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OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON THE

Draft Guidance Document on Aquatic Ecotoxicology (DG VI – 8075/VI/97-Rev.4 of 18.12.1998)

(Opinion expressed by the SCP on 24 September 1999)

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1. Terms of reference

The draft *Guidance Document on Aquatic Ecotoxicology* had been referred to the Scientific Committee on Plants for consultation with the following questions:

1. Which factor of sensitivity would qualify a species as 'clearly the most sensitive' (section 4 of guidance document)?
2. Which are, in the opinion of the SCP, adequate triggers for a life cycle study in fish?
3. Would it be more adequate to base the trigger for a bioconcentration study on the DT50 instead of the DT90 (section 7 of guidance document)?
4. Which is the opinion of the SCP concerning the adequate trigger, method of application and of analytical measurements for studies on sediment dwelling organisms (section 10 of guidance document)?

2. Background

The draft Guidance Document on Aquatic Ecotoxicology had been developed as a working document of the Commission, with the purpose to provide guidance to Member States and to notifiers on the use of the respective sections of Annexes II, III and VI of Directive 91/414/EEC. It should ensure a uniform and harmonised approach of evaluation and aquatic risk assessment of active substances and plant protection products in the EC review and in Member States. They were referred to the Scientific Committee on Plants for opinion. The Committee had also been supplied with the reports of the FOCUS group and with comments from Member States and from ECPA on the guidance documents.

*In the following opinion, the Committee will first discuss more widely a number of scientific issues mentioned in the draft guidance document. For each issue, a brief summary of the main statements of the guidance document is given under the heading ´**Context**´, in order to allow this opinion to be read as a stand-alone document as much as possible. This discussion section provides background to the specific answers of the SCP to the questions listed above. The specific answers are provided at the end of this opinion.*

3. General observations

The guidance document provides a good overview of issues which need to be considered during the evaluation of the aquatic ecotoxicology in the context of Directive 91/414/EEC[1]. It can be expected to contribute to better consistency and transparency in decision-making both at European Community (EC) and Member State level.

[1] OJ No L 230, 19. 8. 1991, p.1.

Basically, the SCP supports most of the views expressed in the document. It strongly endorses the statement on avoiding duplicate animal testing which is made in section 2 of the terrestrial ecotoxicology document, and recommends to include an equivalent statement in the aquatic guidance document.

3.1 Introduction / Overall presentation

The aquatic guidance document should - to the extent possible - cross-reference, and be compatible with, the guidance documents on Terrestrial Ecotoxicology (2021/VI/97), on Persistence in Soil (9188/VI/97), and the document on Relevant Metabolites (under development). A number of issues relevant to more than one of the guidance documents are highlighted in this opinion.

Flow diagrams or tabular listing of the data requirements at each decision node, and the triggers applied would be helpful in summarising how to proceed, and to gain a quick overview. One *example* each of such a diagram and table are attached for consideration.

The document includes a number of technical details which might – for reason of clarity, to ensure necessary detail and easier revision – be better provided for in annexes (e.g., calculation methods for spray drift, runoff and subsurface transport, for TWA[2] concentrations; standard scenarios; calculation of statistical power in toxicity tests).

The current annex on the distribution of tasks between the EC peer review meetings of ecotoxicologists and fate experts may be important for internal reasons, but it is not considered important for notifiers or other users of the guidance document, and should therefore be removed.

3.2 Terminology

Not all the long-term tests discussed in the document are true *chronic* tests (which, by WHO definition, comprise the greater part of the life span of the organism). Therefore, use of the more neutral term *long-term* rather than *chronic* would be preferable throughout the guidance document.

3.3 Areas that may require further consideration

The documents should be reviewed and (when necessary) revised regularly, in order to reflect changes of the scientific knowledge and results of international activities/harmonisation in this area (e.g., test guidelines, FOCUS results).

From current knowledge, it is likely that the following areas will require more detailed guidance in the near future:

- the testing and assessment of endocrine effects (where the currently ongoing work of the OECD EDTA group should be reviewed and possibly implemented)
- the principles of refined risk assessment following a probabilistic approach (where results from the US – EDSTAC initiative will need to be reviewed and possibly adapted)

[2] time-weighted average

- the development of standard methodology for PEC[3] calculations, possibly to be based on the FOCUS work on standard scenarios.

4. Detailed notes

4.1 Acceptable test guidelines/general issues

Both the aquatic (in section 2) and the terrestrial ecotoxicology guidance documents mention some general issues for acceptable guidelines which in the opinion of the SCP apply to both, and should therefore be merged in a common section for both documents:

Several tests in both the aquatic and the terrestrial compartment aim at determining a no-observed-effect-level/concentration (NOEC[4] or NOEL[5]), a concept that has scientifically been challenged (Laskowski 1995; OECD 1998). OECD member countries have agreed to phase out the NOEC and replace it by a regression-based parameter (based on an ECx – design). Currently, the OECD is working on those alternatives for evaluating such tests. The SCP supports the decision to phase out the NOEC. In view of current OECD activities, a common section should be added to both guidance documents on the issue of replacing the NOEC by an ECx to be developed and decided upon under OECD. Such section could be based on section 3. *NOEL-values as summary parameters* of the terrestrial guidance document.

