Research centre
for toxic compounds
in the environment

Ecotoxicological bioassays

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Introduction

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CHEMICAL ENTERS THE ENVIRONMENT

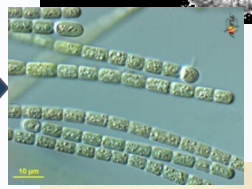
CHEMICAL ENTERS THE ORGANISM

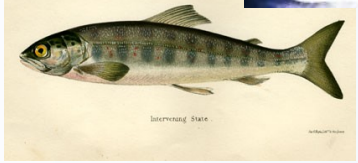
LEVELS, FATE, PROCESSES

Toxikokinetics

biotransformation
bioactivation
excretion / sequestration

Bioavailable fraction





Target site

"EXPOSURE" ?

acute

chronic

Role of ecotoxicology in environmental protection

FOCUS:

Investigate relationships between organisms and contaminants

ROLE:

Scientific basis of env. protection; know-how; rational env. protection

Activities:

Research of:

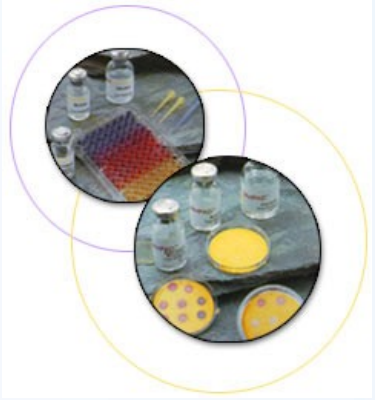
- Principles …
- Mechanisms …
- Fate and bioavailability
- Mixture toxicity
- Biodiversity …

Provide **TOOLS** for praxis:

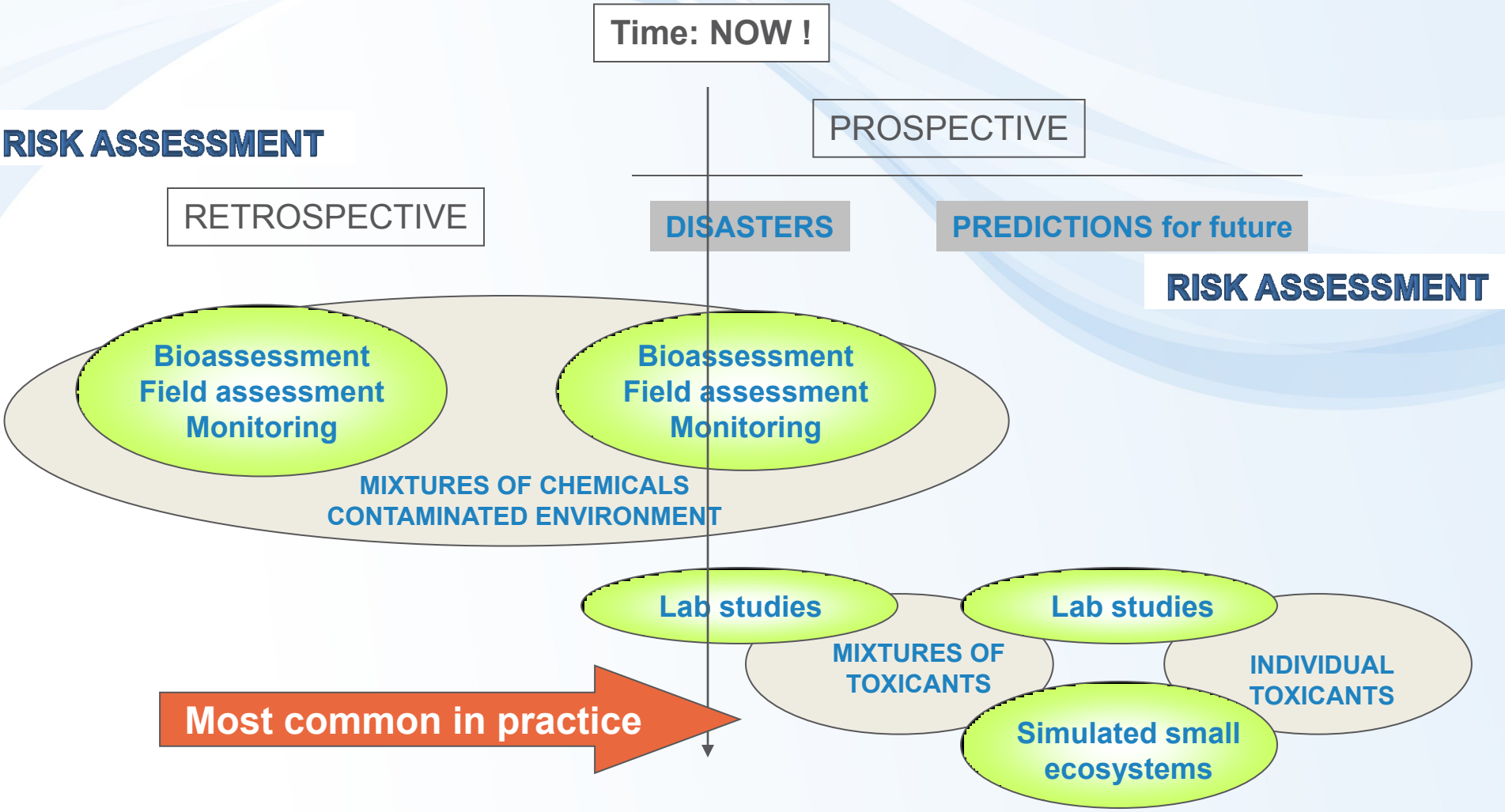
- (Eco)toxicity testing = **bioassays**
- Chemical and pesticides
- Testing wastes, contaminated sites
- Environmental quality assessment

Bioassays, ecotoxicity tests

- **tools (methods) for ecotoxicological research and praxis – for environmental legislation and protection**
- biota (tissue, organism, population, ecosystem …) is exposed to chemicals (and/or other factors), in the lab (controlled conditions) or in the field (less controlled) and effects are evaluated and related to exposure
- **WHY?** To understand the cause-effects relationships (causality, dose-response …)



Testing effects of chemicals and risk assessment



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Why bioassays?

Chemical analyses only are not able to identify risks properly because:

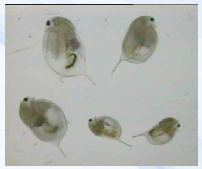
1) Real exposure is different – **bioavailability** in particular situation
2) Pollutant **mixture** - always in real ecosystems
3) **Matrix** itself has effects or interacts with effects of contaminants
4) Analytical methods are **limited** vs. wide spectrum of possibly toxic chemicals

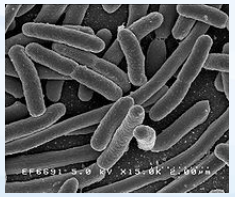
Bioassay development

- **old bioassays** – acute, ecologically irrelevant, testing pure chemicals, pesticides
- **new bioassays** – sublethal endpoints, ecological relevancy, chemical mixtures, miniaturization, simple to measure endpoints

Differenciation of bioassays

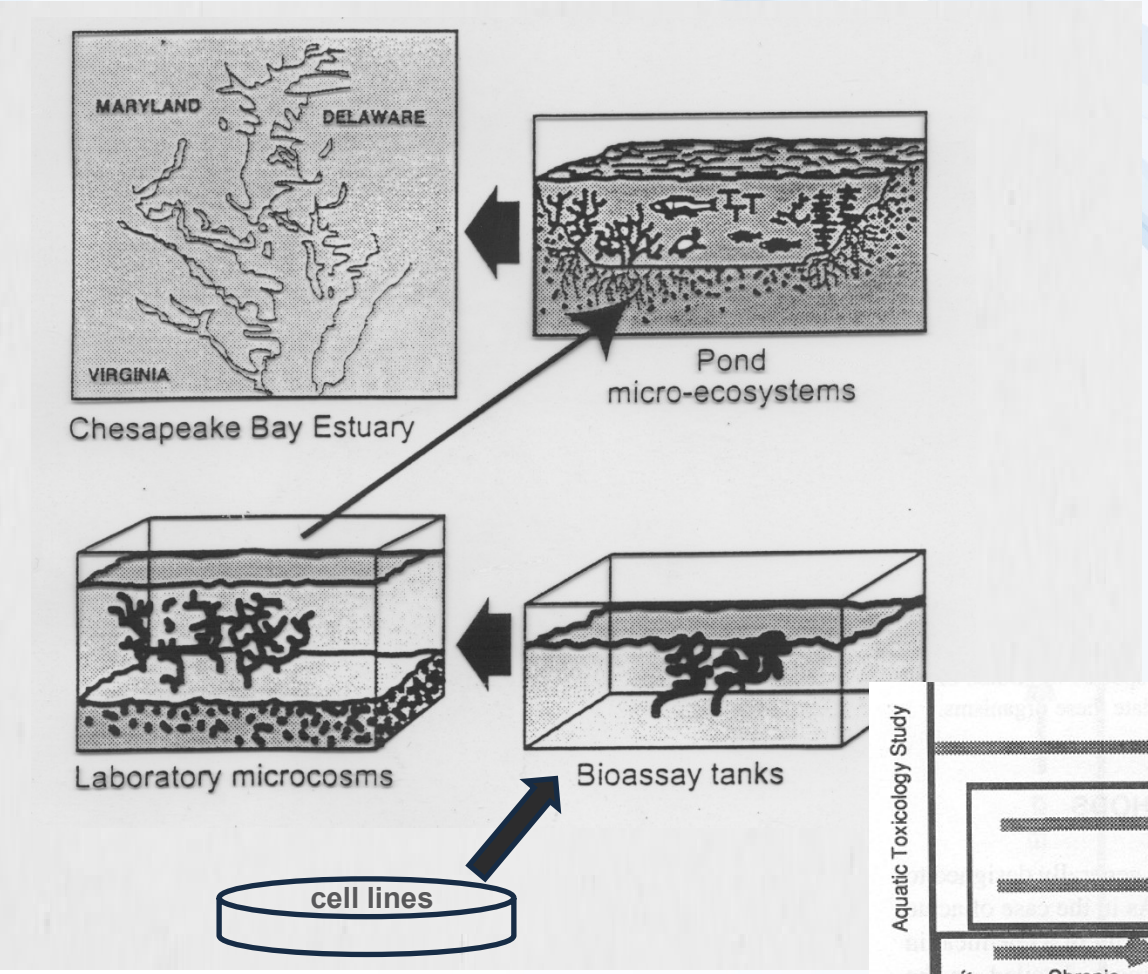
- major trophic levels
  - producers
  - consumers
  - destruents



