Capacity building on compliance with chemicals legislation, with emphasis on REACH/CLP linked to Industrial Emission Directive – Technical aspects
ECRAN - 60146

Environmental risk assessment and ecotoxicological endpoints

Martin Murín, MSc.
Ekotoxikologické centrum Bratislava s.r.o.
Tomášikova 10/F
821 03 Bratislava
Tel/Fax.: +421 45943712 / 45945223
E-mail: ekotox@ekotox.sk
www.ekotox.eu

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RISK ASSESSMENT - roles

- **Legislative background**
  - Industrial Chemicals
  - Pesticides
  - Biocides
  - Pharmaceuticals
  - …contaminated areas (“specific risk assessment”)

- **Assessment of a Priority Chemicals – SVHC**
- **Hazard vs Risk**
- **RISK ASSESSMENT – RISK MANAGEMENT**

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Summary Table of Available Tools for Risk Assessment

Categories		Links to Available Materials	Explanation
Hazard Assessment	**Gathering existing information**	OECD Existing Chemicals database	OECD-wide agreed hazard assessments elaborated in the OECD Co-operative Chemicals Assessment Programme
		eChemPortal	Global Portal to Information on Chemical Substances
		Manual for the Assessment of Chemicals (Chapter 2)	A set of guidance documents for (initial) risk assessment developed for the OECD Co-operative Chemicals Assessment Programme. See chapter 2 for gathering data
	Evaluating existing information	Manual for the Assessment of Chemicals (Chapter 3)	See chapter 3.1 for determining the quality of existing data
	Generating new data	Test guidelines	Test methods for assessing (hazard) properties of chemicals
		The OECD (Q)SAR Project	Guidance and tools for filling data gaps by non-testing methods.
	Assessing the hazards	Manual for the Assessment of Chemicals (Chapter4) & (Chapter 5)	Chapter 4 provides guidance assessing the hazards of chemical substances to man and the environment Chapter 5 provides guidance on elaborating a hazard assessment report.
		Series on Testing and Assessment	Guidance documents and reports related to assessment of several inherent effects

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What is risk assessment?

EPA uses risk assessment to characterize the nature and magnitude of health risks to humans (e.g., residents, workers, recreational visitors) and ecological receptors (e.g., birds, fish, wildlife) from chemical contaminants and other stressors, that may be present in the environment. Risk managers use this information to help them decide how to protect humans and the environment from stressors or contaminants. Note that "risk managers" can be:

- federal or state officials whose job it is to protect the environment,
- business leaders who work at companies that can impact the environment, or
- private citizens who are making decisions regarding risk.

At EPA, environmental risk assessments typically fall into one of two areas:

- Human Health
- Ecological

Risk assessment is, to the highest extent possible, a scientific process. In general terms, risk depends on the following factors:

- How much of a chemical is present in an environmental medium (e.g., soil, water, air),
- How much contact (exposure) a person or ecological receptor has with the contaminated environmental medium, and
- The inherent toxicity of the chemical.

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Risk Assessment

European Union

GENERIC RISK ASSESSMENT

- **Human Health RA**
  - scenarios for workers and consumers
- **Environmental RA**
  - exposure through environ. compartments
  - ecosystems & human beings

USA

SITE SPECIFIC RISK ASSESS.

- **Human Health RA**
  - all exposure routes incl. environ. compartments
- **Ecological RA**
  - ecosystems, endangered species
  - all stressors

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Risk Assessment

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- **Effect Assessment**

Data: toxicological and ecotoxicological data incl. environmental fate
ADI/TDI (UN); RfD (US EPA); DNEL (EU) / PNEC

- **Exposure Assessment**

Data: measured concentrations, monitoring, models
Exposure levels / PEC

- **Risk Characterisation**

Data: toxicity / ecotoxicity, emissions into environment and exposure based on standardised conditions

GOAL: control and management of chemicals

Generic Risk Assessment

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EU legislation - Risk Assessment

- **Guidance Document in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commision Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances**

European Commision, CR-48-96-001-EN-C

REACH Regulation – Guidance documents

http://echa.europa.eu/guidance-documents/guidance-on-reach

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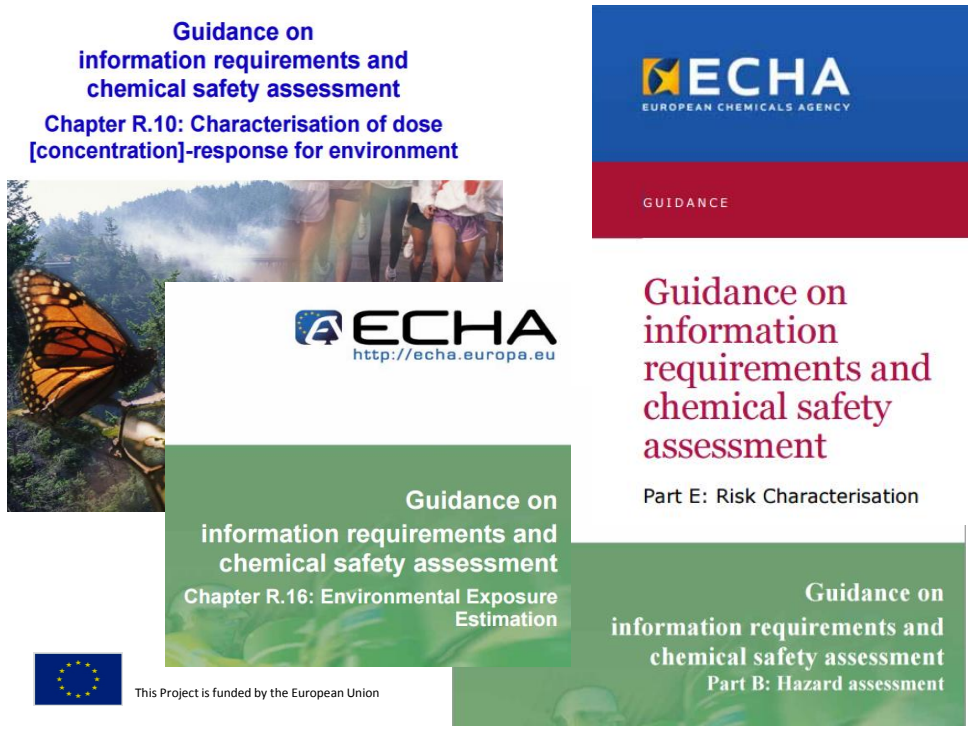
* **RA for Human Health**
* **Use of (Q)SARs / models**
* **RA Report Format**
* **Environmental Risk Assessment**
* **Use Categories**
* **Emmision Scenario Documents**



