

MANUAL FOR INVESTIGATION OF HPV CHEMICALS

CHAPTER 4: INITIAL ASSESSMENT OF DATA

4.2. Guidance for the Initial Assessment of Aquatic Effects¹

4.2.1 Introduction

1. This section provides guidance for the initial assessment of aquatic effects of High Production Volume (HPV) chemicals with a full SIDS. It is based on the Guidance Document on Aquatic Effects Assessment (OECD 1996), which had been developed reflecting the results of three OECD Workshops (OECD 1992a, 1992b and 1992c). These reference documents may be referred to whenever detailed information relating to the assessment procedure presented in this document is required. In particular, examples of effects assessments in OECD (1996) are useful for understanding the procedure and better reporting. The Technical Guidance Document of the European Commission (1996 and 2002) also provides detailed guidance on environmental effects assessment.

NOTE: This section exclusively deals with the assessment of data. For testing requirements within the OECD HPV Chemicals Programme, chapter 2 of this manual should be consulted. It is assumed that for the initial assessment of aquatic effects, the SIDS requirements as described in chapter 2 are fulfilled. Any further existing data on aquatic effects should also be used for the assessment.

2. This section focuses on the initial aquatic effects assessment. In such an assessment, the impact of the chemical is generally assessed against only one or two representative species from each of three trophic levels by means of short-term toxicity tests; i.e. using primary producers (algae), primary consumers (*Daphnia*) and secondary consumers (fish). A more refined assessment uses chronic or sub-chronic test data, as well as data on a larger number of aquatic species or data on terrestrial organisms. At the next stage of comprehensive effects assessment, (semi-) field studies provide the basis for assessments.

3. Therefore, the hazard parameters in SIDS that are relevant to this guidance are the following:

- acute toxicity to fish;
- acute and chronic toxicity to *Daphnia*; and
- toxicity to algae.

Other ecotoxicity information such as toxicity to microorganisms, earthworms, terrestrial plants, birds and benthic organisms is also relevant in environmental effects assessment, but this guidance does not address these information items in detail.

4. The following parameters, which are used in aquatic hazard classification, are also important in initial aquatic effects assessment:

- partition coefficient ($\log K_{ow}$);
- biodegradation; and
- bioaccumulation.

5. In aquatic effects assessments, the "low risk" concentration where no unacceptable adverse effects on the ecosystem are expected (i.e. Predicted No Effect Concentration, PNEC²) is calculated, and it is

¹ This document was prepared by the OECD Secretariat based on the agreements reached in the OECD Existing Chemicals Programme up to October 2002.

compared with the concentrations that are present in the environment, either measured or calculated (i.e. Predicted Environmental Concentration, PEC). When the PEC exceeds the PNEC, further assessment or risk management action needs to be considered. However, in the refocused HPV programme, where priority is given to hazard assessment, quantitative exposure information (i.e. estimation of PEC) is not a part of the process any more. Under the refocused HPV Chemicals Programme, it is also not required to propose PNEC values (see section 5.2.2). Deriving a PNEC value might nevertheless be useful for the interpretation of the available toxicity data.

6. As the aquatic effects assessment proceeds from the initial stage to refined and comprehensive stages, estimation on PNEC becomes more precise with more detailed information made available.

7. This guidance is directly applicable to soluble compounds. Regarding poorly soluble compounds and other chemicals difficult to test, OECD (2000) provides guidance for testing the aquatic toxicity. Also, OECD (2001) provides guidance on hazard classification of such chemicals.

4.2.2 Evaluation of data used for the assessment

8. Before conducting an effects assessment, data should be evaluated for their adequacy. Specific considerations for data evaluation, described in OECD (1995), are summarised below.

Octanol-water Partition Coefficient

9. The octanol-water partition coefficient (K_{OW}) is an important parameter in initial hazard assessment, and therefore should be examined carefully. For example, determination of K_{OW} by the shake flask method is not suitable for highly hydrophobic chemicals ($\log K_{OW} > 5$). For those chemicals, the slow stirring method or generator column method can be used. It should also be noted that $\log K_{OW}$ may not work for surfactants, polymers, inorganics, and organometalics.