- aquatic / soil
- single / multiple species
- acute / chronic effects
- contact bioassays / eluate bioassays / TIE
- legislative / standardized (practical) / experimental (research)
- toxicity / bioaccumulation / biodegradation tests

Differenciation of bioassays



size, complexity

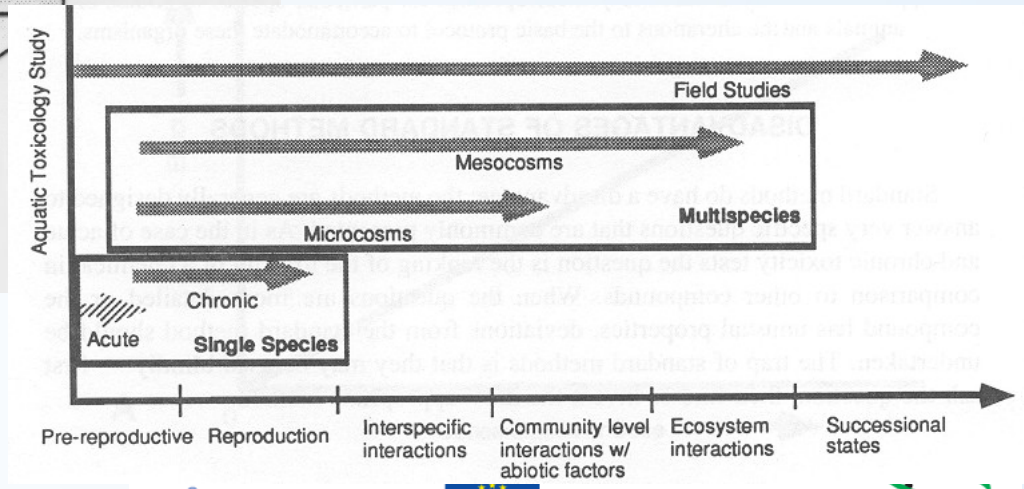
ecological relevancy

controling the conditions

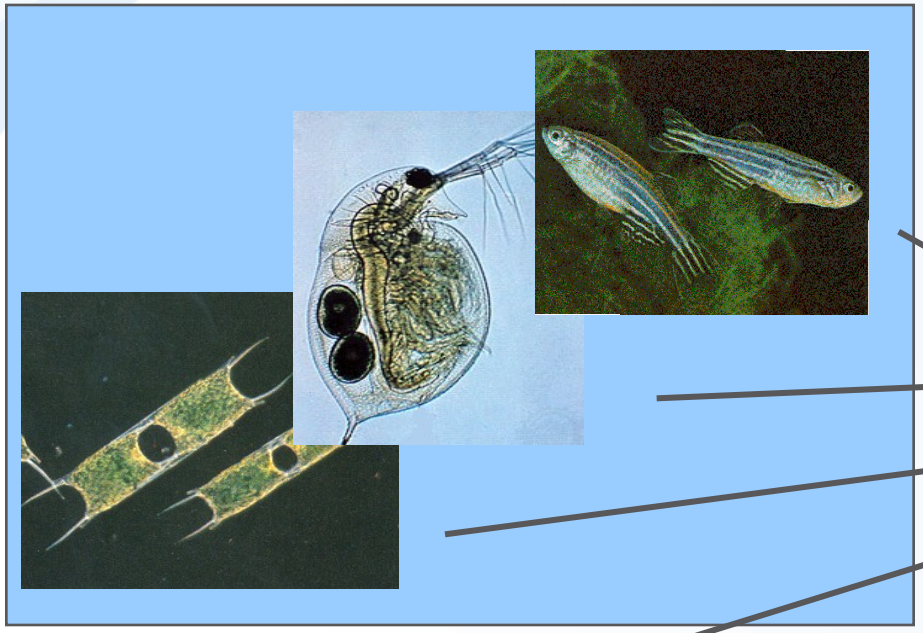
old / fresh contamination

no. of species

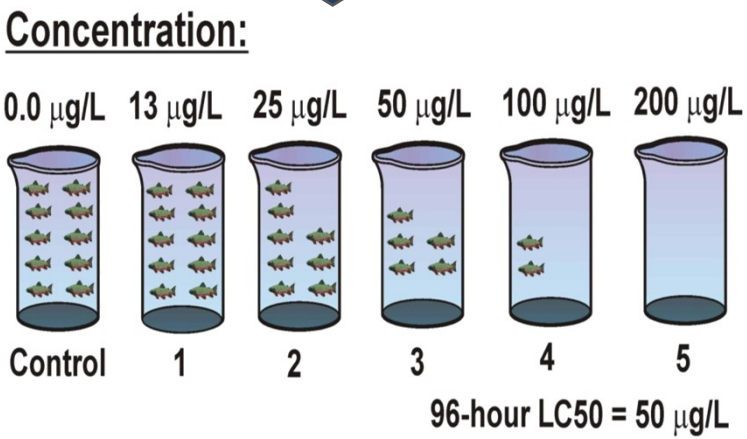
…



1 - Laboratory studies



Cu addition

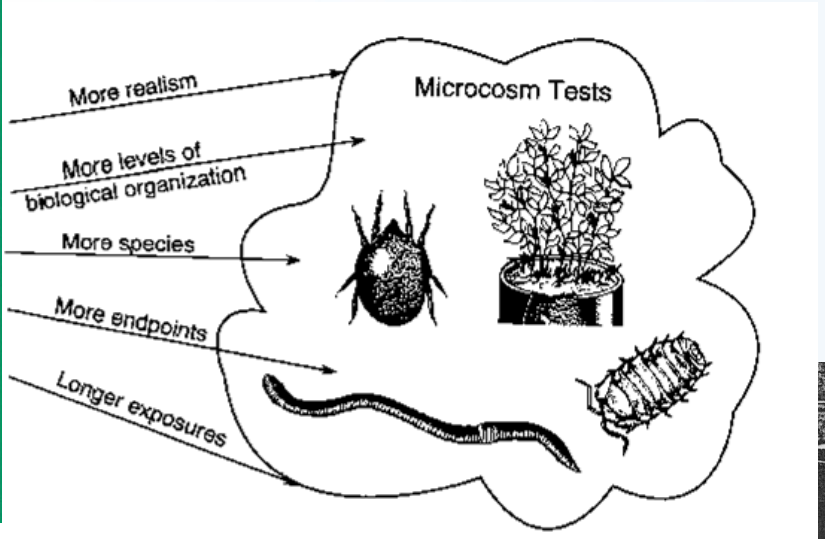


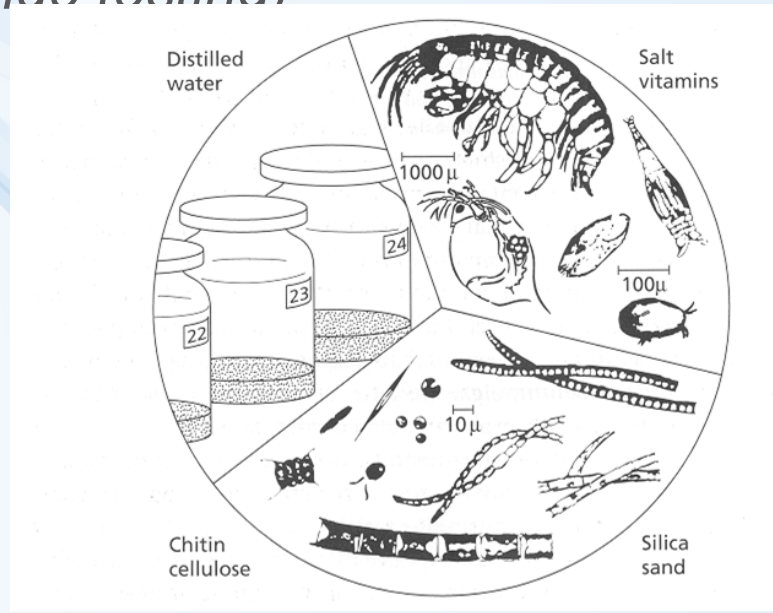
Effect concentrations expressed in total/dissolved Cu

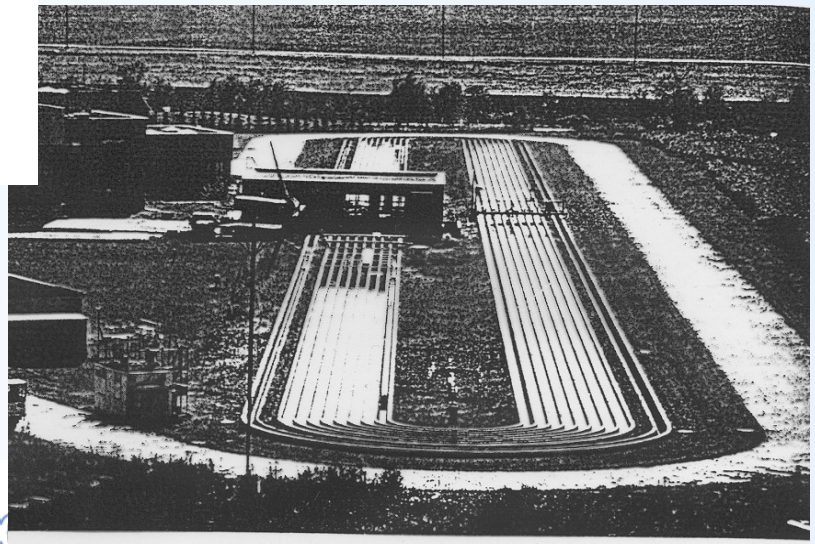
??? Safe concentrations ???

