 dose. If it is intended to establish a benchmark dose level rather than a NOAEL/LOAEL, it
870 may be sensible to increase the number of dose groups. A reduced spacing of dose levels may
871 allow the study to be conducted with fewer animals per subgroup, depending on the statistical
872 requirements for this approach.

873 Possible starting points for setting dose levels are known LD₅₀ values in animals and expected
874 exposure levels in humans. Furthermore, also cytotoxicity data according to NIH Publications
875 No: 01-4500 (1) can be used.

876 In addition, the highest/overall NOAEL from the repeated dose studies using the same animal
877 species could be selected as the low dose and together with one or two of the effect doses
878 from the repeated dose studies. The high dose may be limited to 1000 mg/kg bw/d, unless
879 expected human exposure indicates the need for a higher dose level to be used.

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

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

886

887 **Administration of Doses**

888 The most appropriate dosing would be by gavage in rodents and by capsule in dogs. Gavage
889 should be done in a single dose to fasted animals using a stomach tube or a suitable intubation
890 cannula.

891 The maximum volume of liquid that can be administered at one time depends upon the size of
892 the test animal. The volume should not exceed 1 mL/100 g body weight, except for aqueous
893 solutions, where 2 mL/100 g bw may be used. With the exception of irritating or corrosive
894 substances, which are likely to cause exacerbated effects with higher concentrations,
895 variability in volume should be minimised by adjusting the concentration to ensure a constant
896 dosing volume at all dose levels.

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

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

906

907 **Clinical Observations**

908 Clinical observations should be made in all animals at least once before exposure to the test
909 substance (to allow for within-subject comparisons) and at least 0.5, 1, 2, 4 and 24 hours after

910 dosing. The peak period of the anticipated effects should be considered when determining the
911 time points for clinical observations.

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

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

917 Signs noted should include, but not be limited to, changes in skin, fur, eyes, mucous
918 membranes, occurrence of secretions and excretions and autonomic activity (e.g. lacrimation,
919 piloerection, pupil size, and unusual respiratory pattern).

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

923

924 **Body Weight and Food/Water Consumption**

925 All animals should be weighed on the day of treatment and prior to sacrifice of the subgroup.

926 In addition, the animals of the 48-120-hour subgroups (if present) should also be weighed
927 every 24 hours after treatment.

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

929

930 **Toxicokinetics**

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

938

939 **Functional Observations**

940 If existing data indicate that the critical effect of the compound is neurotoxicity, then the acute
941 neurotoxicity test guideline should be considered (see OECD 424 and OPPTS 870.6200).

942 Alternatively, the elements described in this guideline may be combined with the design of an
943 acute neurotoxicity battery study, as long as none of the requirements of both guidelines are

944 violated by the combination. The parameters included may be tailored based on the extent of
945 existing knowledge.

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

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

953

954 **Haematology**

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

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

963

964 **Clinical Biochemistry**

965 The clinical biochemistry examination is only required if data from repeated dose studies
966 indicate that these parameters are of concern. The parameters evaluated may depend on the
967 species selected (typically rat or dog) and on the results of the repeated dose studies. Clinical
968 biochemistry determinations should be performed on blood samples of all animals taken just
969 prior to or as part of the procedure for killing the animals in each subgroup at the end of the
970 test period. In general, the following investigations of plasma or serum should be included:
971 glucose, total cholesterol, urea, creatinine, total protein, albumin, at least two enzymes
972 indicative of hepatocellular effects (such as alanine aminotransferase, aspartate
973 aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase and sorbitol
974 dehydrogenase). Measurements of additional enzymes and bile acids may provide useful
975 information under certain circumstances.

976 In addition, the investigation of serum markers of acute tissue damage should be considered.

977 These need to be identified for chemicals in certain classes or on a case-by-case basis.

978 If a specific, potentially acute effect of the test substance has been observed using special
979 techniques in repeated dose studies, then these techniques should also be used in this study.

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

988

989 **Urinalysis**

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

995

996 **Pathology**

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

1000 **Gross necropsy**

1001 All animals in the study shall be subjected to a full, detailed gross necropsy which includes
1002 careful examination of the external surface of the body, all orifices, the cranial, thoracic and
1003 abdominal cavities and their contents.