Bioaccumulation

10. Bioaccumulation occurs through multiple routes of exposure including uptake of food and sediment/soil, but for most organic substances uptake from water (bioconcentration) is believed to be the predominant route of exposure. Data on bioconcentration can be obtained through a QSAR equation by using K_{OW} as well as by experiment. It should be noted that simple bioconcentration QSARs often cannot predict the bioconcentration factor (BCF) of extremely hydrophobic chemicals under field conditions. If more than one BCF is available for the same species, the geometric mean for the species could be used; however, the test concentration should be taken into account. BCF values are more often available for fish, but results may also be available for other species (blue mussel, oyster, scallop). Reported BCFs for microalgae should be used with caution. Further guidance on the interpretation of bioaccumulation data can be found in OECD (2001).

Aquatic Toxicity

11. The water solubility of the test substance must be measured or predicted and it should be confirmed that effect concentration derived from the test does not significantly exceed the solubility limit. Test results using solvents should be treated with care. For further guidance of difficult substances, see OECD (2000).

12. For the interpretation of the data, the key aspects of the study methods which affect study quality, such as measured or nominal concentration, control response, use of "insensitive" species, and water quality

² In OECD (1995), "maximum tolerable concentration"(MTC) is used instead of PNEC.

values, should be examined. Endpoints which have direct ecological relevance (e.g. survival, growth, reproduction) should be given more weight than other endpoints (e.g. biochemical parameters). Consideration of test species is also important: for example, chronic studies performed with the most sensitive species in the acute tests have highest relevance compared to results with other species.

13. Chronic toxicity tests are preferred for persistent or bioaccumulative chemicals. For some of these chemicals, a 96-hour exposure in acute tests may not be sufficiently long.

14. If multiple data are available for the same species, the following procedure is proposed for using the data.

- If these data are based on the same effect parameter (endpoint) and the same time period, the geometric mean value should be used. The geometric mean is defined as $GMy = (y_1 * y_2 * y_3 * \dots * y_n)^{1/n}$. The geometric mean minimises, compared to the arithmetic mean, the influence of highly deviating values.
- If different effect parameters or different exposure times are used, only the lowest value from the longest test time should be used taking into account the importance of the endpoints and the exposure periods in the various tests.

4.2.3 Calculation of PNEC - assessment factors

15. The assessment factor method is the method most usually used for the derivation of a PNEC within the OECD HPV Chemicals Programme. A PNEC is calculated using toxicity test data such as LC_{50} , EC_{50} , other $L(E)C_x$ values, NOEC (no observed effect concentration) and LOEC (low observed effect concentration). MATC (maximum allowable toxicant concentration, calculated as $MATC = (NOEC \times LOEC)^{1/2}$) is also used in effects assessment.

16. Assessment factors are used to adjust the effect concentration from a limited data set and to estimate a PNEC. Assessment factors should be applied with care to acute data for substances which are suspected of having a specific mode of action, or which have a high $\log K_{OW}$ or which significantly bioaccumulate. Assessment factors should reflect the following uncertainties and extrapolations:

- intra-species and inter-species variations;
- the extrapolation of short term toxicity towards long term toxicity; and
- the extrapolation of laboratory results towards the field.

17. Several assessment factors proposed so far are summarised in appendix 1. In the following paragraphs, assessment factors to be used in estimating PNEC from SIDS data are proposed. These are summarised in Table 1.

18. When only acute toxicity data in the SIDS are available, an assessment factor of between 100 and 1000 is applied to the lowest $L(E)C_{50}$ [i.e. case (a)]. A factor of 1000 is a conservative and protective factor and applied when only limited data are available, i.e. this value may be reduced to 100 if evidence is available to suggest that this may be a more appropriate factor. Such evidence would include:

- (1) availability of data from a wide variety of species including those which are considered to represent the most sensitive species;
- (2) information, from structurally similar compounds or QSAR, to suggest that the acute to chronic ratio is likely to be low;