2 - Micro & Mesocosms

Expensive & time consuming *(e.g. Pesticide testing)*
Variable results (natural variability …)
Higher ecological relevancy

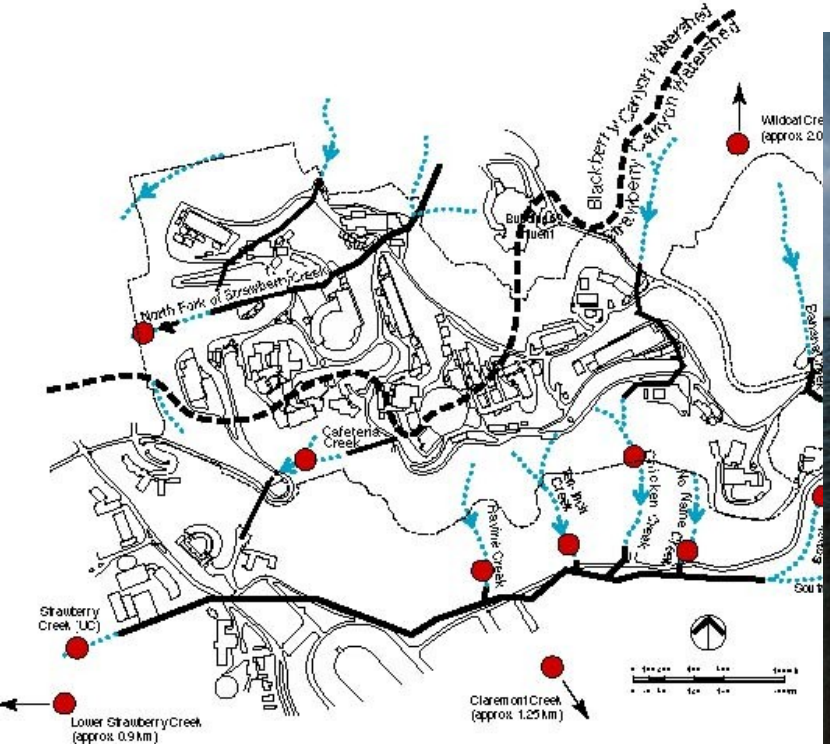






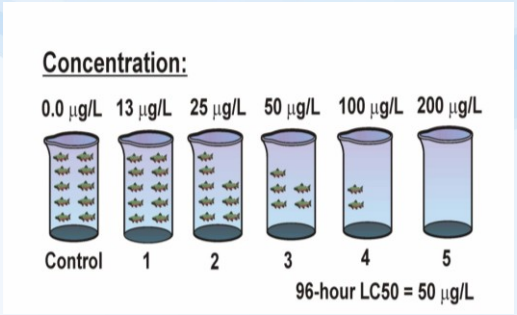
3 - Field assessment / biomonitoring

Complex issue (chemistry, biology, geology, climate, pedology ..)
Ecotoxicology + Ecology
Comparing „contaminated“ to „control“ sites



Use of bioassays

- **Testing chemicals**
  - Traditional / bioassays developed to assess **individual chemicals**
  - Advantage: Standardized approaches
    - OECD – Guideline methods - series „2“ Effects on biota
    - ISO methods
      - E.g. Fish tests - OECD 203 / ISO 7346
      - E.g. D. magna - OECD 202 / ISO 6341
  - Disadvantage: Limited ecological relevance
    - often **acute** tests only
    - „too standardized…“ (? Less representative ?)
    - does not assess/consider bioavailability
    - no consideration of mixture effects
    - no consideration of specific modes of action
    - no consideration of ecological situation



Use of bioassays

- Testing toxicity of natural **contaminated matrices**
  - Rather new in ecotoxicology – many open challenges
    - Whole effluent toxicity testing (WET)
    - Contact soil toxicity assays
  - More complex and more complicated
    - „cause-effects“ often not clear
      - Natural variability in matrices
      - Algal tests - nutrients (Nitrogen, Phosporus) >> Toxic compounds

eluate 1:10

Mixture at different ratios

OECD guidelines

Organization for Economic Cooperation Development

http://www.oecd.org/document/40/0,3746,en_2649_34377_37051368_1_1_1_1,00.html

Aquatic organisms

Test No. 201: Alga, Growth Inhibition Test	11 July 2006
Test No. 221: Lemna sp. Growth Inhabition Test	11 July 2006
Test No. 202: Daphnia sp. Acute Immobilisation Test	23 Nov 2004
Test No. 211: Daphnia magna Reproduction Test	16 Oct 2008
Test No. 203: Fish, Acute Toxicity Test	17 July 1992
Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study	04 Apr 1984
Test No. 210: Fish, Early-Life Stage Toxicity Test	17 July 1992
Test No. 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages	21 Sep 1998
Test No. 215: Fish, Juvenile Growth Test	21 Jan 2000
Test No. 229: Fish Short Term Reproduction Assay	08 Sep 2009
Test No. 230: 21-day Fish Assay	08 Sep 2009
Test No. 231: Amphibian Metamorphosis Assay	08 Sep 2009

Sediment organisms

Test No. 218: Sediment-Water Chironomid Toxicity Using Spiked Sediment	23 Nov 2004
Test No. 219: Sediment-Water Chironomid Toxicity Using Spiked Water	23 Nov 2004
Test No. 233: Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment	23 July 2010
Test No. 225: Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment	15 Oct 2007

OECD guidelines

Organization for Economic Cooperation Development

http://www.oecd.org/document/40/0,3746,en_2649_34377_37051368_1_1_1_1,00.html

Soil organisms

Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test	17 Aug 2006
Test No. 227: Terrestrial Plant Test: Vegetative Vigour Test	17 Aug 2006
Test No. 207: Earthworm, Acute Toxicity Tests	04 Apr 1984
Test No. 220: Enchytraeid Reproduction Test	23 Nov 2004
Test No. 222: Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)	23 Nov 2004
Test No. 228: Determination of Developmental Toxicity of a Test Chemical to Dipteran Dung Flies(Scathophaga stercoraria L. (Scathophagidae), Musca autumnalis De Geer (Muscidae))	16 Oct 2008
Test No. 232: Collembolan Reproduction Test in Soil	08 Sep 2009
Test No. 226: Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil	16 Oct 2008
Test No. 216: Soil Microorganisms: Nitrogen Transformation Test	21 Jan 2000
Test No. 217: Soil Microorganisms: Carbon Transformation Test	21 Jan 2000

Other tests

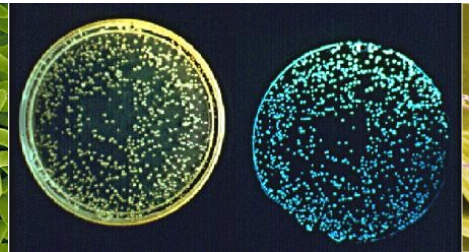
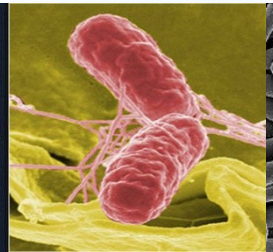
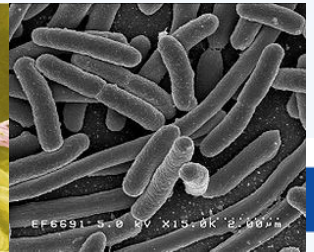
Test No. 213: Honeybees, Acute Oral Toxicity Test	21 Sep 1998
Test No. 214: Honeybees, Acute Contact Toxicity Test	21 Sep 1998
Test No. 205: Avian Dietary Toxicity Test	04 Apr 1984
Test No. 206: Avian Reproduction Test	04 Apr 1984
Test No. 223: Avian Acute Oral Toxicity Test	23 July 2010