1004 The following tissues should be preserved in the most appropriate fixation medium for both
1005 the type of tissue and the intended subsequent histopathological examination: all gross lesions,
1006 brain (representative regions including cerebrum, cerebellum, and pons), spinal cord, stomach,
1007 small and large intestines (including Peyer`s patches), liver, kidneys, adrenals, spleen, heart,
1008 thymus, thyroid, trachea, and lungs (preserved by inflation with fixative and then immersion),
1009 gonads, accessory sex organs (e.g. uterus, prostate), urinary bladder, lymph nodes (preferably
1010 one lymph node covering the route of administration and another one distant from the route of
1011 administration to cover systemic effects), peripheral nerve (sciatic or tibial) preferably in close

1012 proximity to the muscle, and a section of bone marrow (or, alternatively, a freshly mounted
1013 bone marrow aspirate). Specific attention should be paid to likely target organs based on the
1014 known properties of the test substance. If an inhalation study is performed, the respiratory
1015 tissues preserved should be those mentioned in OECD TG 412. Skin should be preserved
1016 when a dermal study is performed.

1017 **Organ weight**

1018 Unless existing data from repeated dose studies with the test substance indicate that an organ
1019 is not a target site, the following organs should be trimmed of any adherent tissue, as
1020 appropriate, and their wet weight should be measured as soon as possible after dissection to
1021 avoid drying: liver, kidneys, adrenals, testes, epididymides, thymus, and spleen.

1022 In addition, if relevant as target organ for acute effects of the test substance, the wet weight
1023 should be determined for the following organs as soon as possible after dissection to avoid
1024 drying: paired ovaries, uterus, seminal vesicles (including coagulating glands), and prostate
1025 (dorsolateral and ventral part combined). Alternatively, seminal vesicles and prostate may be
1026 trimmed after fixation. Clamp or ligature should be present during fixation as leakage of fluid
1027 provokes damage to fine structures in seminal vesicles.

1028 The following organs should be weighed after fixation: thyroid (trimming should also be
1029 performed after fixation in order to avoid tissue damage) and dorsolateral and ventral parts of
1030 the prostate separately after separation.

1031 **Histopathology**

1032 Full histopathology should be carried out on the preserved organs and tissues of all animals in
1033 the control and high dose groups unless existing data from repeated dose studies indicate that
1034 an organ is not a target site. These examinations should be extended to animals of all other
1035 dose groups, if treatment-related changes are observed in the high dose group.

1036 All gross lesions shall be examined.

1037

1038 **DATA AND REPORTING**

1039 Individual animal data should be provided. Additionally, all data should be summarised in
1040 tabular form showing, for each test group, the number of animals at the start of the test, the
1041 number of animals found dead during the test or sacrificed for humane reasons and their
1042 respective cause of death, the number showing signs of toxicity, a description of the signs of
1043 toxicity observed, including time of onset, duration, and severity, the number of animals
1044 showing lesions, the type of lesions and the percentage of animals displaying each type of
1045 lesion.

1046 When possible, numerical results should be evaluated by an appropriate and generally
1047 acceptable statistical method. The statistical method should be selected during the design of
1048 the study.

1049

1050 **Test Report**

1051 The test report must include the following information:

1052 Aim of the study:

- 1053 - Justification for conducting such a single exposure study
- 1054 - Rationale for the specific design (e.g. choice of species and sex, dose selection, endpoint
1055 selection)

1056

1057 Guidelines and Quality Assurance:

- 1058 - Test type (Guideline)
- 1059 - GLP

1060 Test substance:

- 1061 - physical nature, purity and physicochemical properties
- 1062 - identification data

1063 Test animals:

- 1064 - species and strain used
- 1065 - number, age and sex of animals
- 1066 - source, housing conditions, diet etc.
- 1067 - individual weight of animals at the start of the test

1068 Test conditions:

- 1069 - rationale for dose level selection
- 1070 - details of test substance formulation/diet preparation, achieved concentration, stability
1071 and homogeneity of the preparation
- 1072 - details of the administration of the test substance
- 1073 - conversion from diet test substance concentration (ppm) to the actual dose (mg/kg bw/d),
1074 if the test substance was administered via the diet
- 1075 - details of food and water quality

1076 Results:

- 1077 - body weight/body weight changes
- 1078 - food consumption, and water consumption, if applicable
- 1079 - toxic response data by sex and dose level, including signs of toxicity