- (3) information to suggest that the chemical acts in a non-specific or narcotic manner, with little inter-species variation in toxicity; and
- (4) information to suggest that the release of the chemical is short-term or intermittent, and that the chemical would not be persistent in the environment.
19. When chronic toxicity data are available in addition to acute data, an assessment factor of between 10 and 100 is applied to the lowest NOEC [i.e. case (b)], taking the following situation into account:
- (1) If chronic NOEC is available from one or two species representing one or two trophic levels (i.e. fish, *Daphnia* or algae), a factor of 100 or 50 is applied to the lowest NOEC. In this case, a PNEC value derived from chronic data should be compared to that derived from the lowest acute data. It is then the lowest value that is used in the assessment.
- (2) If chronic NOECs are available from three species representing three trophic levels (i.e. fish, *Daphnia* and algae), a factor of 10 is applied to the lowest NOEC. If there is convincing evidence that the most sensitive species has been tested, a factor of 10 may also be applied to the lowest NOEC from two species representing two trophic levels (i.e. fish and/or *Daphnia* and/or algae).
20. Use of different assessment factors should be clearly justified in the assessment report.

Table 1. Summary of Proposed Assessment Factors for Estimating an PNEC

Case	Data available	Range of Assessment factor
(a)	EC ₅₀ algae (72hr) EC ₅₀ <i>Daphnia</i> (24-48hr acute test) LC ₅₀ fish (96hr)	100 - 1000
(b)	NOEC <i>Daphnia</i> (14-21d chronic toxicity test) NOEC algae (72hr) NOEC fish (chronic toxicity test)	10 - 100

[Note]

- In case (a), all three acute data should be included in the SIDS.
- In case (b), NOEC_{algae} is a SIDS element and NOEC_{*Daphnia*} or NOEC_{fish} may also be included in the SIDS for certain chemicals.

4.2.4 Use of QSAR approach

21. In some cases, a SIDS element regarding aquatic toxicity can be filled with (Q)SAR estimations (see chapter 2, section 2.3.1). For further guidance on the use of (Q)SARs, see also chapter 3, section 3.3.
22. QSAR results may also be used for determining the assessment factors for estimating PNEC (see paragraph 18). QSARs can also be used to confirm the validity of test data or to decide which further data are necessary. Further guidance for the use of QSARs for aquatic toxicity is given in appendix 2.
23. When QSARs are used, the approach and its reliability should clearly be described in the assessment report.

4.2.5 Assessment with data beyond SIDS

Statistical Extrapolation Methods

24. If a large data set from long-term tests for different taxonomic groups is available (OECD, 1992), statistical extrapolation methods may be used to derive a PNEC. The main underlying assumptions of the statistical extrapolation methods are as follows (OECD, 1992):

- The distribution of species sensitivities follows a theoretical distribution function;
- The group of species tested in the laboratory is a random sample of this distribution.

25. The effects assessment can be performed with a statistical extrapolation method if the database on Species Sensitivity Distributions (SSDs) is sufficient for its application (Posthuma et al., 2002).

26. In general, long-term toxicity data are log-transformed and fitted according to the distribution function and a prescribed percentile of that distribution is used as a criterion. Several distribution functions have been proposed. The EPA (1985) assumes a log-triangular function, Kooijman (1987) and Van Straalen and Denneman (1989) a log-logistic function, and Wagner and Løkke (1991) a log-normal function. Aldenberg and Slob (1993) refined the way to estimate the uncertainty of the 95th percentile by introducing confidence levels, which was again more refined by Aldenberg and Jaworska (2000).

27. The approach of statistical extrapolation is still under debate and needs further validation. An advantage of these methods is that they use the whole sensitivity distribution of species in an ecosystem to derive a PNEC instead of taking always the lowest long-term NOEC. However, such methods could also be criticised. Among the major drawbacks, the reasons put forward are: the lack of transparency by using this method compared to the standard approach, the question of the representativity of the selected test species, the comparability of endpoints, the arbitrary choice of a specific percentile and a statistical confidence level, etc.