ISO guidelines

International Standardization Organization

Aquatic microorganisms

ISO 10712:1995	Water quality -- Pseudomonas putida growth inhibition test (Pseudomonas cell multiplication inhibition test)
ISO 11348-1:2007	Water quality -- Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) -- Part 1: Method using freshly prepared bacteria
ISO 11348-2:2007	Water quality -- Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) -- Part 2: Method using liquid-dried bacteria
ISO 11348-3:2007	Water quality -- Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) -- Part 3: Method using freeze-dried bacteria
ISO 13641-1:2003	Water quality -- Determination of inhibition of gas production of anaerobic bacteria -- Part 1: General test
ISO 13641-2:2003	Water quality -- Determination of inhibition of gas production of anaerobic bacteria -- Part 2: Test for low biomass concentrations
ISO 13829:2000	Water quality -- Determination of the genotoxicity of water and waste water using the umu-test
ISO 16240:2005	Water quality -- Determination of the genotoxicity of water and waste water -- Salmonella/microsome test (Ames test)
ISO/DIS 11350	Water quality -- Determination of the genotoxicity of water and waste water -- Salmonella/microsome fluctuation test (Ames fluctuation test)
ISO 15522:1999	Water quality -- Determination of the inhibitory effect of water constituents on the growth of activated sludge microorganisms
ISO 21338:2010	Water quality -- Kinetic determination of the inhibitory effects of sediment, other solids and coloured samples on the light emission of Vibrio fischeri (kinetic luminescent bacteria test)
ISO 8192:2007	Water quality -- Test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation
ISO 9509:2006	Water quality -- Toxicity test for assessing the inhibition of nitrification of activated sludge microorganisms

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ISO guidelines

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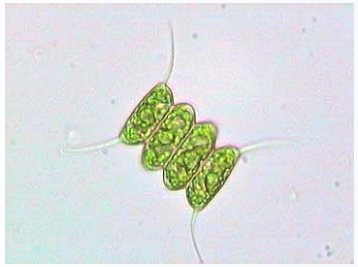
Aquatic plants

ISO 20079:2005	**Water quality -- Determination of the toxic effect of water constituents and waste water on duckweed (Lemna minor) -- Duckweed growth inhibition test**
ISO 8692:2004	**Water quality -- Freshwater algal growth inhibition test with unicellular green algae**
ISO/CD 16191	**Water quality - Determination of the toxic effect of sediment and soil on the growth behaviour of Myriophyllum aquaticum - Myriophyllum test**
ISO 10253:2006	**Water quality -- Marine algal growth inhibition test with Skeletonema costatum and Phaeodactylum tricornutum**
ISO 10710:2010	**Water quality -- Growth inhibition test with the marine and brackish water macroalga Ceramium tenuicorne**
ISO 14442:2006	**Water quality -- Guidelines for algal growth inhibition tests with poorly soluble materials, volatile compounds, metals and waste water**
ISO/DIS 13308	**Water quality -- Toxicity test based on reproduction inhibition of the green macroalga Ulva pertusa**
ISO/TR 11044:2008	**Water quality -- Scientific and technical aspects of batch algae growth inhibition tests**



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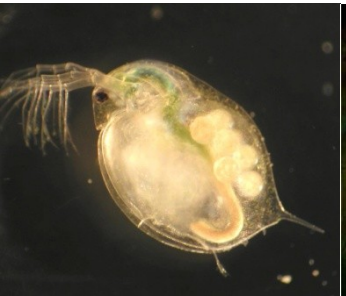
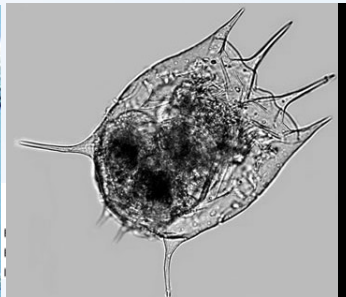
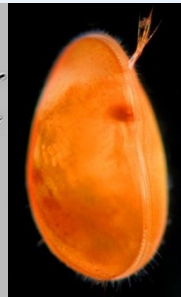
Aquatic invertebrates

ISO 6341:1996	**Water quality -- Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) -- Acute toxicity test**
ISO 10706:2000	**Water quality -- Determination of long term toxicity of substances to Daphnia magna Straus (Cladocera, Crustacea)**
ISO/DIS 14380	**Water quality -- Determination of the acute toxicity to Thamnocephalus platyurus (Crustacea, Anostraca)**
ISO/CD 16303	**Water quality -- Determination of toxicity of fresh water sediments using Hyalella azteca**
ISO 10872:2010	**Water quality -- Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of Caenorhabditis elegans (Nematoda)**
ISO 16712:2005	**Water quality -- Determination of acute toxicity of marine or estuarine sediment to amphipods**
ISO 20665:2008	**Water quality -- Determination of chronic toxicity to Ceriodaphnia dubia**
ISO 20666:2008	**Water quality -- Determination of the chronic toxicity to Brachionus calyciflorus in 48 h**
ISO 14669:1999	**Water quality -- Determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea)**
ISO/DIS 14371	**Water quality -- Determination of freshwater-sediment subchronic toxicity to Heterocypris incongruens (Crustacea, Ostracoda)**
ISO 7828:1985	**Water quality -- Methods of biological sampling -- Guidance on handnet sampling of aquatic benthic macro-invertebrates**
ISO 8265:1988	**Water quality -- Design and use of quantitative samplers for benthic macro-invertebrates on stony substrata in shallow freshwaters**
ISO 8689-1:2000	**Water quality -- Biological classification of rivers -- Part 1: Guidance on the interpretation of biological quality data from surveys of benthic macroinvertebrates**
ISO 8689-2:2000	**Water quality -- Biological classification of rivers -- Part 2: Guidance on the presentation of biological quality data from surveys of benthic macroinvertebrates**
ISO/DIS 10870	**Water quality -- Guidelines for the selection of sampling methods and devices for benthic macroinvertebrates in fresh waters**
ISO/WD 16778	**Water quality -- Calanoid copepod development test with Acartia tonsa**





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Aquatic vertebrates

ISO 15088:2007	**Water quality -- Determination of the acute toxicity of waste water to zebrafish eggs (Danio rerio)**
ISO 7346-1:1996	**Water quality -- Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] -- Part 1: Static method**
ISO 7346-2:1996	**Water quality -- Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] -- Part 2: Semi-static method**
ISO 7346-3:1996	**Water quality -- Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] -- Part 3: Flow-through method**
ISO 10229:1994	**Water quality -- Determination of the prolonged toxicity of substances to freshwater fish -- Method for evaluating the effects of substances on the growth rate of rainbow trout (Oncorhynchus mykiss Walbaum (Teleostei, Salmonidae))**
ISO 12890:1999	**Water quality -- Determination of toxicity to embryos and larvae of freshwater fish -- Semi-static method**
ISO 21427-1:2006	**Water quality -- Evaluation of genotoxicity by measurement of the induction of micronuclei -- Part 1: Evaluation of genotoxicity using amphibian larvae**
ISO 21427-2:2006	**Water quality -- Evaluation of genotoxicity by measurement of the induction of micronuclei -- Part 2: Mixed population method using the cell line V79**
ISO 23893-1:2007	**Water quality -- Biochemical and physiological measurements on fish -- Part 1: Sampling of fish, handling and preservation of samples**
ISO/TS 23893-2:2007	**Water quality -- Biochemical and physiological measurements on fish -- Part 2: Determination of ethoxyresorufin-O-deethylase (EROD)**
ISO/CD 23893-3	**Water quality -- Biochemical and physiological measurements on fish -- Part 3: Determination of vitellogenin**

ISO guidelines

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Soil microorganisms

ISO 10381-6:2009	**Soil quality -- Sampling -- Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory**
ISO 14240-1:1997	**Soil quality -- Determination of soil microbial biomass -- Part 1: Substrate-induced respiration method**
ISO 14240-2:1997	**Soil quality -- Determination of soil microbial biomass -- Part 2: Fumigation-extraction method**
ISO 16072:2002	**Soil quality -- Laboratory methods for determination of microbial soil respiration**
ISO 17155:2002	**Soil quality -- Determination of abundance and activity of soil microflora using respiration curves**
ISO 15685:2004	**Soil quality -- Determination of potential nitrification and inhibition of nitrification -- Rapid test by ammonium oxidation**
ISO 14238:1997	**Soil quality -- Biological methods -- Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes**
ISO 23753-1:2005	**Soil quality -- Determination of dehydrogenase activity in soils -- Part 1: Method using triphenyltetrazolium chloride (TTC)**
ISO 23753-2:2005	**Soil quality -- Determination of dehydrogenase activity in soils -- Part 2: Method using iodotetrazolium chloride (INT)**
ISO/DIS 11063	**Soil quality -- Method to directly extract DNA from soil samples**
ISO/TS 29843-1:2010	**Soil quality -- Determination of soil microbial diversity -- Part 1: Method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis**
ISO/PRF TS 29843-2	**Soil quality -- Determination of soil microbial diversity -- Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method**
ISO/TS 10832:2009	**Soil quality -- Effects of pollutants on mycorrhizal fungi -- Spore germination test**
ISO/TS 22939:2010	**Soil quality -- Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates**
ISO 11266:1994	**Soil quality -- Guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions**
ISO 15473:2002	**Soil quality -- Guidance on laboratory testing for biodegradation of organic chemicals in soil under anaerobic conditions**
ISO 14239:1997	**Soil quality -- Laboratory incubation systems for measuring the mineralization of organic chemicals in soil under aerobic conditions**