The statement on avoiding duplicate animal testing (section 2 of terrestrial guidance document) is also relevant to both ecotoxicology documents. The same applies to the statements made in section 2 of the aquatic document concerning internationally accepted guidelines and the responsibility of notifiers.

4.2 Exposure assessment

Context: Section 3 of the aquatic guidance document focuses largely on spray drift but remains unclear on the exposure assessment regarding runoff and subsurface transport (e.g., drainage). Although these routes of exposure are mentioned as important, and reference to the work of FOCUS is made, it is stated that only spray drift should be considered in a standard risk assessment. However, no guidance is given on when and how to proceed from a standard to a refined risk assessment.

Opinion: In the view of the SCP, it is not appropriate to base the standard risk assessment solely on spray drift. Such practise might lead to an underestimation of exposure e.g., for granular applications where spray drift would be considered to be negligible. Instead, all possible routes of entry should be considered equally at the same stages of exposure and risk assessments, i.e., for the initial and the TWA PEC values. This does not exclude the use of different exposure models or scenarios at different stages, e.g., simple screening-type (worst case) exposure estimates as the first step. Progress made in FOCUS on those other routes of exposure should be incorporated into the guidance document as soon as possible.

[3] Predicted environmental concentration
[4] No-observed-effect-concentration
[5] No-observed-effect-level

4.3 Metabolites

Context: Section 4 provides triggers for the testing of metabolites but also mentions another guidance document on relevant metabolites which is currently under development. The guidance document on aquatic ecotoxicology recommends toxicity testing for those metabolites which occur at levels > 10% of the applied radioactivity in the water phase of water/sediment studies. In addition, other fate or toxicity information and expert judgement shall be applied to decide on the testing of any metabolite.
The guidance document further recommends to restrict metabolite toxicity tests to the taxonomic group (i.e., test organism) which was clearly (by a factor of 100 or more) the most sensitive one in acute tests with the active substance. Acute tests shall be conducted first. Long-term tests should only be performed if the metabolite was more acutely toxic than the active substance. Long-term tests should also be restricted to the most sensitive taxonomic group.

Opinion:

10%-trigger: Consistency between the different documents with regard to such triggers is obviously important for clear guidance, as will be a clarification on the relationship between the two documents. As to the 10%-trigger, the SCP supports this as a pragmatic screening approach. However, it is recognised that metabolites occurring at lower levels may well be ecotoxicologically relevant. Hence, all available information and expert judgement should be used to assess if metabolites < 10% give rise to particular concern. Such metabolites should then also be subjected to a risk assessment rather than a 'specific justification'.

most sensitive taxonomic group: For practical reasons, it is desirable to predict the toxicant sensitivity of a wide range of species from the measured responses of only few standard test species. However, a number of studies has demonstrated that the degree of variability among species is highly chemical dependent. For example, in an analysis of 22 test species and 15 chemical substances, the ratio of the highest acute L(E)C50 to the lowest acute L(E)C50 varied from about 10 to almost 9000 (Sloof et al. 1983). In a later study comparing a total of 1190 interspecies extrapolation factors, Sloof et al. (1986) found over a 3-order of magnitude span of values, ranging from 1 to 3200.

In summary, while there appears to be broadly significant statistical correlations in the toxicity of chemicals between species, relationships between these values are not predictable within several orders of magnitude. Selecting a single value, such as the proposed factor of 100, for such extrapolations is therefore not scientifically justifiable and could only be taken as a pragmatic, statistical approach to reduce testing, with an uncertain degree of error.

Therefore, for the individual case of a metabolite, all relevant information needs to be taken into account. For example, if the metabolite in question still possesses the biologically active part of the parent molecule, it is reasonable to assume a similar activity and species sensitivity as was determined for the parent molecule. Absence of that part would, however, not exclude toxicity to non-target organisms. Furthermore, exposure should also be considered before waiving tests on a specific taxonomic group.

long-term tests: With regard to the issue of most sensitive species, see discussion above. As for the relationship between acute and long-term effects, it is not possible to generalise, as this depends on the substance-specific mode of action, the organism and the associated endpoints. Statistical correspondence (correlation) has been used for pragmatic reasons to minimise testing (and costs), but the precision of such predictions may actually be very low: Kenaga (1979) found acute-to-chronic ratios (ACRs) ranging from 1.1 to 11000. Ratios for pesticides and metals seem to be higher than for other groups of chemical substances. For pesticides as a whole, Kenaga (1982) reported 59% of a total of 27 pesticides to have ACRs above 25 and 19% to have ACRs above 125, but again the percentages were shown to vary for different classes of pesticides (e.g., herbicides, chlorinated hydrocarbon insecticides, cholinesterase inhibitors). The highest ACR was found for the herbicide, propanil, which had a value of 18100 for the fathead minnow. This same species had much lower ACRs for other herbicides (e.g., 4 for propachlor, 3 for butralin, 435 for diuron).

Therefore, the requirement for long-term testing should be based on likelihood of exposure together with other information (e.g., toxicity of substances with similar modes of action, structure-activity considerations) rather than on acute toxicity.

For the individual case of a metabolite, all relevant information needs to be taken into account. For example, if the metabolite in question still possesses the biologically active part of the parent molecule, it is reasonable to assume a similar ACR as was determined for the parent molecule. Absence of that part would, however, not exclude toxicity to non-target organisms. Furthermore, exposure would have to be considered before waiving tests on a specific taxonomic group.