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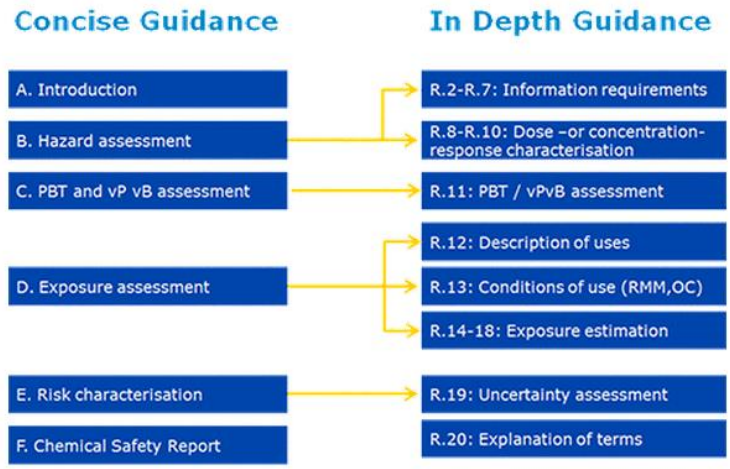
EU Risk Assessment Guidance



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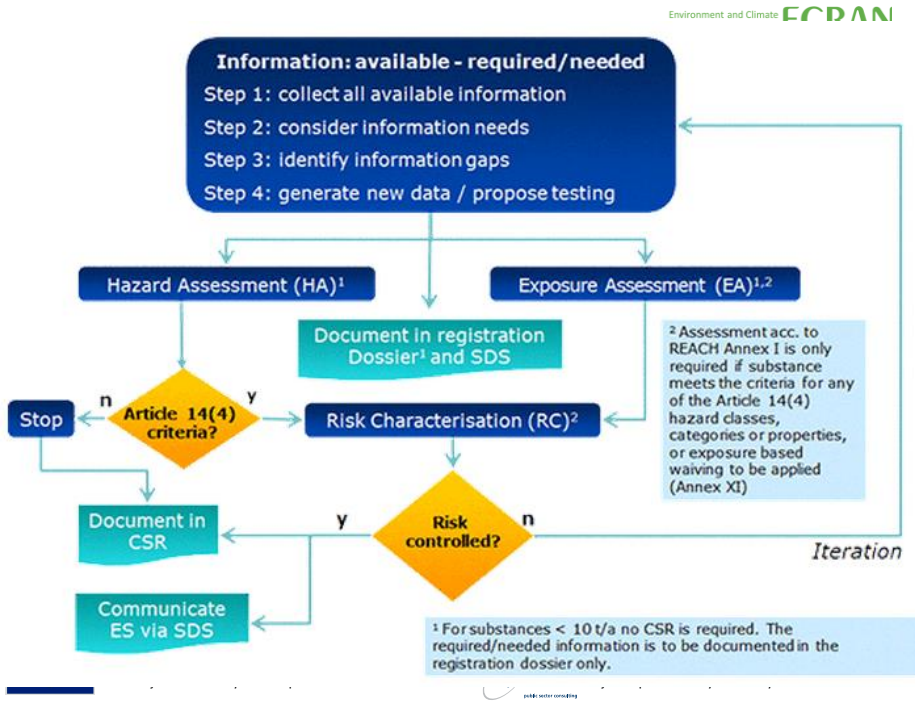

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Effects Assessment

- **Hazard Identification**
  - **classification / other hazard**
- **Dose (concentration) - response (effect) assessment**
  - **Predicted No-Effect Concentration (PNEC)**
    - **ecosystem sensitivity depends on the most sensitive species**
    - **protecting ecosystem structure protects community function**

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Table R.10-1 Overview of toxicity test endpoints and guidance on derivation of L(E)C50 and NOEC values

Short-term studies:

If a test report does not indicate the L(E)C50 values but the raw data are presented, the L(E)C50 should be calculated, for example by regression analysis. If only one toxicity value lies between the L(E)C0 and the L(E)C100, the L(E)C50 cannot be calculated e.g. by Probit analysis. Instead, the L(E)C50 may be estimated by, e.g., linear regression.

If results are presented as >L(E)C10 and <L(E)C50, they can be rated as L(E)C50 while results clearly above a L(E)C50 can only be used as an indication of the short-term toxicity of the chemical considered.

Table R.10-1 Overview of toxicity test endpoints and guidance on derivation of L(E)C50 and NOEC values

Long-term studies:

An EC10 for a long-term test which is obtained using an appropriate statistical method (usually regression analysis) will be used preferentially. The **NOEC** (no observed effect concentration) is defined as "the highest concentration tested at which the substance is observed to have no statistically significant effect ($p<0.05$) when compared with the control, within a stated exposure period" (OECD 211, 1998b) or the test concentration immediately below the LOEC, which then compared with the control has no statistically significant effect ($p<0.05$) within a stated period (OECD 211, 1998b). There has to be a concentration-effect relationship.

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Assessment Factors to derive a PNEC (simplified training set)

Data availability	Assessment factor
At least one short-term L(E)C_{50} from each of three trophic levels of the base-set (fish, Daphnia and	1000
One long-term NOEC (either fish or Daphnia)	100
Two long-term NOECs from species representing two trophic levels (fish and/or Daphnia and/or algae)	50
Long-term NOECs from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10
Field data or model ecosystems	Reviewed on a case by case basis

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Table R.10-2. Relationship between different targets of the risk characterisation for different inland compartments

Target	Medium of exposure (PEClocal / PECregional)	Section	PNEC	Section
Aquatic organisms	Surface water	R.16.5.6.2. R.16.5.6.8	$PNEC_{water}$	R.10.3
Benthic organisms	Sediment	R.16.5.6.3 R.16.5.6.8	$PNEC_{sed}$	R.10.5
Terrestrial Organisms	Agricultural soil	R.16.5.6.6 R.16.5.6.8	$PNEC_{soil}$	R.10.6
Fish-eating Predators	Fish	R.16.5.7	PNECoral from $NOAEL_{avian/mammalian}$	R.10.8
Worm-eating Predators	Earthworms	R.16.5.7	PNECoral from $NOAEL_{avian/mammalian}$	R.10.8
Microorganisms	STP aeration tank	R.16.5.5	$PNEC_{microorganisms}$	R.10.4