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Soil invertebrates

ISO 11268-1:1993	Soil quality -- Effects of pollutants on earthworms (Eisenia fetida) -- Part 1: Determination of acute toxicity using artificial soil substrate
ISO 11268-2:1998	Soil quality -- Effects of pollutants on earthworms (Eisenia fetida) -- Part 2: Determination of effects on reproduction
ISO 11268-3:1999	Soil quality -- Effects of pollutants on earthworms -- Part 3: Guidance on the determination of effects in field situations
ISO 11267:1999	Soil quality -- Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants
ISO 16387:2004	Soil quality -- Effects of pollutants on Enchytraeidae (Enchytraeus sp.) -- Determination of effects on reproduction and survival
ISO 15952:2006	Soil quality -- Effects of pollutants on juvenile land snails (Helicidae) -- Determination of the effects on growth by soil contamination
ISO 20963:2005	Soil quality -- Effects of pollutants on insect larvae (Oxythyrea funesta) -- Determination of acute toxicity
ISO 17512-1:2008	Soil quality -- Avoidance test for determining the quality of soils and effects of chemicals on behaviour -- Part 1: Test with earthworms (Eisenia fetida and Eisenia andrei)
ISO/DIS 17512-2	Soil quality -- Avoidance test for determining the quality of soils and effects of chemicals on behaviour -- Part 2: Test with collembolans (Folsomia candida)
ISO 23611-1:2006	Soil quality -- Sampling of soil invertebrates -- Part 1: Hand-sorting and formalin extraction of earthworms
ISO 23611-2:2006	Soil quality -- Sampling of soil invertebrates -- Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina)
ISO 23611-3:2007	Soil quality -- Sampling of soil invertebrates -- Part 3: Sampling and soil extraction of enchytraeids
ISO 23611-4:2007	Soil quality -- Sampling of soil invertebrates -- Part 4: Sampling, extraction and identification of soil-inhabiting nematodes
ISO/DIS 23611-5	Soil quality -- Sampling of soil invertebrates -- Part 5: Sampling and extraction of soil macro-invertebrates
ISO/DIS 23611-6	Soil quality -- Sampling of soil invertebrates -- Part 6: Guidance for the design of sampling programmes with soil invertebrates





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Plants

ISO 11269-1:1993	**Soil quality -- Determination of the effects of pollutants on soil flora -- Part 1: Method for the measurement of inhibition of root growth**
ISO 11269-2:2005	**Soil quality -- Determination of the effects of pollutants on soil flora -- Part 2: Effects of chemicals on the emergence and growth of higher plants**
ISO 17126:2005	**Soil quality -- Determination of the effects of pollutants on soil flora -- Screening test for emergence of lettuce seedlings (Lactuca sativa L.)**
ISO 22030:2005	**Soil quality -- Biological methods -- Chronic toxicity in higher plants**
ISO/CD 29200	**Soil quality -- Assessment of genotoxic effects on higher plants -- Micronucleus test on Vicia faba**

Research centre for toxic compounds in the environment

US EPA

http://www.epa.gov/ocspp/pubs/frs/home/draftguidelines.htm

US Environmental Protection Agency

Aquatic

850.1010 - Aquatic Invetebrate Acute Toxicity, Test, Freshwater Daphnids (PDF) (10 pp, 36K)
850.1020 - Gammarid Acute Toxicity Test (PDF) (11 pp, 36K)
850.1025 - Oyster Acute Toxicity Test (Shell Deposition) (PDF) (9 pp, 32K)
850.1035 - Mysid Acute Toxicity Test (PDF) (10 pp, 34K)
850.1045 - Penaeid Acute Toxicity Test (PDF) (9 pp, 32K)
850.1055 - Bivalve Acute Toxicity Test (Embryo Larval) (PDF) (7 pp, 27K)
850.1075 - Fish Acute Toxicity Test, Freshwater And Marine (PDF) (13 pp, 45K)
850.1085 - Fish Acute Toxicity Mitigated By Humic Acid (PDF) (10 pp, 35K)
850.1300 - Daphnid Chronic Toxicity Test (PDF) (12 pp, 42K)
850.1350 - Mysid Chronic Toxicity Test (PDF) (10 pp, 36K)
850.1400 - Fish Early-Life Stage Toxicity Test (PDF) (15 pp, 66K)
850.1500 - Fish Life Cycle Toxicity (PDF) (4 pp, 16K)
850.1710 - Oyster BCF (PDF) (14 pp, 50K)
850.1730 - Fish BCF (PDF) (25 pp, 74K)
850.1735 - Whole Sediment Acute Toxicity Invertebrates, Freshwater (PDF) (19 pp, 65K)
850.1740 - Whole Sediment Acute Toxicity Invertebrates, Marine (PDF) (14 pp, 50K)
850.1790 - Chironomid Sediment Toxicity Test (PDF) (16 pp, 57K)
850.1800 - Tadpole/Sediment Subchronic Toxicity Test (PDF) (15 pp, 49K)
850.1850 - Aquatic Food Chain Transfer (PDF) (4 pp, 16K)
850.1900 - Generic Freshwater Microcosm Test, Laboratory (PDF) (28 pp, 76K)
850.1925 - Site-Specific Aquatic Microcosm Test, Laboratory (PDF) (21 pp, 91K)
850.1950 - Field Testing For Aquatic Organisms (PDF) (7 pp, 21K)
850.4400 - Aquatic Plant Toxicity Test Using Lemna Spp., Tiers I and II (PDF) (10 pp, 36K)
850.4450 - Aquatic Plants Field Study, Tier III (PDF) (9 pp, 30K)
850.5400 - Algal Toxicity, Tiers I and II (PDF) (11 pp, 42K)
850.6800 - Modified Activated Sludge, Respiration Inhibition Test for Sparingly Soluble Chemicals (PDF) (9 pp, 37K)

US EPA

http://www.epa.gov/ocspp/pubs/frs/home/draftguidelines.htm

US Environmental Protection Agency

Soil

850.2450 - Terrestrial (Soil-Core) Microcosm Test (PDF) (19 pp, 123K)
850.4000 - Background-Nontarget Plant Testing (PDF) (15 pp, 50K)
850.4025 - Target Area Phytotoxicity (PDF) (15 pp, 51K)
850.4100 - Terrestrial Plant Toxicity, Tier I (Seedling Emergence) (PDF) (8 pp, 29K)
850.4150 - Terrestrial Plant Toxicity, Tier I (Vegetative Vigor) (PDF) (8 pp, 28K)
850.4200 - Seed Germination/Root Elongation Toxicity Test (PDF) (8 pp, 29K)
850.4225 - Seedling Emergence, Tier II (PDF) (10 pp, 36K)
850.4230 - Early Seedling Growth Toxicity Test (PDF) (9 pp, 33K)
850.4250 - Vegetative Vigor, Tier II (PDF) (10 pp, 35K)
850.4300 - Terrestrial Plants Field Study, Tier III (PDF) (8 pp, 27K)
850.4600 - Rhizobium-Legume Toxicity (PDF) (14 pp, 73K)
850.4800 - Plant Uptake and Translocation Test (PDF) (13 pp, 35K)
850.5100 - Soil Microbial Community Toxicity Test (PDF) (11 pp, 46K)
850.6200 - Earthworm Subchronic Toxicity Test (PDF) (13 pp, 43K)

Other

850.2100 - Avian Acute Oral Toxicity Test (PDF) (11 pp, 38K)
850.2200 - Avian Dietary Toxicity Test (PDF) (12 pp, 42K)
850.2300 - Avian Reproduction Test (PDF) (16 pp, 53K)
850.2400 - Wild Mammal Acute Toxicity (PDF) (5 pp, 18K)
850.2500 - Field Testing For Terrestrial Wildlife (PDF) (43 pp, 115K)
850.3020 - Honey Bee Acute Contact Toxicity (PDF) (8 pp, 27K)
850.3030 - Honey Bee Toxicity of Residues on Foliage (PDF) (6 pp, 23K)
850.3040 - Field Testing for Pollinators (PDF) (5 pp, 18K)

Testing strategy

- **Battery of assays**
  - Fast screening tests (Vibrio fisheri bioluminiscence, 30 min toxicity)
  - Standardized acute toxicity tests
  - Further studies with chronic assays
  - **Combine trophic levels! Combine exposure routes!**

- **Various purposes -> guidelines and recommendations**
  - REACH (EU - Registration, Evaluation and Authorisation of Chemicals)
  - Plant protection products + biocides
  - Veterinary and human pharmaceuticals
  - Waste materials …

- **The most common set ups**
  - algae / D. magna / fish for aquatic environment
  - earthworm (enchytraeid/springtail) / plant for soil environment

Testing strategy

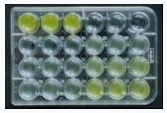
ISO 15799 (2003): Guidance on the ecotoxicological characterization of soils and soil materials

ISO 17616 (2008): Guidance on the choice and evaluation of bioassays for ecotoxicological characterization of soils and soil materials

Retention function – Biotests with eluates

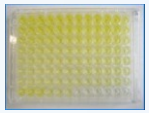
Ecotoxic contents

Luminescent bacteria test



Algal inhibition test

Genotoxic contents



Umu-test

Habitat function - Biotests with solids

Site inherent test organisms

Respiration test

Nitrification test

Added test organisms

Bacteria contact test

Earthworm avoidance test

Plant test

Earthworm test

Collembolan test

General scheme of bioassay

1) Prepare the organism

Culture media, standardized numbers, age, etc.