4.4 Persistence trigger for long-term fish tests

Context: The guidance document (section 5) proposes that long-term fish studies not be performed for unstable substances. Unless repeated applications lead to long-term exposure even with unstable substances, tests shall not be performed if the water-phase DT50 in a water/sediment study is ≤ 2 days with a pH in the range of 6 - 9.

Opinion: The SCP supports the intention to avoid unnecessary fish testing. Within the environmentally relevant pH-range of 6 to 9, however, DT50 values for active substances often vary widely. If such a DT50 should be used for waiving long-term toxicity tests with Daphnia or fish, as proposed in section 5, the SCP considers it necessary to establish that the chosen DT50 is the same or very similar over the whole pH-range of 6 - 9 which is environmentally relevant.

4.5 Appropriate long-term fish tests

Context: The guidance document (section 6) states that long-term fish tests should be triggered by the likelihood of exposure, not by the results of acute toxicity tests. From the various test designs available, a combination of OECD Test Guideline 204 and the new OECD Juvenile Growth Test is recommended. Fish Early Life Stage or Life Cycle studies are

proposed where one or more of various triggers is breached (acute toxicity, BCF[6], elimination rate, water/sediment DT90[7], expert judgement.)

Opinion: As stated above, the correspondence between acute and long-term effects cannot be well established. Hence, the use of the LC50[8] as a trigger for long-term tests (as stated in the current version of Annex II of the directive 91/414/EEC) does not appear to be appropriate. Again, exposure considerations should drive this decision.

As to the different long-term fish tests, the OECD 204 test protocol is clearly of rather limited value. As pointed out in the guidance document, at least the additional endpoints from the juvenile growth test need to be covered. Hence, effects on reproduction and the endocrine system would not be detected. Therefore, it should be considered to replace the standard long-term fish test by a test with more relevant endpoints, like the early-life-stage or the full-life-cycle test. Of these two, the guidance document states correctly that only the full-life-cycle test can adequately cover endocrine disrupters. It should therefore be the preferred method for test guideline development and harmonisation.

In addition, regardless of exposure, a bioaccumulating substance with low depuration should be tested with one of the tests covering reproduction, preferably the full-life-cycle study. The criteria mentioned in the guidance document seem appropriate.

Two activities of SETAC, the HARAP (1998) and CLASSIC (1999) workshops are likely to provide further valuable guidance with regard to higher-tier aquatic testing and refined risk assessment. The guidance document should provide adequate reference and, preferably, brief summaries of the workshop conclusions as annexes.

4.6 Fish bioconcentration study

Context: Annex II states a log Pow[9] ≥ 3 to trigger a fish bioaccumulation study, unless exposure leading to possible bio-accumulation is unlikely. The guidance document (section 7) specifies this by recommending that only substances with a water/sediment (whole system) DT90 ≥ 10 days should be tested, unless repeat applications might cause sufficiently continuous exposure.

Opinion: The SCP supports the triggers proposed in the guidance document. The Committee is aware that for substances with a log Pow > 3, the time to reach steady-state is usually in the range of 5-10 days. Therefore, for a *single* application, the proposed trigger value of DT90 < 10 days can be used to avoid unnecessary testing for substances which degrade faster than would be necessary to reach steady-state under natural conditions. In the case of *repeat* applications, the situation may be different, and the presence of the substance in the water body more prolonged. Therefore, SCP also supports the views of the guidance document that in deciding on the necessity for the study, the DT90 should then be used in conjunction with the intended use pattern as the indication of the overall exposure.

[6] Bio-concentration factor
[7] Disappearance time for first 90% of compound
[8] Lethal concentration 50%
[9] Octanol/water partition coefficient

4.7 Long-term tests on invertebrates other than *Daphnia*

Context: The guidance document (section 8) requires testing of insect species if there is evidence that *Daphnia* is not representative for insects for that substance. Other taxonomic groups (e.g., gastropods) should be tested when direct application to surface waters is proposed.

Opinion: Although, at least as far as **acute** toxicity is concerned, *Daphnia* has been shown to be among the more sensitive of the aquatic organisms for a number of active substances, there are also cases where other taxa (e.g., mayfly, caddisfly and stonefly larvae for pyrethroids) are clearly more sensitive. Appropriate **long-term** data for other taxa are lacking for most active substances. Hence, very often, the evidence for the non-representativeness of *Daphnia*, as required by the guidance document, will not exist. This however, cannot be taken as proof for its representativeness, in the absence of data. Therefore at present, it is difficult to provide clearer guidance on the need for such tests, apart from the case of IGR's (Insect Growth Regulators) where such tests should be obligatory because of their mode of action, and which should be added to the guidance document. For insecticides in general, testing of a standard insect species would be desirable.