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Table R.10-3. Relationship between different targets of the risk characterisation for different marine compartments

Target	Medium of exposure (PEClocal / PECregional)	Section	PNEC	Section
Aquatic organisms	Seawater	R.16.5.6.4	$PNEC_{water}$	R.10.3.2.3
Benthic organisms	Marine sediment	R.16.5.6.5	$PNEC_{marine\ sed}$	R.10.5.3
Fish-eating predators	Fish	R.16.5.7	$PNECoral_{predators}$	R.10.8
Top predators	Fish-eaters	R.16.5.7	PNECoral, top predators	R.10.8

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Chemicals bevaviour

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- Physico-chemical parameters that determine which compartment a chemical will go:

- Aboitic compartment
  - **Air: - Henry constant (H), The pressure of a gas above a solution is proportional to the concentration of the gas in the solution**
  - **water: - water solubility (S)**
  - **Soil/sediment:- soil sorption coefficient (Koc)**

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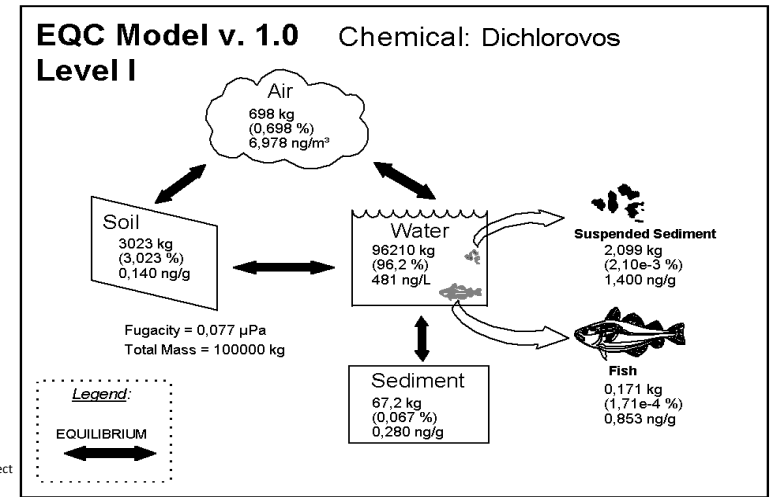
Fate in the environmental compartments - models

- Donald Mackay



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Hazard Assessment Starting-point:

A. physico-chemical inherent properties of a compound

- molecular weight
- distribution coefficient octanol / water (Kow)
- water solubility
- vapor presure
- boiling point

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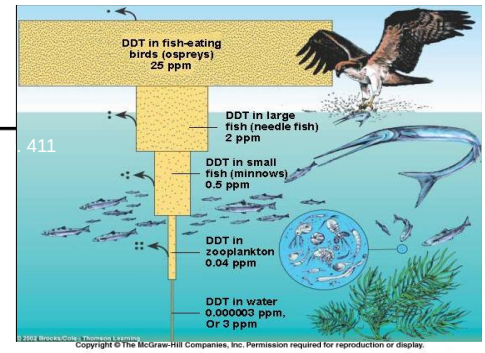
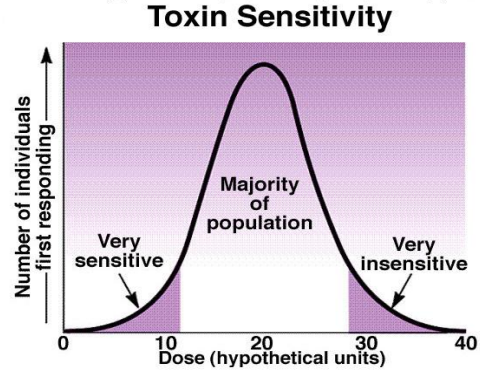
Toxicology

- Toxicity
- Dosage
- Bioaccumulation
- Biomagnification
- Synergism
- Response
- Acute effect
- Chronic effect





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TOXICOLOGY: Assessing Chemical Hazards

Toxicity: measure of how harmful a substance is in causing injury, illness, or death to living organisms.

FACTORS AFFECTING TOXICITY:

1) Dose: the amount of substance ingested, inhaled or absorbed...

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Toxicology and Ecotoxicology are similar but not identical.

Toxicology	***Ecotoxicology***
Absorption	Release into the environment
Distribution	Fate and Disposition
Metabolism	Metabolism
Elimination	No counterpart!

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Tox / Ecotox

Toxicology	***Ecotoxicology***
• Host defense mechanisms	• **Bioaccumulation**
• Individual susceptibility states	• **Bioconcentration (in water)**
• Single effects	• **Biomagnification**
• Cumulative exposure	• **(Never) single effects**
	• **Movement between media (air, water)**

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Acute Toxicity

- **Acute toxicity:** It involves lethal concentrations and short-term exposures
- Acute effects of a toxin appear immediately after exposure.
- The end point is usually death (lethal), hence it is used to derive $LD_{50,}$ LC_{50}
- An LD_{50} / LC_{50} is a dose / concentration of a toxic chemical that kills half of the population.
- LD_{50} is obtained by plotting, for a given dose the proportion of the population that responded to that dose and all lower doses
- Other end-points:

  EC = effective concentration IC = inhibitory concentration

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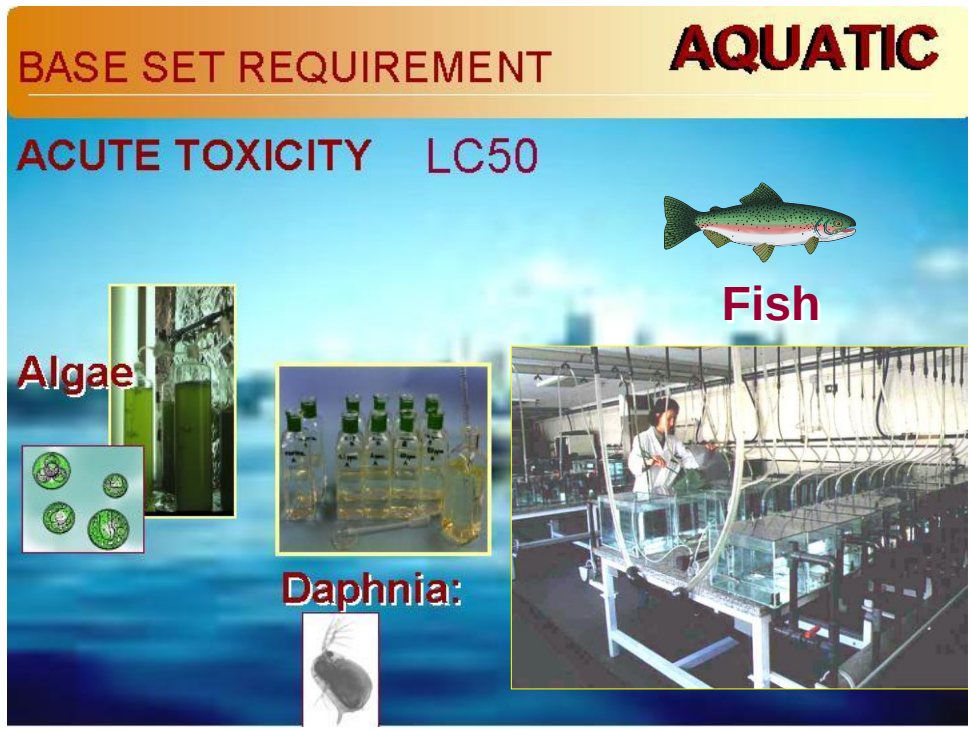
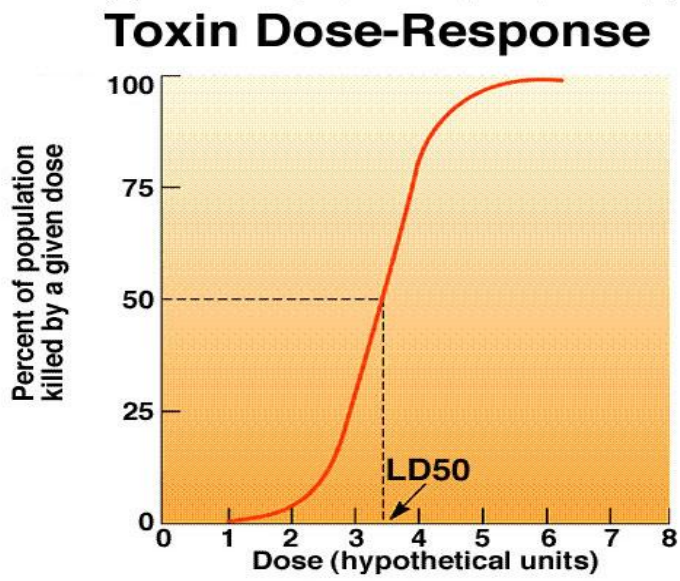


CHEMICALS: Major Types of Toxicity



Dose-Response Curves

- Dose-response
- Nonthreshold
- Threshold

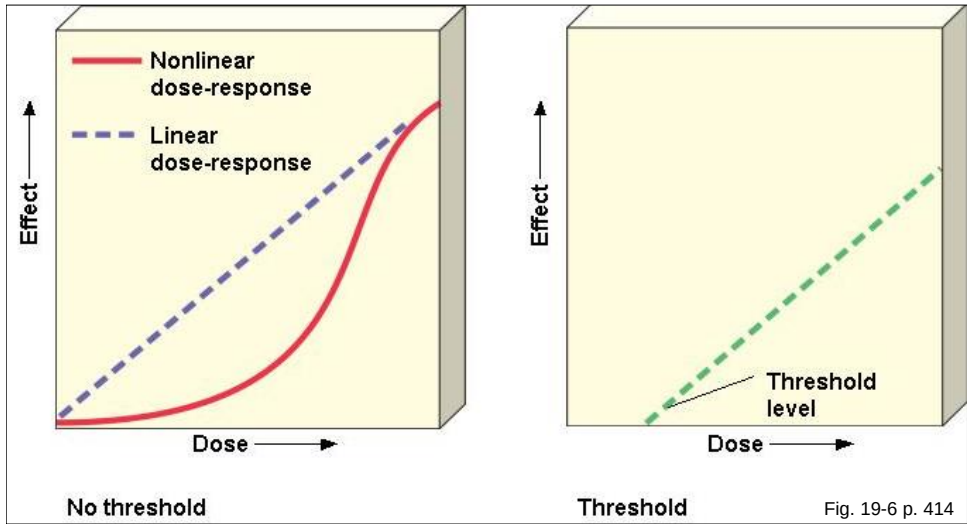
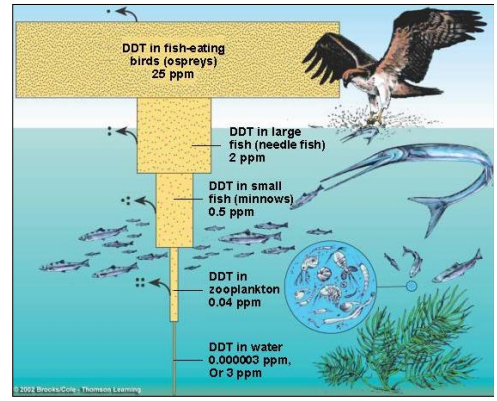


Fig. 19-6 p. 414

Factors Affecting Harm Caused By A Substance

1) Solubility (water soluble move through environment easily)
2) Fat Soluble (can accumulate in body tissue and cells)
3) Persistence (how long before it breaks down)

- Bioaccumulation
- Biomagnifications



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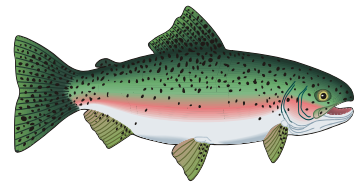
C.1. ACUTE TOXICITY FOR FISH

- LC = lethal concentration

acute lethal toxicity of a substance to fish in fresh water



- acute toxicity is expressed as the median lethal concentration (LC50), that is the concentration in water which kills 50% of a test batch of fish within a continuous period of exposure which must be stated.