2) Prepare the sample

Dilution series

water/culture media – direct organism exposure

Include BLANK (medium only)

solvent for organic compounds – minimum to be added

Include SOLVENT CONTROL

3) Expose organisms

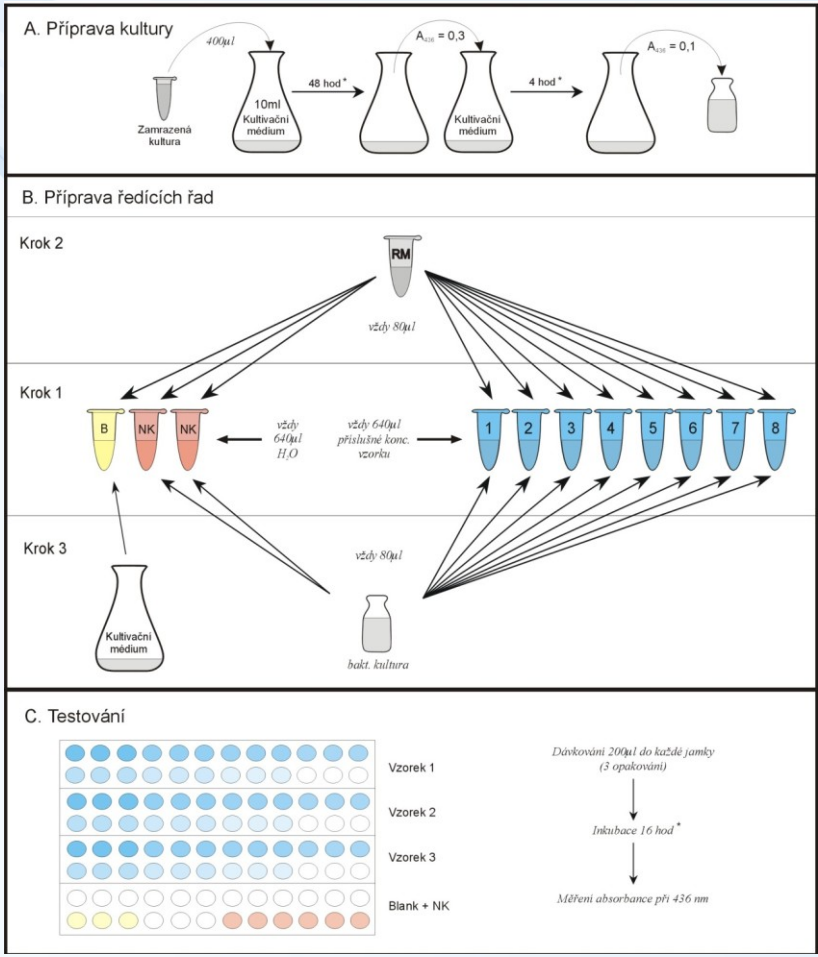
… for appropriate time, number of repetitions,
under specified conditions

4) Evaluate and report results

measure the endpoint / count organisms

validity criteria

statistical evaluation (means, ANOVA, dose-response …)



Research centre
for toxic compounds
in the environment

Aquatic ecotoxicology bioassays

cetocoen

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

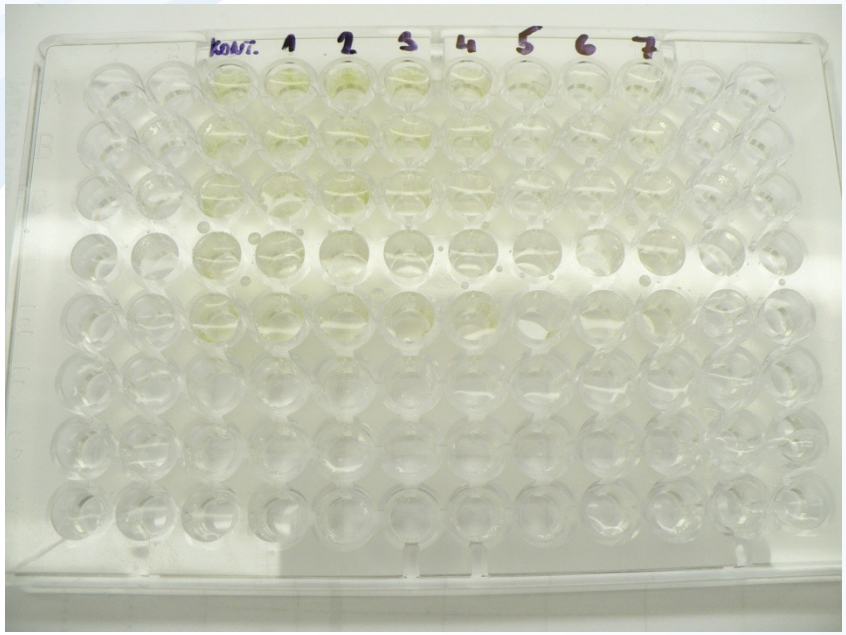
2007-13
OP Research and
Development for Innovation

Algal growth inhibition test (ISO 8692)

Organisms	Freshwater species: *Selenastrum capricornutum, Scenedesmus subspicatus, Chlorella vulgaris, Microcystis aeruginosa, Anabaena flos-aquae, Navicula pelliculosa;* Saltwater species: *Skeletonema costatum, Thalassiosira pseudonana,* and *Dunaliella tertiolecta*
Number of organisms per chamber (±10%)	*Selenastrum capricornutum* and other freshwater green algae: 2×10^4 cells/ml *Navicula pelliculosa*: 2×10^4 cells/ml *Microcystis aeruginosa*: 5×10^4 cells/ml *Anabaena flos-aquae*: 2×10^4 cells/ml Saltwater species: 2×10^4 cells/ml
Experimental design	
Test vessel type and size	Sterile Erlenmeyer flasks of borosilicate glass, any size
Test solution volume	Not to exceed 50% of the flask volume for tests conducted on a shaker, and not more than 20% of the flask volume for tests not conducted on a shaker
Number of replicate chambers per sample	2 or more
Test duration	96 h
Physical and chemical parameters	
Water temperature	24 ± 2°C for freshwater green and blue-green algae 20 ± 2°C for *Navicula pelliculosa* and other saltwater algae
Light quality	Continuous "cool-white" fluorescent
Light intensity	Should not vary by more than ±15%: 60 µE m^{-2}/s^{-1} (4300 lm/m^2) for freshwater diatoms and green algae 30 µE m^{-2}/s^{-1} (2150 lm/m^2) for freshwater blue-green algae 82–90 µE m^{-2}/s^{-1} (5900 to 6500 lm/m^2) for *Thalassiosira* 60 µE m^{-2}/s^{-1} (4300 lm/m^2) for *Skeletonema*
Photoperiod	14 h light/10 h dark for *Skeletonema*
Test solution pH	7.5 ± 0.1 for freshwater 8.0 ± 0.1 for saltwater
Endpoint	Biomass, cell number, area underneath the growth curve

Algal growth inhibition test (ISO 8692)

- Miniaturization

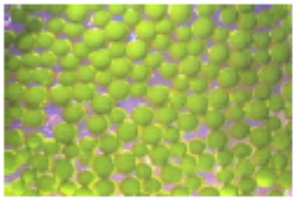


- Alternative: Algaltoxkit

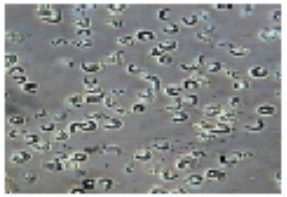
ALGALTOXKIT F™

MICROBIOTESTS

Cost-effective, culture/maintenance free* bioassays with the micro-algae *Selenastrum capricornutum*
(renamed *Raphidocoelis subcapitata/Pseudokirchneriella subcapitata*)



Algal beads (2 mm)
> 1 million algal cells per bead



Algal cells

The micro-algae are included in the kits in "algal beads" from which they can be set free "on demand"

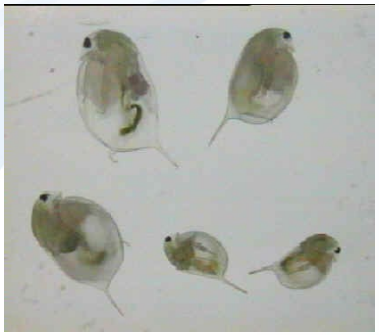
Each Algaltoxkit contains all the materials to perform two 72h growth inhibition tests

Duckweed bioassay (ISO 20079)

- *Lemna minor*
- 10 leaves per 1 beaker
- pH 6.5; 10 000 lx; 24°C
- 96 hours
- growth, biomass, no. of leaves
- image analysis possible
- validity:
  - 8x increase in control
  - IC_{50} for $K_2Cr_2O_7$ 10-60 mg/L

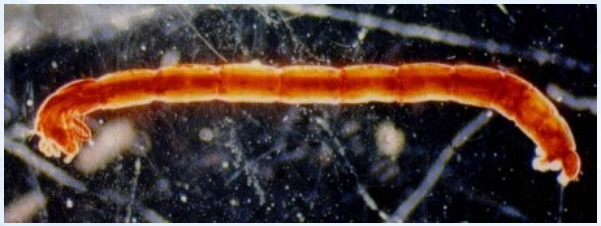
Aquatic consumers - invertebrates

Daphnia magna

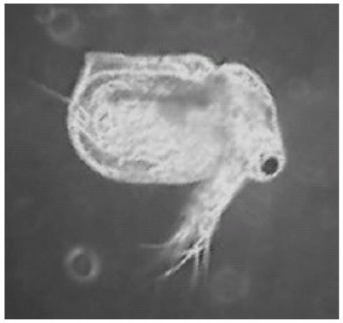


Artemia salina

Chironomus riparius



Ceriodaphnia dubia



Gammarus

Tamnocephalus platyurus

Potamopyrgus antipodarum

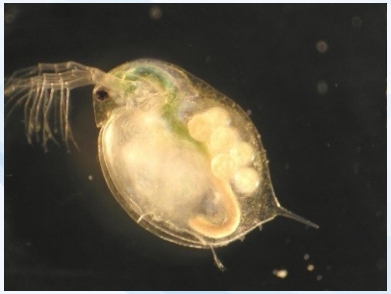
Tubifex tubifex

Lumbriculus variegatus

Hyalella azteca

Daphnia magna test (ISO 6341)