For herbicides and fungicides, the SCP hesitates to recommend standard testing of an insect species. The SCP rather recommends to carefully analyse all the existing long-term data, in order to allow the development of clearer guidance to notifiers and regulators. It would be valuable if a comparative analysis of the existing long-term data could be undertaken, using both regulatory files and published literature. The study should compare long-term *Daphnia* data with similar data on other aquatic invertebrates. The SCP is especially aware of three main types of data which could be valuable:

- *Chironomus:* Data on *Chironomus* have been required for some time, especially in some EC countries, and should be available to regulatory authorities.
- *Amphipods:* Data on amphipods like *Hyalella* and *Gammarus* are available, especially in the US and in the literature.
- *Mesocosms:* Micro-and mesocosm studies submitted to regulatory authorities usually contain data on the populations of a number of invertebrate taxa.

4.8 Sediment organisms

Context: Annex II of directive 91/414 requires unspecified tests with sediment organisms where an active substance is likely to partition and to persist in aquatic sediments. The guidance document (section 10) specifies triggers and test design: Substances where (a) ≥ 10% of the applied radioactivity occur in the sediment after day 14, and (b) with an NOEC < 0.1 mg/L from a long-term *Daphnia* or insect test should be tested with *Chironomus.* The ´spiked water´ design (where the test substance is applied to the water phase of the complete test system and allowed to partition) is generally (with exceptions and flexibility) preferred over the ´spiked sediment´ design (where the test substance is applied to a sediment slurry and allowed to adsorb, before water, sediment and organisms are combined in the test system). Analytical measurements from the water/sediment fate study may be transferred to the *Chironomus* test if conditions are shown to be comparable.

Opinion:

Triggers: As suggested by the guidance document, benthic organisms should be tested when the active substance partitions into the sediment. In addition to the proposed trigger (10% radioactivity in the sediment after 14 days), the use pattern and the persistence should be taken into account by calculating a TWA concentration in the sediment over the time period of all the intended applications per season. This second trigger would cover the case of repeat applications which could lead to a build-up in the sediment (slower than in 14 days).
As additional trigger, the guidance document proposes a *Daphnia* long-term NOEC < 0.1 mg/l. This seems to be an arbitrary choice since it does not take account varying dose rates and exposure (if this figure is derived from a screening-type scenario, this should be provided in an annex for clarity and transparency). The SCP recommends a TER[10]-based approach, as also mentioned in the guidance document. In the absence of the comparative study on species sensitivity which was suggested above, but taking note of existing experience within regulatory agencies, the SCP acknowledges that a preliminary TER of 10 (based on the sediment TWA as above and the long-term *Daphnia* NOEC) could serve as a trigger for pragmatic reasons especially in cases where a persistent metabolite does not exceed 10% (but see above discussion on uncertainties with regard to species-to-species extrapolation for comparable endpoints)..

Design: Of the two methods of applying the test substance under discussion, the spiked water test is considered to provide the more realistic total exposure to the test organisms for most cases. This is clearly the case for entry via spray drift or in the dissolved state via water (e.g., subsurface transport). As to particle runoff, it will also enter water bodies as water carrying particles. In this slurry, partitioning of the substance between the adsorbed and the dissolved phase will have taken place to a certain degree, hence creating a scenario closer to the spiked water test than to the spiked sediment test. Possible exceptions include the case mentioned in the guidance document when slow build-up in the sediment may finally lead to considerably higher concentrations in the sediment than a single application (as in the test).

Analytical measurements: Under comparable conditions (e.g., with regard to water and sediment type, light and temperature) and method of application, the partitioning of the substance should be similar in the water/sediment fate study and the *Chironomus* toxicity study (both spiked water tests). Although there is evidence that bioturbation (i.e., the mixing or movement of sediment by burrowing animals like oligochaetes) can influence the partitioning, this appears to be negligible in the case of the *Chironomus* test in question. Hence, the reasoned transfer of analytical measurements from the water/sediment fate study to the *Chironomus* test seems appropriate. It should be noted that this may need to be re-established when/if test conditions change (i.e., for the new OECD-versions of those test guidelines which are currently in the final stages of adoption).

4.9 Aquatic plants

Context: Annex II requires a test on aquatic plants for herbicides. The guidance document (section 11) specifies available test guidelines for *Lemna*.

[10] Toxicity exposure ratio

Opinion: The SCP strongly supports the inclusion of aquatic plants into the effects assessment. Clearly, herbicides have to undergo such testing due to their mode of action being targeted at plant metabolism. In addition, other active substances which show a high toxicity to algae should also be tested. Until more data become available, this should be required if algae are the most sensitive test organisms. Again, as with the issue of insects and crustacea, this area requires more research and experience.

Like for animals, substances which partition and remain in the sediment may pose a different exposure as can be tested in the *Lemna* study. The risk assessment for such substances with regard to aquatic plants rooted in the sediment needs more attention by the scientific community. Non-standard test methods like micro- or mesocosms have been applied by researchers, and should be considered as higher-tier tests in cases of high herbicidal activity , given exposure, and partitioning and stability in the sediment.

4.10 Microcosms/mesocosms

Context: The guidance document (section 13) discusses the merits of micro- and mesocosm studies especially where the TER values of Annex VI are breached and the 'unless-clause' applies. The SETAC guidance is recommended for the study design, but case-by-case discussion with regulatory authorities is necessary.