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C.1. Information need

- water solubility, vapour pressure, chemical stability, dissociation constants and biodegradability of the substance to help in the selection of the most appropriate test method

  (static, semi-static or flow-through)
- Additional information (for instance structural formula, degree of purity, nature and percentage ofsignificant impurities, presence and amounts of additives, and n-octanol/water partition coefficient) should be taken into consideration in both the planning of the test and interpretation of the results.

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C.1. Test principle

- A limit test may be performed at 100 mg per litre in order to demonstrate that the LC50 is greater than this concentration.
- The fish are exposed to the test substance added to water at a range of concentrations for a period of 96 hours.
- Mortalities are recorded at least at 24-hour intervals, and the concentrations killing 50% of the fish (LCso) at each observation time are calculated where possible.

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C.1. Quality Criteria

- The quality criteria shall apply to the limit test as well as the full test method.
- The mortality in the controls must not exceed 10% (or one fish if less than ten are used) by the end of the test.
- The dissolved oxygen concentration must have been more than 60% of the air-saturation value throughout.
- The concentrations of the test substance shall be maintained to within 80% of the initial concentrations throughout the duration of the test.
- The pH should not vary by more than 1 unit.
- For substances which dissolve easily in the test medium, yielding stable solutions i.e. those which will not to any significant extent volatilize, degrade, hydrolyze or adsorb, the initial concentration can be taken as being equivalent to the nominal concentration. Evidence shall be presented that the concentrations have been maintained throughout the test and that the quality criteria have been satisfied.

C.1. Quality Criteria

- For substances that are:
  - **(i) poorly soluble in the test medium, or**
  - **(ii) capable of forming stable emulsions or dispersions, or**
  - **(iii) not stable in aqueous solutions,**
- the initial concentration shall be taken as the concentration measured in solution (or, if technically not possible, measured in the water column) at the start of the test. The concentration shall be determined after a period of equilibration but before the introduction of the test fish.
- In any of these cases, further measurements must be made during the test to confirm the actual exposure concentrations or that the quality criteria have been met.

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C.1. Test Procedures

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- Static test:
  - Toxicity test in which no flow of test solution occurs. (Solutions remain unchanged throughout the duration of the test.)
- Semi-static test:
  - Test without flow of test solution, but with regular batchwise renewal of test solutions after prolonged periods (e.g. 24 hours).
- Flow-through test:
  - Toxicity test in which the water is renewed constantly in the test chambe rs, the chemical under test being transported with the water used to renew the test medium.

C.1. Chemicals with low solubility

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- Ultrasonic dispersion, organic solvents, emulsifiers or dispersants may be used as an aid to prepare stock solutions of substances with low aqueous solubility or to help to disperse these substances in the test medium.
- When such auxiliary substances are used, all test concentrations should contain the same amount of auxiliary substance, and additional control fish should be exposed to the same concentration of the auxiliary substance as that used in the test series. The concentration of such auxiliaries should be minimized, but in no case should exceed 100 mg per litre in the test medium.


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C.1. Test conditions

- duration: 96 hours
- number of animals: at least 7 per concentration,
- test concentration: At least five concentrations differing by a constant factor not exceeding 2,2 and as far as possible spanning the range of 0 to 100 % mortality,
- light: 12 to 16 hours illumination daily,
- temperature: appropriate to the species (Appendix 2) but within ± 1 °C within any particular test,
- dissolved oxygen concentration: not less than 60 % of the air-saturation value at the selected temperature,
- feeding: none.

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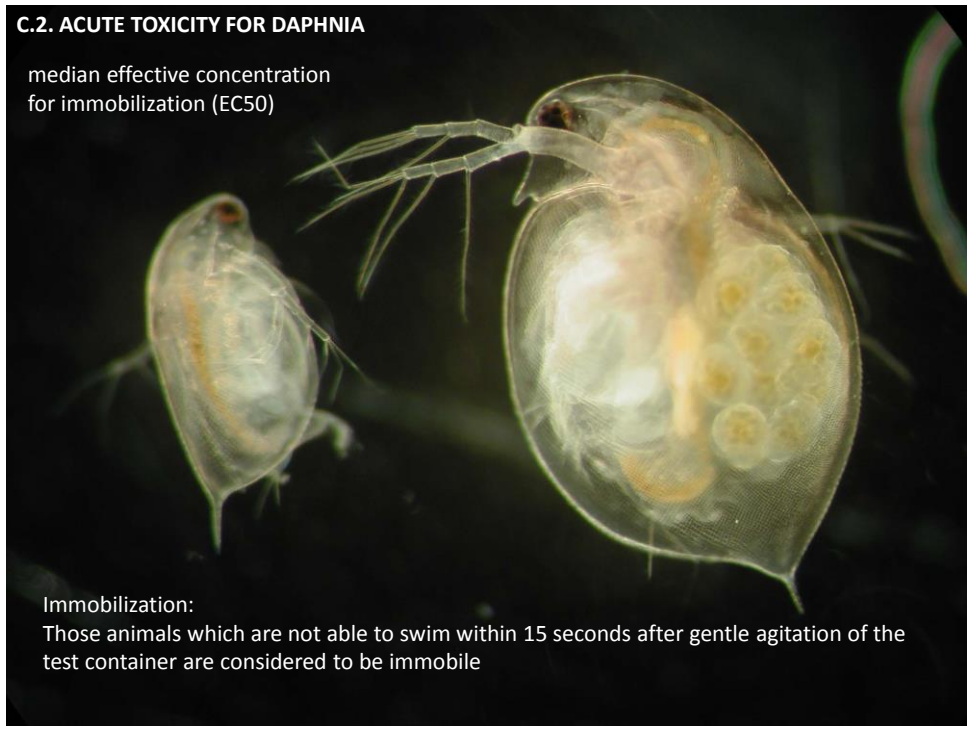
Recommended species	Recommended range of test temperature (°C)	Recommended total length of test animal (cm)
Brachydanio rerio (Teleostei, Cyprinidae) (Hamilton-Buchanan) Zebrab-fish	20 to 24	3,0 ±0,5
Pimephales promelas (Teleostei, Cyprinidae) (Rafinesque) Fathead minnow	20 to 24	5,0 ±2,5
Cyprinus carpio (Teleostei, Cyprinidae) (Linneaus 1758) Common carp	20 to 24	6,0 ±2,0
Oryzias latipes (Teleostei, Poeciliidae) Cyprinodontidae (Tomminck and Schlege 1850) Red killifish	20 to 24	3,0 ±1,0
Poecilia reticulata (Teleostei, Poeciliidae) (Peters 1859) Guppy	20 to 24	3,0 ±1,0
Lepomis macrochirus (Teleostei, Centrarchidae) (Rafinesque Linneaus 1758) Bluegill	20 to 24	5,0 ±2,0
Onchorhynchus mykiss (Teleostei, Salmonidae) (Walbaum 1988) Rainbow trout	12 to 17	6,0 ±2,0
Leuciscus idus (Teleostei, Cyprinidae) (Linneaus 1758) Golden Orfe	20 to 24	6,0 ±2,0