28. When using a statistical extrapolation method to derive a PNEC, the following issues need to be addressed:

- Clarification of the type of input data, i.e. preferably reliable NOECs from chronic/long-term studies, full life-cycle or multigeneration studies;
- Information on the mode of action of the substance that may help to identify and to evaluate the need to include possible sensitive taxonomic groups or to exclude possible overrepresentation of certain taxonomic groups;
- The minimum species requirements, e.g. representative species from the following taxonomic groups: fish, crustaceans, insects, algae, higher plants, other groups not already represented. It is recognised that for some taxa mentioned above, no internationally standardised test guidelines for long-term tests are currently available. The requirement can be adapted based on knowledge/reasoning about sensitive endpoints and species as well as knowledge on structure – activity and mode of action.
- The minimum sample size (number of data). This issue is subject of an ongoing debate. While OECD (1992) proposes a minimum of 8 NOECs on species from different taxonomic groups, EC (2002) recommends 10 NOECs (and preferably more than 15) on species from 8 taxonomic groups. Similar proposals have been made by Gibbons and Coleman (2001) or de Bruijn et al. (1999).
- How multiple data for one species are dealt with, e.g. averaging comparable data, or selecting the most sensitive endpoint when various data are available;

- Statistical fitting procedures, i.e. the method must be mentioned and explained, where the log-normal distribution is the preferred one for pragmatic reasons. In addition, a statistical method is to be used to test the goodness of fit. In addition to the Kolmogorov-Smirnov test, the Anderson–Darling goodness of fit test can be used as a criterion for the choice of a parametric distribution for data-rich data sets, because it gives more weight to the tails of the distribution. Results should be discussed in regards to the graphical representation of the species distribution. If the data do not fit any distribution, the left tail of the distribution (the lowest effect concentrations) should be analysed more carefully. Any choice of a specific distribution function should be clearly explained;
- Estimated parameter, i.e. the concentration corresponding with the point in the species sensitivity distribution (SSD) profile below which 5% of the species occur may be derived with a 50% confidence interval associated with this concentration, as an intermediate value in the determination of the PNEC;
- Estimation of the PNEC, i.e. the intermediate value may be divided by an appropriate assessment factor, if needed, to reflect the further uncertainties identified. If mesocosm studies are available, they should also be evaluated to decide on the assessment factor;
- Deviations from these recommendations can be made on a case by case basis, through consideration of sensitive endpoints, sensitive species, mode of toxic action and/or knowledge from structure activity considerations;

29. The PNEC should also be derived by applying the assessment factor approach (see section 4.2.3) on the same database.

(Semi-) Field Test

30. Results from (semi-) field studies, (including short-term multi-species trials and long-term mesocosm trials), will not be available for many HPV chemicals. Where they are available and are considered appropriate, they provide the basis for a comprehensive effects assessment in combination with chronic toxicity data. The assessment factor to be used will need to be reviewed on a case-by-case basis. Criteria for judging the applicability of these results for estimation of a PNEC in the comprehensive assessment are recommended in OECD (1995).

Consideration of Indirect Effects Assessment and Assessment on Benthic Organisms

31. In addition to the effects assessments using pelagic aquatic organisms, assessments of indirect effects on birds and mammals through the ingestion of aquatic organisms and effects on benthic organisms (OECD 1992c) could be done if information on the chemical suggests possible hazard. However, these are thought to be beyond the scope of the initial assessment of HPV chemicals with SIDS. Some methods mentioned in OECD (1995), USEPA (1984) and European Commission (1996, 2002), namely an approach using BCF for indirect effects and the equilibrium partitioning method for benthic organisms, could be considered. However, assessments carried out using only data available in SIDS may be very uncertain.

4.2.6 Reporting and identification of further work

32. In SIDS initial assessment reports, the assessment approaches, test data, exposure estimates and assumptions for estimation should be clearly stated.

33. If the conclusions of the initial assessment of a chemical suggest a concern with regard to aquatic effects, a more precise assessment by elaborating exposure assessment, or by further testing, could be considered and proposed. For example, in cases where an estimated PNEC was derived from the results of acute toxicity tests and assessment factors, performing chronic tests with appropriate species (e.g. most

sensitive species in acute tests) would be considered as one of the possible further activities. Also if there is a possibility of indirect effects on birds and mammals or a possible hazard to benthic organisms, assessments on these could be considered and proposed for the next phase.