Opinion: Such studies are clearly very valuable tools in the aquatic risk assessment. They can be used best if the design addresses the specific problems of the active substance in question. The design should therefore, as recommended by the document, be discussed with regulatory authorities on the basis of a risk assessment. Their interpretation is complex, and requires careful use of adequate statistical tools. Although MANOVA is often used, the statistical power and the proof of (required) normal distribution of data are often lacking. Multivariate statistical techniques for non-normal data have become more widely available with increased (and cheap) computer power, and should be considered for the analysis (for a recent review, see ET&C 18 No 2, 1999, pp. 111 – 166, seven related articles under the heading *´Multivariate Statistics`*).

Specifically with regard to **recovery** which can be observed in such studies, it should be pointed out that recovery needs to be carefully interpreted, taking into account (if not done by the study design):

- repeated applications of the substance in question during the period necessary for recovery
- the timing of the applications in relation to the timing of the life cycle
- applications of other products which would be used under normal agricultural practice during the period necessary for recovery.

As mentioned above, recent international activities like the SETAC HARAP Workshop should be summarised in an annex.

4.11 Long-term tests with formulated products

Context: Annex III requires studies with the formulated product where its toxicity cannot be predicted from data with the active substance. The guidance document (section 14) discusses

the lack of appropriate fate data for formulations. It recommends long-term tests with the formulated product where it is acutely more toxic (factor 10 or more) than the active substance.

Opinion: The SCP shares the concerns expressed in the guidance document with regard to the lack of knowledge concerning the fate and toxicity of formulations as a whole or of important parts. Issues include:

- interactions of two or more active substances in the formulation
- co-formulant(s) may change fate, distribution or toxicity of the active substance
- co-formulant(s) may exhibit own toxicity in environmentally relevant concentrations.

With respect to interactions between several active substances, current scientific knowledge does not allow to predict the combined toxicity from the effects of the individual substance. Experience shows that the effects are additive or dominated by one active substance in most cases, rather than synergistic. It would, however, be very valuable if a comparative analysis of existing data from regulatory files be undertaken.

The current version Annex III of directive 91/414/EEC requires the formulation to be tested on **acute** toxicity in cases where formulation toxicity cannot be predicted. However, considering the remaining uncertainties with predicting long-term effects from acute toxicity studies (see above), the SCP considers it more appropriate if instead of the acute tests the long-term tests were undertaken. Thus, far more knowledge and certainty in the assessment could be gained.

In the absence of regular testing, co-formulants should nevertheless receive more attention in the assessment. Relevant information especially concerning the effect of specific co-formulants on the fate of the active substance can possibly be gained from the reason for the use of the specific co-formulant (i.e., if it is explicitly used to stabilise the active substance in water). Such information could be required and used more routinely in the assessment.

4.12 Options when TER trigger values are breached

Context: Section 15 of the guidance document discusses options for those cases where the TER values of Annex VI are breached and the 'unless-clause' applies. Those options include:

- refining the exposure assessment
- using micro- or mesocosm studies
- consider potential for recovery.

Opinion: The SCP supports the general approach to refine the exposure assessments and to use higher-tier toxicity studies in this case. Micro-and mesocosm studies (designed case-specifically; see discussion above in section 4.10) are expected to be crucial for decision-making in many cases. As to the issue of **recovery**, the aspects mentioned in this opinion in the section on mesocosms apply here as well. With regard to **refined risk assessment** once trigger values are breached, the concept of probabilistic risk assessment seems to be promising approach, if properly performed. Here, the results of the US ECOFRAM initiative should be reviewed in the European context.

5. Executive Summary

5.1 Answers of the SCP to the specific questions

1. Which factor of sensitivity would qualify a species as 'clearly the most sensitive' ?
Determining differences in sensitivity among species to the same chemical can be complicated sometimes by substantial inter-laboratory variation in results for the same test (same chemical and endpoint). From a statistical perspective, species can be defined as different in sensitivity if the effect endpoints (e.g., LC50, ECx) are at least one order of magnitude apart, with non-overlapping 95% confidence intervals. For NOEC's where confidence intervals cannot be estimated, statistical comparisons cannot be made. However, as discussed above (section 4.3), on the basis of the published literature, there appears to be little scientific justification for expecting consistency in the relative sensitivities among species to parent compounds and their metabolites. In general, metabolites which retain the active part of the parent molecule can be expected to behave relatively similarly to the parent in terms of toxicity. However, there may be exceptions to this. A scientifically conservative approach would therefore argue for testing of the same taxonomic groups for both parent and relevant metabolites.
The Committee is aware that data on metabolite toxicity have already been created for regulatory purposes. A thorough analysis of existing published and those unpublished data could provide a scientific basis for restricting testing of metabolites to a selected subset of taxonomic groups.

2. Which are, in the opinion of the SCP, adequate triggers for a life cycle study in fish?
As stated above, acute effects cannot reliably predict long-term effects. Hence, the use of the LC50 as a trigger for long-term tests is not considered to be appropriate. The main trigger for long-term studies including life cycle tests should be exposure.

As to the different long-term fish tests, the OECD 204 test protocol is clearly of rather limited value. As pointed out in the guidance document, at least the additional endpoints from the juvenile growth test need to be covered. However, effects on reproduction and the endocrine system would not be detected. It should therefore be considered to replace the standard long-term fish test by a test with more relevant endpoints, like the early-life-stage or the full-life-cycle test. Of these two, the guidance document states correctly that only the full-life-cycle test can adequately cover endocrine disrupters, and it should therefore be used whenever there are indications of endocrine activity of the substance. The full-life-cycle test should be the preferred method for test guideline development and harmonisation.