- 1080 - nature, severity and duration of clinical signs
- 1081 - functional observations (e.g., sensory reactivity, grip strength, motor activity assessments)
- 1082 - haematological tests with relevant base-line values
- 1083 - clinical biochemistry tests with relevant base-line values
- 1084 - body weight at sacrifice and organ weight data
- 1085 - gross necropsy findings
- 1086 - a detailed description and tabulation of all histopathological findings
- 1087 - statistical treatment of results
- 1088 **Summary and discussion of results**
- 1089 **Conclusions, Critical effects, NO(A)EL, LO(A)EL (or benchmark dose, if applicable)**
- 1090
- 1091

1092 **Appendix to Annex 2**

1093

1094 **A retrospective analysis of ARfD values of pesticides in the European Union**

1095

1096 **Introduction**

1097

1098 In 2002 an analysis of the ARfD values set by several regulatory bodies was performed (1, 2).
1099 There were large differences in the ARfD values between the analysed regulatory bodies (up
1100 to 2500-fold for some individual pesticides).

1101 In result of this analysis it was concluded “that the current database of toxicological studies is
1102 not optimal for the derivation of the ARfD. More specific information on the acute toxicity
1103 other than lethality is often needed for setting an adequate ARfD. The development of an
1104 acute study design that produces more comprehensive toxicological and toxicokinetic data for
1105 setting ARfDs was considered to be of high value.”

1106 In 2002 the ARfD value was still relatively new and no harmonised guidance for the
1107 derivation of an ARfD was available. In the mean time the regulatory authorities made more
1108 comprehensive experiences with the derivation of ARfD values for all pesticides, which were
1109 evaluated in the last six years. Notifiers and authorities made also the first practical
1110 experiences with the application of specific additional studies which were designed and
1111 performed for the derivation of ARfDs. Therefore, a new retrospective analysis was
1112 considered necessary in order to identify the toxicological studies on which the ARfD values
1113 are based in 2008. This analysis was recommended as a supportive basis for a harmonized
1114 guidance on how to use all available data on ARfD derivation and also for the development of
1115 an ARfD study design. This analysis should also identify how often such a specific single
1116 exposure test was submitted, what were the tested parameters, that such a study can be
1117 performed in future according to a harmonised OECD test procedure.

1118 The current retrospective analysis was based on the data of active pesticide substances which
1119 have been evaluated and peer-reviewed in Europe and included in Annex I of EU directive
1120 91/414/EWG between 2000 and 2008.

1121 The reason to use this EU data base for the ARfD analysis was that in the EU all ARfD values
1122 are especially well intensively discussed in a long peer review process by the regulatory
1123 authorities of all member states of the European Union and the results of this discussion are
1124 published regularly.

1125

1126

1127 **Material and Methods**

1128

1129 The European Commission maintains a tabular list with all existing and new active pesticide
1130 substances. This table contains information about the stage in which the active substance is
1131 evaluated, the Rapporteur Member State, the current status of each substance as well as
1132 further data such as ARfD and ADI including the source for the derivation of these threshold
1133 values and the year.

1134 The current analysis of the ARfD values was based on the last revision of this annotated list of
1135 active pesticide substances which was published by the European Food Safety Authority
1136 (EFSA) on the EFSA website.³

1137 Substances of this list have only been considered for the retrospective analysis if they have
1138 already been included in Annex I of EU directive 91/414/EWG. This means that the risk
1139 assessment process on basis of a draft assessment report and a peer review by the EU Member
1140 States has been finalised.

1141 Furthermore, substances of the EFSA list have only been considered if a statement on the
1142 ARfD was given. Microbial pesticides and other not clearly chemically defined substances
1143 like plant extracts have been sorted out. Finally 198 active substances (existing and new
1144 pesticide substances) have been considered for the analysis.

1145 The sources for the ARfD derivation according to SANCO 3010 have been sorted into 8
1146 groups of studies: 1. Special ARfD studies (i.e. single dose studies which have been
1147 performed to derive a refined ARfD; these are additional studies to the usual data
1148 requirements which have not been performed according to OECD guidelines), 2. Acute
1149 Neurotoxicity studies, 3. Repeated dose studies, 4. Multigeneration studies, 5. Developmental
1150 toxicity studies, 6. Developmental neurotoxicity studies (DNT), 7. Human studies, 8. No
1151 ARfD derived (i.e. considered not necessary because of low acute toxicity according to the
1152 guidance on *the principles for not setting an ARfD*).