Appendix 1: assessment factors proposed in literature

34. Several sets of assessment factors have been proposed to date. At an OECD workshop, (OECD 1992b), a factor of 10 is suggested for each extrapolation step described in paragraph 16. This approach is a modification of a method proposed in USEPA (1984).

35. Assessment factors proposed in the EU technical Guidance Document (European Commission 1996; 2002) depend on the properties of the chemical and the conditions of testing (such as use of the most sensitive species). In Heger et al. (1995), a factor of 100 between the E(L)C₅₀ of acute toxicity and NOEC of chronic toxicity has been shown by measured data to be generally justifiable.

36. The proposals from the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC 1993) are based on comparisons of toxicity data. An acute: chronic ratio of 40, a chronic: ecosystem ratio of 5, and an ecosystem : field ratio of 1 are suggested.

37. Table 2 summarises these proposals. These factors can be modified under certain conditions (e.g. an assessment factor of 1000 in the EU Technical Guidance Document can be lowered to 100 with certain evidence). The original reference should be referred to for detailed explanation of such modifications.

Table 2. Proposed Assessment Factors for Application to Aquatic Toxicity Data for Estimating a PNEC

Available information applied	Assessment factor applied to the lowest value (modifications not included)		
	(a) OECD Workshop	(b) EU Technical Guidance Document	(c) ECETOC proposal
One acute L(E)C ₅₀ for acute toxicity from one trophic level	1000	-	-
At least one acute ³ L(E)C ₅₀ from each of three trophic levels of the base-set (fish, <i>Daphnia</i> and algae)	100	1000	200
One chronic NOEC (either fish or <i>Daphnia</i>)	-	100	-
Two chronic NOECs from species representing two trophic levels (fish and/or <i>Daphnia</i> and/or algae)	-	50	5
Chronic NOECs from at least three species (normally fish, <i>Daphnia</i> and algae) representing three trophic levels	10	10	
Field data or model ecosystems	-	case-by-case	1

³ In the EU Technical Guidance Document, "short-term toxicity" and "long-term toxicity" are used instead of "acute toxicity" and "chronic toxicity".

Appendix 2: Applicability of QSAR for aquatic toxicity

38. QSARs based on chemical classes are used widely (for example see USEPA (1988)). OECD (1994) compared the QSAR models used in USEPA and the test data in EC, and demonstrated good agreement between predicted and measured toxicity for *Daphnia* and fish. Proper selection and use of a model for a given chemical can be carried out on a case-by-case basis by using computerised systems such as ECOSAR (USEPA 2000).

39. QSARs can also be applied to chemicals with a common mode of toxic action, such as narcosis where the mechanism is dependent on a chemical's hydrophobicity (e.g. $\log K_{OW}$). The OECD Utrecht Workshop (OECD 1992a) concluded that adequate QSAR predictions of aquatic toxicity could only be made for chemicals classified under Class I (inert chemicals, baseline toxicity) or Class II (less inert chemicals), shown in Table 2. For a Class I chemical, QSARs may be used to estimate the toxicity for fish, *Daphnia* and algae. For a Class II chemical, estimation by QSAR can be done for acute toxicity to fish. It should be noted that QSARs are valid only for liquids at room temperature and for solids on which data on water solubility are available.

Table 2. Categorisation of Chemicals for QSARs for Approach by Common Mode of Action

Class	Structure	Available QSARs	Reliability
Class I	aliphatic alcohols, aliphatic ketones, aliphatic ethers, alkoxyethers, aliphatic halogenated hydrocarbons, saturated alkanes and halogenated benzenes (only C,H,N,O,F,Cl,Br could be included)	acute and chronic tox. to fish and to <i>Daphnia magna</i> , chronic tox. to algae (for only non-polar narcotics)	concentration can be predicted
Class II	non-or weakly acidic phenols, aromatic amines and anilines, aliphatic primary amines, weakly basic pyridines	acute tox. to fish (phenol and primary aromatic amines)	a range can be predicted

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