In addition to given exposure, a bioaccumulating substance with low depuration should be tested in any case with one of the tests covering reproduction, preferably the full-life-cycle study. The Committee supports the criteria mentioned in the guidance document (BCF > 1000, elimination < 95% in 14 days, DT90 > 100 d in water/sediment systems)

3. Would it be more adequate to base the trigger for a bioconcentration study on the DT50 instead of the DT90?
No. The trigger here would distinguish between substances which degrade almost completely in a short period of time from those which do not degrate so fast. The DT50 value alone,

without information on the reaction kinetic, does not allow a conclusion on when most of the substance would be degraded. The DT90 does provide this information.

4. Which is the opinion of the SCP concerning the adequate trigger, method of application and of analytical measurements for studies on sediment dwelling organisms?
Such studies should be performed when the active substance (or a metabolite) partitions into the sediment (> 10% in 14 days or over one season), taking into account the stability and the use pattern. *Daphnia* toxicity might be used as an additional trigger in a TER, especially to cover persistent, highly toxic metabolites at levels below 10%. For most cases (type of pesticides and their methods of application), the spiked-water design is considered the most appropriate simulation of the different routes of exposure to a benthic organism. Analytical measurements from a comparable water/sediment study can be used.

5.2 Observations and recommendations of the SCP to other issues

- The documents should be reviewed/revised regularly.

- The following areas are currently likely to require more detailed guidance in the near future:
  - the testing and assessment of endocrine effects
  - the principles of refined risk assessment following a probabilistic approach
  - standard methodology for PEC calculations

- The SCP strongly endorses attempts to avoid duplicate animal testing. It is expected that in some cases, a thorough analysis of already existing but yet unpublished data could help establishing criteria for waiving tests, e.g., with metabolites or long-term tests.

- Instead of the term *chronic,* the more neutral term *long-term* should be used.

- Technical details might better be provided in annexes to the guidance document.

- It is not considered appropriate to base the standard risk assessment solely on spray drift. All possible routes of entry should be considered equally at the same stages of exposure and risk assessments.

- The SCP supports the 10%-value for metabolites as a pragmatic screening approach, but metabolites occurring at lower levels may well be ecotoxicologically relevant. Hence, all available information and expert judgement should be used to assess if metabolites give rise to particular concern. Such metabolites should then also be subjected to a risk assessment.

- If lack of stability of the substance is to be used for waiving long-term toxicity tests, the SCP considers it necessary to establish that the chosen trigger (e.g., DT50) is the same or very similar over the whole pH-range of 6 - 9 which is environmentally relevant

- Long-term testing should be based on likelihood of exposure together with other information rather than on acute toxicity. This should also be considered for the next revision of Annex II of the directive 91/414/EEC.

- Two activities of SETAC, the HARAP (1998) and CLASSIC (1999) workshops are likely to provide further valuable guidance with regard to higher-tier aquatic testing and refined risk assessment.

- Insect species testing should be required for IGR's (Insect Growth Regulators). For insecticides in general, testing of a standard insect species would be desirable. For herbicides and fungicides, the SCP recommends to carefully analyse all the existing long-term data, in order to allow the development of clearer guidance.

- The SCP strongly supports the inclusion of aquatic plants into the effects assessment.

- The SCP regards micro- and mesocosm studies as very valuable tools in the aquatic risk assessment. They need to be carefully designed and interpreted.

- The SCP shares the concerns with regard to the lack of knowledge on fate and toxicity of formulated products. Considering the remaining uncertainties with predicting long-term effects from acute toxicity studies, the SCP considers it more appropriate if instead of the acute tests the long-term tests were undertaken.

6. Acknowledgements

The Committee wishes to acknowledge the contribution of the SCP working group that prepared the initial draft opinion. The membership of the group was as follows:

Professor A. Hardy (Chairperson), and Committee Members Dr. H. G. Nolting and Prof. A. Silva Fernandes and invited experts Drs V. Forbes, J. Boesten, A. Carter and T. Sherratt and Mr H. Koep.

7. References:

Laskowski, R. (1995) Some good reasons to ban the use of NOEC, LOEC and related concepts in ecotoxicology. *Oikos* **73**, 140-144.

Kenaga, E.E. (1979) Aquatic test organisms and methods useful for assessment of chronic toxicity of chemicals. In: K.L. Dickson, A.W. Maki and J. Cairns, Jr., Analyzing the Hazard Evaluation Process. American Fisheries Society, pp. 101-111.

Kenaga, E.E. (1982) Predictability of chronic toxicity from acute toxicity of chemicals in fish and aquatic invertebrates. Environ. Toxicol. Chem. 1: 347-358.

OECD (1998) Report of the OECD Workshop on Statistical Analysis of Aquatic Toxicity Data. OECD Environmental Health and Safety Publications, Series on Testing and Assessment No 10, Paris.

Slooff, W., Canton, J.H., Hermens, J.L.M. (1983) Comparison of the susceptibility of 22 freshwater species to 15 chemical compounds. I.

(sub)acute toxicity tests. Aquat. Toxicol. 4: 113-128.