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C.2. Information need

- water solubility, vapour pressure, chemical stability , dissociation constants and biodegradability of the substance before starting the test.
- Additional information (for instance structural formula, degree of purity, nature and percentage ofsignificant impurities, presence and amounts of additives, and n-octanol/water partition coefficient) should be taken into consideration in both the planning of the test and interpretation of the results.

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C.2. Test principle

- A limit test may be performed at 100 mg per litre in order to demonstrate that the EC50 is greater than this concentration.
- The *Daphnia* are exposed to the test substance added to water at a range of concentrations for 48 hours. If a shorter test is used, justification should be given in the test report.
- Under otherwise identical test conditions, and an adequate range of test substance concentrations, different concentrations of a test substance exert different average degrees of effect on the swimming ability of *Daphnia* . Different concentrations result in different percentages of *Daphnia* being no longer capable of swimming at the end of the test. The concentrations causing zero or 100 % immobilization are derived directly from the test observations whereas the 48-hour EC50 is determined by calculation if possible.

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C.2. Quality Criteria

- Immobilization in the controls must not exceed 10% at the end of the test.
- Test *Daphnia* in the control groups must not have been trapped at the surface of the water.
- It is desirable that concentration of dissolved oxygen in the test vessels should remain above 3 mg l-1 throughout the course of the test. However, in no circumstances should the dissolved oxygen concentration fall below 2 mg l-1.
- The concentration of the test substance shall be maintained to within 80% of the initial concentration throughout the duration of the test.
- For substances which dissolve easily in the test medium, yielding stable solutions i.e. those which will not to any significant extent volatilize, degrade, hydrolyze or adsorb, the initial concentration can be taken as being equivalent to the nominal concentration.
- Evidence shall be presented that the concentrations have been maintained throughout the test and that the quality criteria have been satisfied.

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C.2. Test conditions

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- duration: 48 hours
- number of animals: at least 20 animals at each test concentration preferably divided into four batches
- test concentration: the test solution should be prepared immediately before introduction of the *Daphnia*, preferably without using any solvent other than water. The concentrations are made up in a geometric series, at a concentration ratio not exceeding 2.2. Concentrations sufficient to give 0 and 100% immobilization after 48 hours and a range of intermediate degrees of immobilizations permitting calculation of the 48 hour EC50 should be tested together with controls,
- light: a light-dark cycle is optional,
- temperature: temperature: the test temperature should be between 18 and 22 °C, but for each single test it should be constant within ± 1 °C,
- aeration: the test solutions must not be bubble-aerated,
- Volatile compounds should be tested in completely filled closed containers, large enough to prevent lack of oxygen.
- feeding: none.

C.3. Algal Inhibition Test

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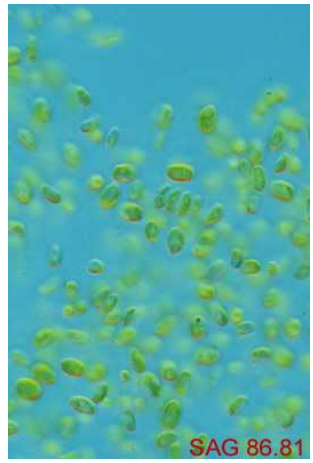

- End-point – inhibition of growth
- effects of a substance on the growth of a unicellular green algal species.

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C.3. Information need

- water solubility, vapour pressure, chemical stability , dissociation constants and biodegradability of the substance before starting the test.
- Additional information (for instance structural formula, degree of purity, nature and percentage ofsignificant impurities, presence and amounts of additives, and n-octanol/water partition coefficient) should be taken into consideration in both the planning of the test and interpretation of the results.

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C.3. Test principle

- A limit test may be performed at 100 mg per litre in order to demonstrate that the EC50 is greater than this concentration.
- Exponentially-growing cultures of selected green algae are exposed to various concentrations of the test substance over several generations under defined conditions.
- The test solutions are incubated for a period of 72 hours, during which the cell density in each solution is measured at least every 24 hours. The inhibition of growth in relation to a control culture is determined.

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C.3. Quality Criteria

- The cell density in the control cultures should have increased by a factor of at least 16 within three days.
- The concentrations of the test substance shall be maintained to within 80 % of the initial concentrations/ throughout a time corresponding to the duration of the test.
- For substances which dissolve easily in the test medium, yielding stable solutions i.e. those which will not to any significant extent volatilize, degrade, hydrolyze or adsorb, the initial concentration can be taken as being equivalent to the nominal concentration. Evidence shall be presented that the concentrations have been maintained throughout the test and that the quality criteria have been satisfied.

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C.3. Test conditions

- duration: 72 hours
- For the test, at least five concentrations are made up in a geometric series at a concentration ratio not exceeding 2,2. The lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50 % relative to the control and, preferably, stop growth completely.
- Test cultures containing the desired concentrations of test substance and the desired quantity of algal inoculum are prepared by adding aliquots of stock solutions of the test substance to suitable amounts of algal pre-cultures (see Appendix 1).
- The culture flasks are shaken and placed in the culturing apparatus. The algal cells are kept in suspension by shaking, stirring or bubbling with air, in order to improve gas exchange and reduce pH variation in the test solutions. The cultures should be maintained at a temperature in the range of 21 to 25 °C, controlled at ± 2 °C.
- The cell density in each flask is determined at least at 24, 48 and 72 hours after the start of the test. Filtered algal medium containing the appropriate concentration of the test chemical is used to determine the background when using cell density measurements other than direct counting methods.