- 5 individuals per replicate (min 2 ml)
- no food
- 20°C; dark or 16h light / 8h dark
- 24h, 48h
- medium
  - $CaCl_2 \cdot 2H_2O$ 11,76 g/l
  - $MgSO_4 \cdot 7H_2O$ 4,93 g/l
  - $NaHCO_3$ 2,59 g/l
  - KCl 0,23 g/l
  - 25 ml each to 1 L
  - pH 7.8
  - aeration



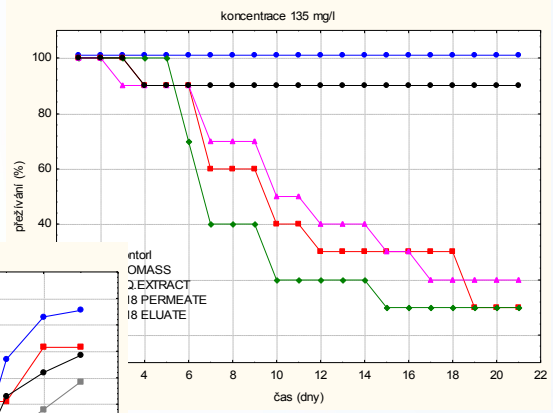
- no. of immobilized individuals
- validity:
  - O_2 > 80 % (2 mg/l)
  - mortality in control 10 %

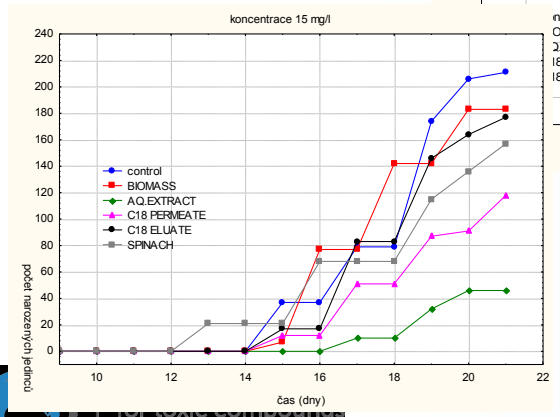
Daphnia magna chronic test (ISO 10706)

- 10 juveniles (24h old) per replicate
- 50ml medium
- 3 times per week medium change
- 21 days

- 20 2 C
- pH 7-9
- disolved O_2 > 3mg/l
- 16 h light / 8 h dark
- food - algae
- week controls: O_2, T, pH

Mortality, survival
Reproduction
Juveniles
Behavior



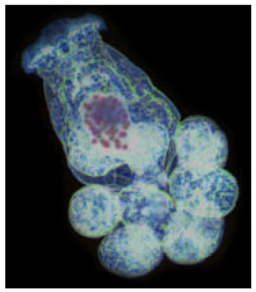


in the environment

Daphnia magna acute vs chronic test

Test type	Chronic (partial life cycle)	Acute 48 h
Organisms	*D. magna*	*D. magna*
Age of test organisms	24-h old	24-h old
Number of organisms per chamber	10	10 (minimum)
Experimental design		
Test vessel type and size	100 ml beakers	250 ml
Test solution volume	80 ml	200 ml
Number of replicates per sample	2 (minimum)	3 (minimum)
Feeding regime	Various combinations of trout chow, yeast, alfalfa, green algae, and diatoms given in excess	Do not feed
Test duration	21 days	48 hr
Physical and chemical parameters		
Water temperature	20°C	20 ± 2°C
Light quality	Ambient laboratory levels	Ambient laboratory levels
Light intensity	Up to 600 lux	540 to 1080 lux
Photoperiod	16 h light and 8 h dark (with 15- to 30-min transition)	16 h light and 8 h dark
pH range	7.0–8.6	7.0–8.6
DO concentration	40–100%	60–100%
Aeration	Not necessary	none
Endpoint	Survival, growth, and reproduction	Immobilization

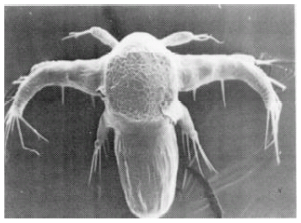
Alternative: microbiotests



**Test organisms are included in the kits as "dormant eggs (cysts)" which can be hatched "on demand"*

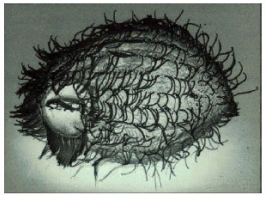
THAMNOTOXKIT F™
MICROBIOTESTS

With the crustacean *Thamnocephalus platyurus*



PROTOXKIT F™
MICROBIOTESTS

With the ciliate protozoan *Tetrahymena thermophila*



ROTOXKIT F
chronic

Contains all the materials to perform three 48h reproduction assays

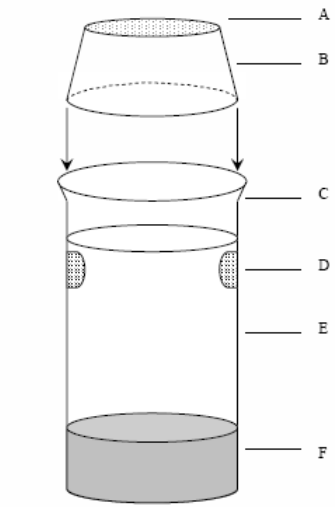
OSTRACODTOXKIT F™
MICROBIOTESTS

FOR SEDIMENT TOXICITY TESTING

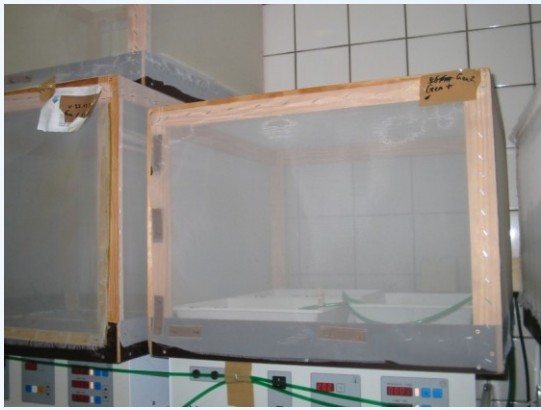
With the benthic crustacean *Heterocypris incongruens*

Chironomus riparius test (OECD 218)

- 10 larvae (cca 10d old) per beaker
- OECD sediment
- 100 ml sediment / 175 ml water
- 20 ± 2°C; food, aeration
- 16h light / 8h dark; controled pH, O_2
- 10 d
- survival and growth



A: the nylon screen
B: the inverted plastic cups
C: the lipless exposure beaker
D: the water exchange screen ports
E: water
F: sediment



Fish bioassays

Guppy, *Poecilia reticulata*

acute tests (96 h)
prolonged tests
embryolarval tests
chronic tests
- reproduction
- growth

Specific endpoints – genotoxicity, endocrine disruption

Zebrafish, *Danio rerio* (syn. *Brachydanio rerio*)

(Rainbow) trout *(Onchorhynchus sp.)*

Fathead minnow, *Pimephales promelas (USA)*

Medaka, *Oryzias latipes*

Nile tilapia, *Oreochromis niloticus*

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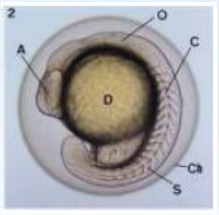
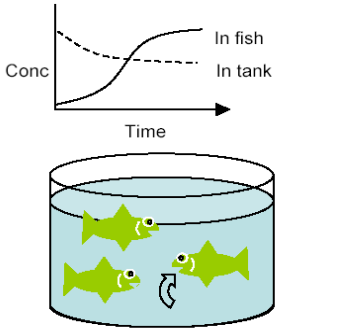
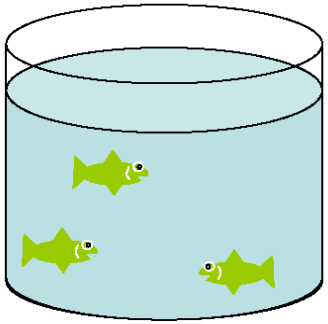
Fish bioassay acute (ISO 7346 1-3)

- *Brachydanio rerio, …*
- 26°C
- medium:
  - pH 7,8 ± 0,2
  - $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, $NaHCO_3$, KCl
  - disolved O_2 > 90%
- 24, 48, 72, 96 h
- validity: mortality in control < 10%, O_2 etc.