Slooff, W., van Oers, J.A.M., de Zwart, D. (1986) Margins of uncertainty in ecotoxicological hazard assessment. Environ. Toxicol. Chem. 5: 841-852.

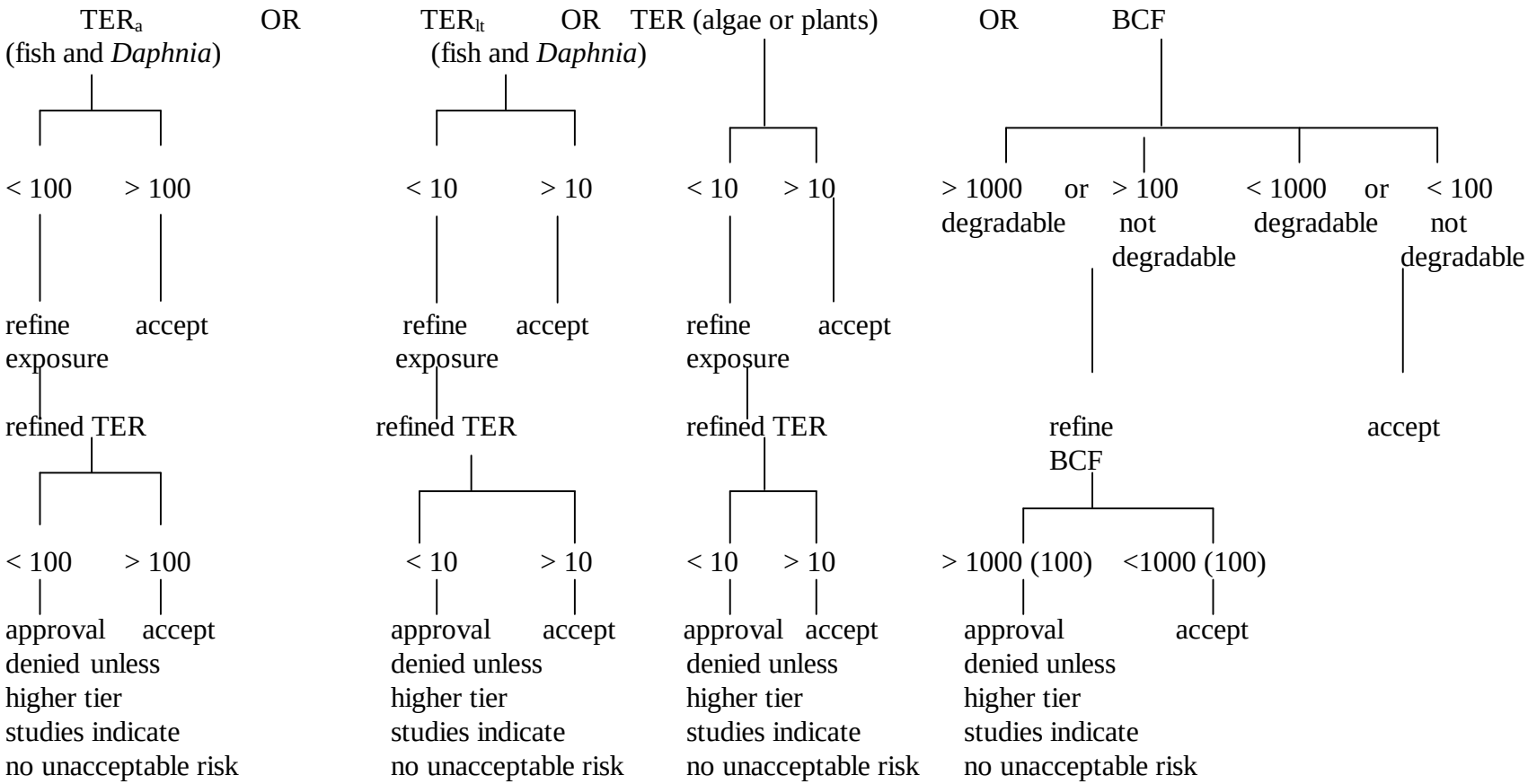
WHO (1978) Principles and Methods for Evaluating the Toxicity of Chemicals, Part I. Environmental Health Criteria 6, World Health Organization, Geneva.

8. Abbreviations

CLASSIC	SETAC-Europe/OECD/EC/BBA/UBA-Workshop ***C**ommunity **L**evel **A**quatic **S**ystem **S**tudies **I**nterpretation **C**riteria*, Schmallenberg, Germany, 29 May –2 June 1999
ECOFRAM	US-EPA *Ecological Committee on FIFRA Risk Assessment Methods*
ECPA	European Crop Protection Association
EDSTAC	US-EPA *Endocrine Disruptors Testing and Assessment ...*
EDTA	OECD Working Group *Endocrine Disruptor Testing and Assessment*
ET&C	Environmental Toxicology and Chemistry (the scientific journal published by SETAC)
FOCUS	Forum for the Coordination of Pesticide Fate Models and their Use
HARAP	SETAC-Europe/OECD/EC-Workshop ***H**igher-tier **A**quatic **R**isk **A**ssessment for **P**esticides*, Lacanau Ocean, France, 19-22 April 1998
OECD	Organisation for Economic Co-operation and Development, Paris
SETAC	Society of Environmental Toxicology and Chemistry
WHO	World Health Organisation, Geneva