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Test organisms

- It is suggested that the species of green algae used be a fast -growing species that is convenient for culturing and testing. The following species are preferred:
- -*Selenastrum capricornutum* , e.g. ATCC 22662 or CCAP 278/4,
- -*Scenedesmus subspicatus*, e.g. 86.81 SAG,

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C.4. DETERMINATION OF 'READY' BIODEGRADABILITY

- A solution, or suspension, of the test substance in a mineral medium is inoculated and incubated under aerobic conditions in the dark or in diffuse light. The amount of DOC in the test solution due to the inoculum should be kept as low as possible compared to the amount of DOC due to the test substance.
- Allowance is made for the endogenous activity of the inoculum by running parallel blank tests with inoculum but without test substance, although the endogenous activity of cells in the presence of the substance will not exactly match that in the endogenous control. A reference substance is run in parallel to check the operation of the procedures.

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C.4. DETERMINATION OF 'READY' BIODEGRADABILITY

- In order to select the most appropriate method, information on the chemical's **solubility, vapour pressure** and **adsorption** characteristics is essential.
- The chemical structure or formula should be known in order to calculate theoretical values and/or check measured values of parameters, e.g. ThOD, ThCO2, DOC, TOC, COD (see Annexes I and II).

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C.4. DETERMINATION OF 'READY' BIODEGRADABILITY

Test	Analytical Method	Suitability for substances which are:		
		poorly soluble	volatile	adsorbing
DOC Die-Away	Dissolved organic carbon	—	—	+ / –
Mod. OECD Die-Away	Dissolved organic carbon	—	—	+ / –
CO_2 Evolution	Respirometry: CO_2 evolution	+	—	+
Manometric Respirometry	Manometric respirometry: oxygen consumption	+	+ / –	+
Closed Bottle	Respirometry: dissolved oxygen	+ / –	+	+
MITI	Respirometry: oxygen consumption	+	+ / –	+

Test	DOC Die-Away	CO_2 Evolution	Manometric Respirometry	Modified OECD Screeing	Closed Bottle	MITI (I)
Concentration of Test Substance as mg/l mg DOC/l mg ThOD/l	10-40	10-20	100 50-100	10-40	2-10 5-10	100
Concentration of Inoculum (in cells/l, approximatively)	≤ 30 mg/l SS or ≤ 100 ml effluent/l ($10^7 - 10^8$)			0,5 ml secondary effluent/l (10^5)	≤ 5 ml of effluent/l ($10^4 - 10^6$)	30 mg/l SS ($10^7 - 10^8$)
Concentration of elements in mineral medium (in mg/l) P N Na K Mg Ca Fe	116 1,3 86 122 2,2 9,9 0,05-0,1				11,6 0,13 8,6 12,2 2,2 9,9 0,05-0,1	29 1,3 17,2 36,5 6,6 29,7 0,15
pH	7,4 ± 0,2					preferably 7,0
Temperature	22 ± 2 °C					25 ± 1 °C

DOC = Dissolved organic Carbon ThOD = Theoretical Oxygen Demand SS = Suspended Solids

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C.4 Ready biodegradability

- Normally, the test lasts for **28 days**. Tests however may be ended before 28 days, i.e. as soon as the biodegradation curve has reached a plateau for at least 3 determinations. Tests may also be prolonged beyond 28 days when the curve shows that biodegradation has started but that the plateau has not been reached day 28.
- Inocula may be pre-conditioned to the experimental conditions, but not pre-adapted to the test chemical. Pre-conditioning consists of aerating activated sludge in mineral medium or secondary effluent for 5-7 days at the test temperature. Pre-conditioning sometimes improves the precision of the test methods by reducing blank values. It is considered unnecessary to pre-condition MITI inoculum.

C.4. Test validity

- A test is considered valid if the difference of extremes of replicate values of the removal of test chemical at the plateau, at the end of the test or at the end of the 10 -day window, as appropriate, is less than 20% and if the percentage degradation of the reference substance has reached the level for ready biodegradability by 14 days.

  <u>If either of these conditions is not met, the test should be repeated.</u>
- Because of the stringency of the methods, low values do not necessarily mean that the test substance is not biodegradable under environmental conditions, but indicates that more work will be necessary to establish biodegradability. If in a toxicity test, containing both the test substance and a reference chemical, less than 35% degradation (based on DOC) or less than 25 % (based on ThOD or ThCO2) occurred in 14 days, the test chemicals can be assumed to be inhibitory (see also Annex IV). The test series should be repeated, if possible using a lower concentration of test chemical and/or a higher concentration of inoculum, but not greater than 30 mg solids/litre.

C. 9. BIODEGRADATION ZAHN -WELLENS TEST

- The purpose of the method is the evaluation of the potential ultimate biodegradability of water-soluble, non-volatile organic substances when exposed to relatively high concentrations of micro-organisms in a static test.
- The substances to be studied are used in concentrations corresponding to DOC-values in the range of **<u>50 to 400 mg/litre</u>** or COD-values in the range of 100 to 1000 mg/litre (DOC = dissolved organic carbon; COD = chemical oxygen demand). These relatively high concentrations have the advantage of analytical reliability. Compounds with toxic properties may delay or inhibit the degradation process.
- In this method, the measure of the concentration of dissolved organic carbon or the chemical oxygen demand is used to assess the ultimate biodegradability of the test substance.
- Activated sludge in an amount corresponding to 0,2 to 1,0 g/litre dry matter in the final mixture.

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Effects Assessment - steps

- **Hazard identification**: The aim of the hazard identification is to identify the effects of concern. The aim is also to review the classification of the;
- **Dose (concentration) - response (effect) assessment:** At this step the predicted no effect concentration (PNEC), shall, where possible, be determined.

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No GLP Studies?

NO GLP data may be used for the risk assessment, if valid conclusions can be drawn from them.

This means that the data, and the test methods used to generate them, must be evaluated in order to determine whether they are of sufficient quality for use in risk assessment. Such an evaluation will require the use of expert judgement, but the determination of data as being valid or not valid must be both justified and transparent.