1153 The portion of every group was calculated in per cent of all studies.

1154

1155 **Results**

1156 The percentages of the 8 groups of studies in this analysis of the data basis for the ARfD
1157 derivation of 198 substances are summarised in Table 1. For 95 pesticides, approximately the
1158 half of all substances (i.e. 48 %), no ARfD was considered necessary because of low acute
1159 toxicity of these pesticides. For 103 of the analysed substances (i.e. 52 %) an ARfD was
1160 established. Most of these ARfD values have been derived from developmental toxicity

1161 studies in rats or rabbits (26.8%). 10.1% of the ARfD values are based on acute neurotoxicity
 1162 studies. The portion of special ARfD studies applied for an ARfD derivation is very low. Only
 1163 4% of the ARfDs are based on such studies. The use of DNT and human studies is negligible
 1164 in the European Union.

1165 For 19 pesticides (< 10 %) the ARfD can be considered as conservative, since it was based on
 1166 repeated dose toxicity or multigeneration studies.

1167

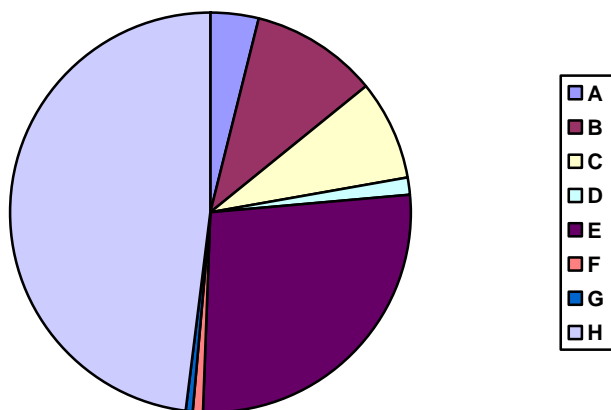
1168 **Table 1 ARfD derivation in the EU pesticide evaluation program (198 active substances)**
 1169

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

1170

1171

1172 **Figure 1 ARfD derivation in the EU pesticide evaluation program**
 1173



1174

1175

1176 **Discussion and Conclusion**
 1177

1178 The results of the retrospective analysis of the ARfD values in the EU in 2008 are
 1179 approximately in the same range of the ARfD values set by several regulatory bodies in 2002.
 1180 Solecki et al. (1) concluded that 23% of the ARfD values were based on single dose studies.
 1181 The majority of these acute studies were acute neurotoxicity studies in rats. 39% of the
 1182 analysed ARfD values were based on maternal and /or developmental effects in

³ (www.efsa.europa.eu) with the specification SANCO 3010, rev 10/11/2008

1183 developmental toxicity studies in rats or rabbits. The conclusion that an ARfD is unnecessary
1184 varied between 14% and 54% of the analysed substances in 2002.

1185 The conclusion of Solecki et al. (1) “that the current database of toxicological studies is not
1186 optimal for the derivation of the ARfD” is not so applicable to the analysis of the EU ARfD
1187 values in 2008. The majority of ARfD values were based on studies in which specific acute
1188 alerts (i.e. developmental toxicity and neurotoxicity) was investigated. For these 75 substances
1189 no refinement of the ARfD with a special study is necessary.

1190 Only for the 19 pesticides in which the ARfD was based on repeated dose toxicity or
1191 multigeneration studies a refinement with a specific study might be considered, if the ARfD is
1192 exceeded by the acute intake assessment.

1193 For a small portion of pesticides (4 %) special acute studies were submitted for the ARfD
1194 derivation. These special acute ARfD studies do not belong to the basic requirements and
1195 guidance papers do not exist. The only available acute studies apart from the LD₅₀-studies are
1196 currently the acute neurotoxicity studies which are used to derive approximately 20% of the
1197 ARfD values, but if neurotoxicity is not the most relevant acute alert, no specific study design
1198 are available.

1199 Special studies to evaluate the acute toxicity as a basis for the ARfD derivation are mostly
1200 performed additionally to the basic data requirements in the process of discussion of the
1201 toxicological assessment between notifiers and authorities. These studies may be required if
1202 the evaluation of acute toxicity and the derivation of an ARfD is difficult on the basis of
1203 routinely required studies. However, in some cases such submitted studies are not acceptable
1204 by the authorities because of quality deficiencies as a result of a missing guidance paper.
1205 Therefore, in the EU peer review process some of the submitted special ARfD studies have
1206 not been used for the ARfD derivation.

1207 The results confirm once more that the development of an acute study design that produces
1208 more comprehensive toxicological data for setting ARfDs would be of high value. One the
1209 other hand this analysis has shown that such a special ARfD study was considered necessary
1210 only for very few pesticides.