Annex 1: Example for flowchart

From Uniform Principles (Annex VI, section 2.5.2.2)



Annex 2: Example for overview table

Aquatic Ecotoxicology Guidance Summary

For Active Substances: (from Annex II, section 8, Directive 91/414/EC)

Test	When Required (Directive text)	Trigger (GD text)	Protocol Required[1] or Recommended
Rainbow Trout Acute Toxicity	Always	----------	67/548/EEC Method C1
Warm-Water Fish Acute Toxicity	Always	----------	67/548/EEC Method C1
Daphnia Acute Toxicity	Always	----------	92/69/EEC Method C2
Algal Growth	Always	----------	92/69/EEC Method C3
Algal Growth (2nd Species)	For Herbicides	----------	92/69/EEC Method C3
Aquatic Plant Toxicity	For Herbicides	----------	
Sediment-Dweller Acute Toxicity (expert judgment to decide btw. acute or chronic)	If partitioning to and persistence in sediment	See chronic	Spiked water or spiked sediment test with *Chironomus*
Insect Acute Toxicity	If Applied to Surface Water	----------	
Crustacean (not Daphnia) Acute Toxicity	If Applied to Surface Water	----------	
Gastropod Acute Toxicity	If Applied to Surface Water	----------	
Chronic fish tests in general	If Continued or Repeated Exposure and if no mesocosm/microcosm study available	If DT50 in water from sediment-water study > 2 d; for multiple applications if lack of prolonged/chronic exposure cannot be demonstrated - the meso/ microcosm study would have to include chronic fish endpoints	
Juvenile Fish Chronic Toxicity			OECD 204 but with 28 d exposure & survival, growth, & behaviour endpts.
Fish Early Life-Stage Test	Acute LC50 < 0.1 mg/L **or** 100 <BCF <1000 with elimination < 95% after 14 d		OECD Method 210
Fish Life Cycle Test	BCF >1000, elimination < 95% after 14d **or** DT90 > 100 d	Acute LC50 < 0.1 mg/L or if special concern e.g. endocrine disrupter	*
Fish Bioaccumulation	If likely to partition to fatty tissue (e.g. LogPow $\geq$ 3) unless no exposure	If LogPow $\geq$ 3 but not if DT90 in sediment-water study <10 d and no long-term exposure from multiple applications	OECD 305E

Daphnia Chronic Toxicity	If Continued or Repeated Exposure	If DT50 in water from sediment-water study > 2 d; for multiple applications if lack of prolonged/chronic exposure cannot be demonstrated	OECD 202, Part II
Insect Chronic Toxicity	If Continued or Repeated Exposure	Notifier to justify why this is not warranted *	
Gastropod Chronic Toxicity	If Continued or Repeated Exposure	Notifier to justify why this is not warranted *	
Sediment-Dweller Chronic Toxicity	If partitioning to and persistence in	If 10% of AR in sed. after 14 d and NOEC for daphnia < 0.1 mg/l *	
Effects on Sewage Treatment	If Exposure in Sewage Treatment		

[1]In the GD it is mentioned that all internationally recognized guidelines should be accepted

*But see opinion of SCP on GD

For Metabolites: (from Annex II, section 8, Directive 91/414/EC)

Test	When Required (Directive text)	Trigger (Guidance Document (GD) text)	Protocol Required or Recommended
Ecotox,. studies in general	Where metabolites can constitute a relevant risk, and where effects cannot be evaluated by the available results for a.s.		
Acute toxicity to fish, Daphnia, algae		If metabolite > 10% of applied radioactivity in water phase or other reasons for concern; if acute L(E)C50 of fish, Daphnia or algae < 100 x other groups only most sensitive group tested.	Same as for active substance
Acute tests on additional species from one or more taxonomic groups		If metabolite more toxic than a.s. in the above test then all groups	
Bioaccumulation	as for a.s.		
Chronic Tests on most sensitive taxonomic group		If metabolite is persistent and if metabolite is more acutely toxic than active substance; or if active substance is unstable and metabolite is persistent. If DT50 in water from sediment-water study > 2 d; for multiple applications if lack of prolonged/chronic exposure cannot be demonstrated.	
Sediment-Dweller Toxicity		If metabolite partitions to sediment Metabolite > 10% AR in sediment phase after 14 days* or other reasons for concern	

*But see opinion of SCP on GD

For Formulations: (from Annex III, section 10, Directive 91/414/EC)

Test	When Required	Trigger	Protocol Required or Recommended
Acute Toxicity to Fish, Daphnia, and algae	Always for at least one of the species; when toxicity cannot be predicted from a.s. or when applied to surface water all 3 must be tested*	All 3 species must be tested if $\geq$2 a.s. or formulants or if Lowest L(E)C50 $\leq$ 100 x next lowest	Same as for a.s.
Microcosm or Mesocosm Study	If initial risk assessment unacceptable	If TERa $\leq$100 or TERlt $\leq$ 10 expert judgement required	
Fish Residue Data	If bioconcentration expected	Expert judgement based on bioconcentration test for a.s.	
Fish Chronic Toxicity	If not possible to extrapolate from a.s.		
Daphnia Chronic Toxicity	If not possible to extrapolate from a.s.		

*But see opinion of SCP on GD