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Calculation of PNEC

- **assumptions are made concerning the aquatic environment which allow, however uncertain, an extrapolation to be made from single-species short-term toxicity data to ecosystem effects.**

It is assumed that:

- **ecosystem sensitivity depends on the most sensitive species, and;**
- **protecting ecosystem structure protects community function.**

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Uncertainties & Extrapolation

- **intra- and inter-laboratory variation of toxicity data;**
- **intra- and inter-species variations (biological variance);**
- **short-term to long-term toxicity extrapolation;**
- **laboratory data to field impact extrapolation (additive, synergistic and antagonistic effects from the presence of other substances may also play a role here).**

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Table 16 Assessment factors to derive a $PNEC_{aquatic}$

Available data	Assessment factor
At least one short-term L(E)C50 from each of three trophic levels of the base-set (fish, Daphnia and algae)	1000 [a]
One long-term NOEC (either fish or Daphnia)	100 [b]
Two long-term NOECs from species representing two trophic levels (fish and/or Daphnia and/or algae)	50 [c]
Long-term NOECs from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10 [d]
Species sensitivity distribution (SSD) method	5-1 (to be fully justified case by case) [e]
Field data or model ecosystems	Reviewed on a case by case basis [f]

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Effects Assessment for Microorganisms

Table 17 Test systems for derivation of $PNEC_{microorganisms}$

Test	Available value	Assessment factor
Respiration inhibition tests	NOEC or EC10	10
EU Annex V C.11; OECD 209 (1984f) ISO 8192 (1986)	EC50	100
Inhibition control in standardised biodegradation tests - Ready biodegradability tests EU Annex V C.4 A-F; OECD 301A-F (1992f) 92/69/EEC C4 (1992) ISO-7827 (1994), -9439 (1999), -10707 (1994), -9408 (1999) - Inherent biodegradability tests EU Annex V C.9; OECD 302 B-C (1981d-1992g) 88/302/EEC (1988) ISO-9888 (1999)	The tested concentration at which toxicity to the inoculum can be ruled out with sufficient reliability (cf. corresponding text section above) could be considered as a NOEC for the toxicity to microorganisms of a STP	10
Inhibition of nitrification	NOEC or EC10	1
ISO-9509 (1989)	EC50	10
Ciliate growth inhibition tests	NOEC or EC10	1
(preferably with *Tetrahymena*, cf. OECD, 1998a) [1]	EC50	10
Activated sludge growth inhibition tests	NOEC or EC10	10
ISO-15522	EC50	100

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Effects Assessment for Microorganisms in Sewage Treatment Plants (STP) 1

Pilot scale activated sludge simulation tests OECD 303A (2001b) ISO-11733	Based on case-by-case expert judgement, the tested concentration not impairing proper functioning of the CAS 2) unit could be considered as NOEC for microorganisms in STPs	Case-by-case down to 1
Growth inhibition test with *Pseudomonas putida* NF EN ISO 10712 (1995)	NOEC or EC10	1
	EC50	10
(Bringmann and Kühn, 1980)	to be used if no other tests are available	
Pseudomonas fluorescens (Bringmann and Kühn, 1960)	Not usable as it uses glucose as substrate	
Escherichia coli (Bringmann and Kühn, 1960)	Not usable as it uses glucose as substrate	
Vibrio fischeri (MICROTOX) NF EN ISO 11348-1, -2, -3 (1999)	Not relevant for STP as the bacterium is a saltwater species	

Notes to Table 17:

1) Ciliate testing would be required as the guideline becomes available
2) CAS: Continuous Activated Sludge

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PNECsed

- **when no toxicity test results are available for sediment organisms, the equilibrium partitioning method is applied to identify a potential risk to sediment organisms. This method is regarded as "screening approach" and is explained in Section 3.5.3;**
- when only acute toxicity test results for benthic organisms are available (at least one) the risk assessment is performed both on the basis of the test result of the most sensitive species using an assessment factor of 1000 and on the basis of the equilibrium partitioning method. The lowest PNECsed is then used for the risk characterisation;
- when long-term toxicity test data are available for benthic organisms the PNECsed is calculated using assessment factors for long-term tests and this result should prevail in the risk assessment.

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Secondary poisoning

- **Standard assays of ecotoxicological effects usually provide information about the direct toxic effects of a substance. Chemicals showing bioaccumulation and biomagnification may pose an additional threat due to exposure of organisms higher in the food chain, e.g. top predators.**
- **If this is the case, the oral intake of a chemical via fish or worms (PECoralfish and PECoralworm) is compared to a PNEC for fish- or worm-eating mammals or birds.**

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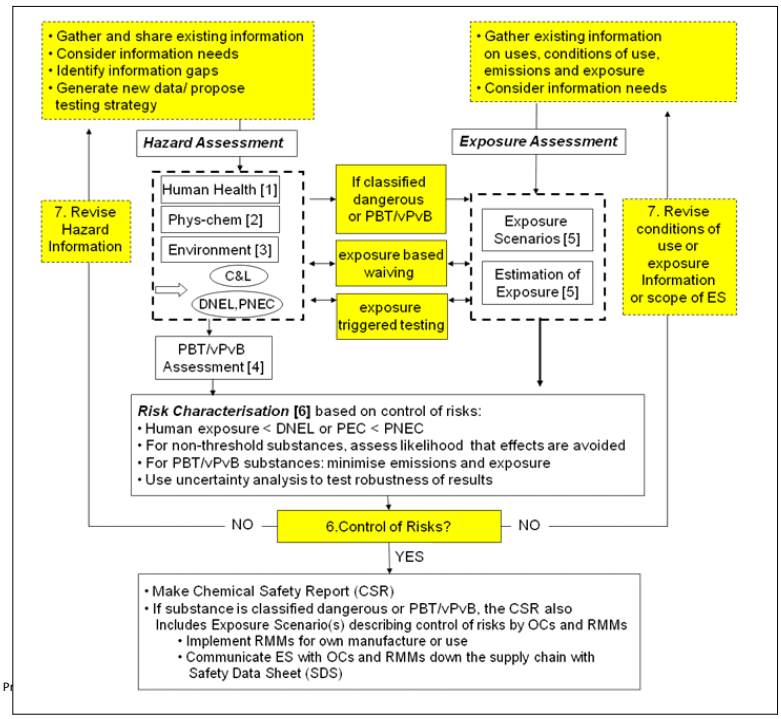

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Environmental Risk Characterisation

PEC / PNEC

Predicted Environmental Concentration / Predicted No Effect Concentration

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