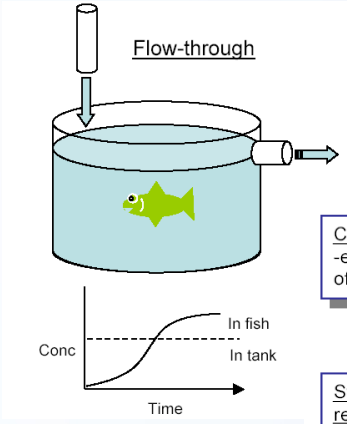




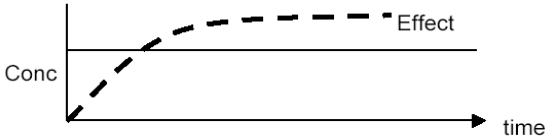
Uptake by organisms depletes chemical in solution



Chemical depleted slowly



Continuous:
-exposure is function of duration



Static renewal, with recovery:
-exposure is function of max conc'n

Static renewal, no recovery:
-exposure is function of cumulative concentration

Research centre for toxic compounds in the environment

Prolonged and chronic fish tests

Prolonged

- OECD 204 – 14d, ISO 10229 - 21 d
- semistatic or flow through
- food; controled pH, O_2
- endpoints: breathing, gills, behavior, orientation, mortality
- 14-21d LC_{50}, NOEC, LOEC

Chronic

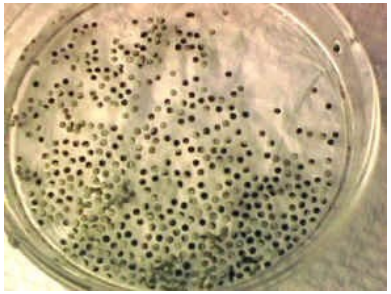
- OECD 210; US EPA OPPTS 850.1500
- 7 - 200 days
- starting with eggs or embryos or juveniles
- endpoints: survival, behavior, feeding, lenght, growth, weight, biochemical parameters, bioaccumulation

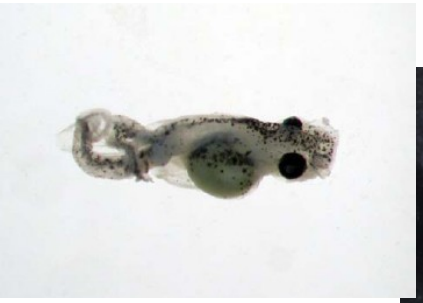
Embryolarval test (ISO 12890, OECD 210, 212)

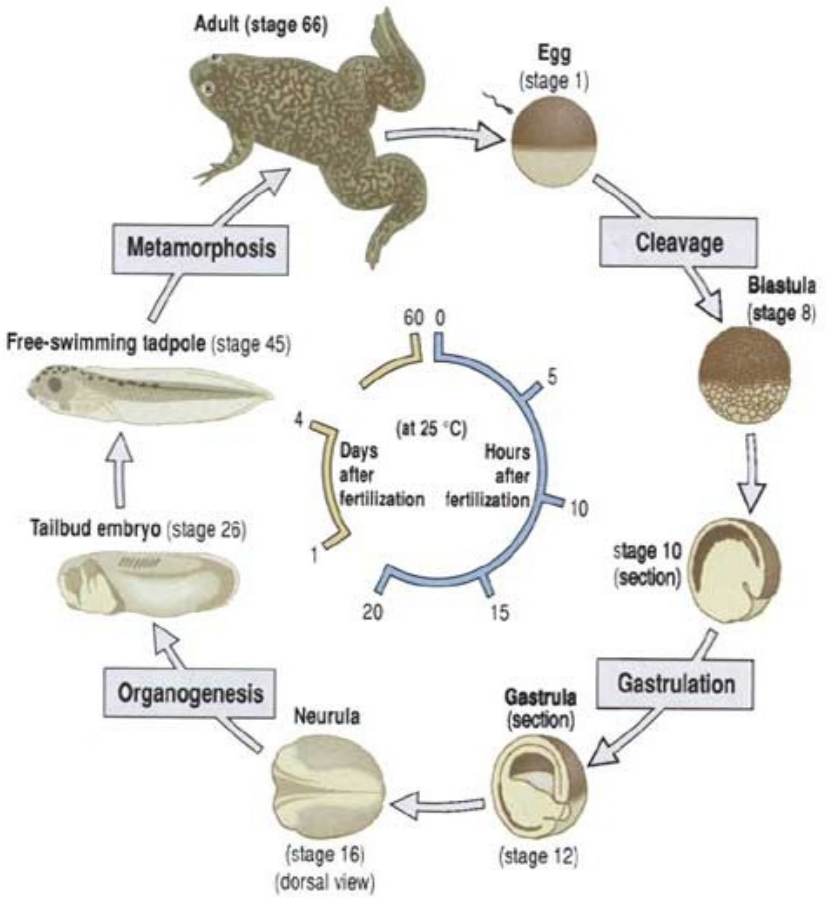
- 100 embryos per replicate
- 6-7 days
- no food
- T, pH, O_2
- Endpoints: hatching, survival, morphology, bahavior, weight, lenght, anomalies

FETAX – Frog Embryo Teratogenicity Assay Xenopus

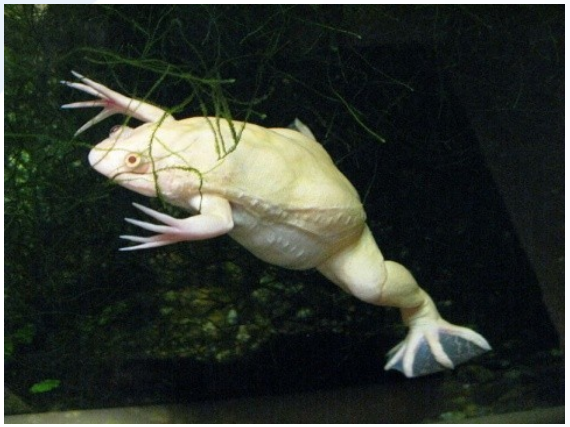
- Choriogonadotropin → eggs after 9-12h
- 25 embryos per Petri dish, 10 ml test solution
- 24°C; pH 6.5-9
- 96h
- validity:
  - 6-aminonicotinamid
    LC50 2,23 mg/ml, EC50 0,005 mg/ml
- mortality, growth, abnormalities
- Atlas of Abnormalities – John A. Bantle







FETAX



NaCl	625 mg
NaHCO3	96 mg
KCl	30 mg
CaCl2	15 mg
CaSO4.2H2O	60 mg
MgSO4	75 mg
H2O	1000 ml
pH	7,6 – 7,9

Table 4.11 The Frog Embryo Teratogenesis Assay: Xenopus (FETAX)

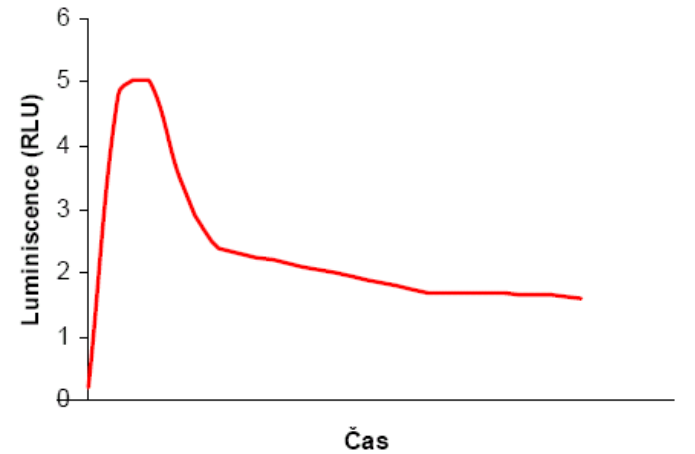
Test type	96 h static renewal
Organism	*Xenopus laevis*
Age of parent organism	Adult male: at least 2 years of age Adult female: at least 3 years of age
Size of parent organism	Adult male: 7.5–10 cm in crown-rump length Adult female: 10–12.5 cm in length
Feeding	Adult: three feedings per week of ground beef liver; liquid multiple vitamins should be added to the liver in concentrations from 0.05–0.075 cc/5 g liver
Experimental design	
Test vessel type and size	Adults: large aquarium or fiberglass or stainless steel raceways; side of tank should be opaque and at least 30 cm high. Breeding adults: 5- or 10-gallon aquarium fitted with a 1-cm mesh suspended approximately 3 cm from the bottom of the tank; nylon or plastic mesh is recommended; aquarium should be fitted with a bubbler to oxygenate the water; the top of aquarium should be covered with an opaque porous material such as a fiberglass furnace filter Embryos: 60-mm glass or 55-mm disposable polystyrene Petri dishes
Test solution volume	Adults: water depth should be 7-14 cm Embryos: 10 ml per dish
Exposure to test substance	Continuous throughout test
Replacement of test material	Every 24 h
Number of concentrations	5
Number of replicates per sample	2
Number of organisms per chamber	Adults: 4–6 per 1800 cm^2 of water surface area Breeding adults: 2 Embryos: 25
Test duration	96 h
Physical and chemical parameters	
Temperature	Adult: 23 ± 3°C Embryos: 24 ± 2°C
Photoperiod	12 h light / 12 h dark
pH range	6.5 to 9
TOC	10 mg/l
Alkalinity and hardness	Between 16 and 400 mg/l as $CaCO_3$
Endpoint	Acute (mortality) and subacute (teratogenesis)

Vibrio fisheri test (ISO 11348)

- 5-30 min
- luminiscence inhibition

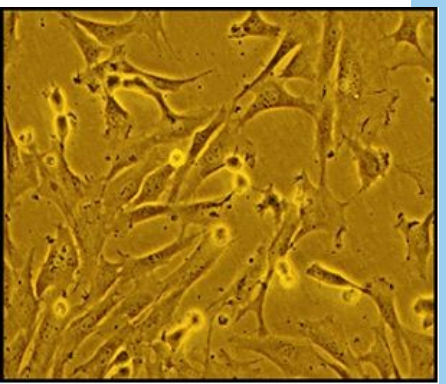
- problem with particles and colour

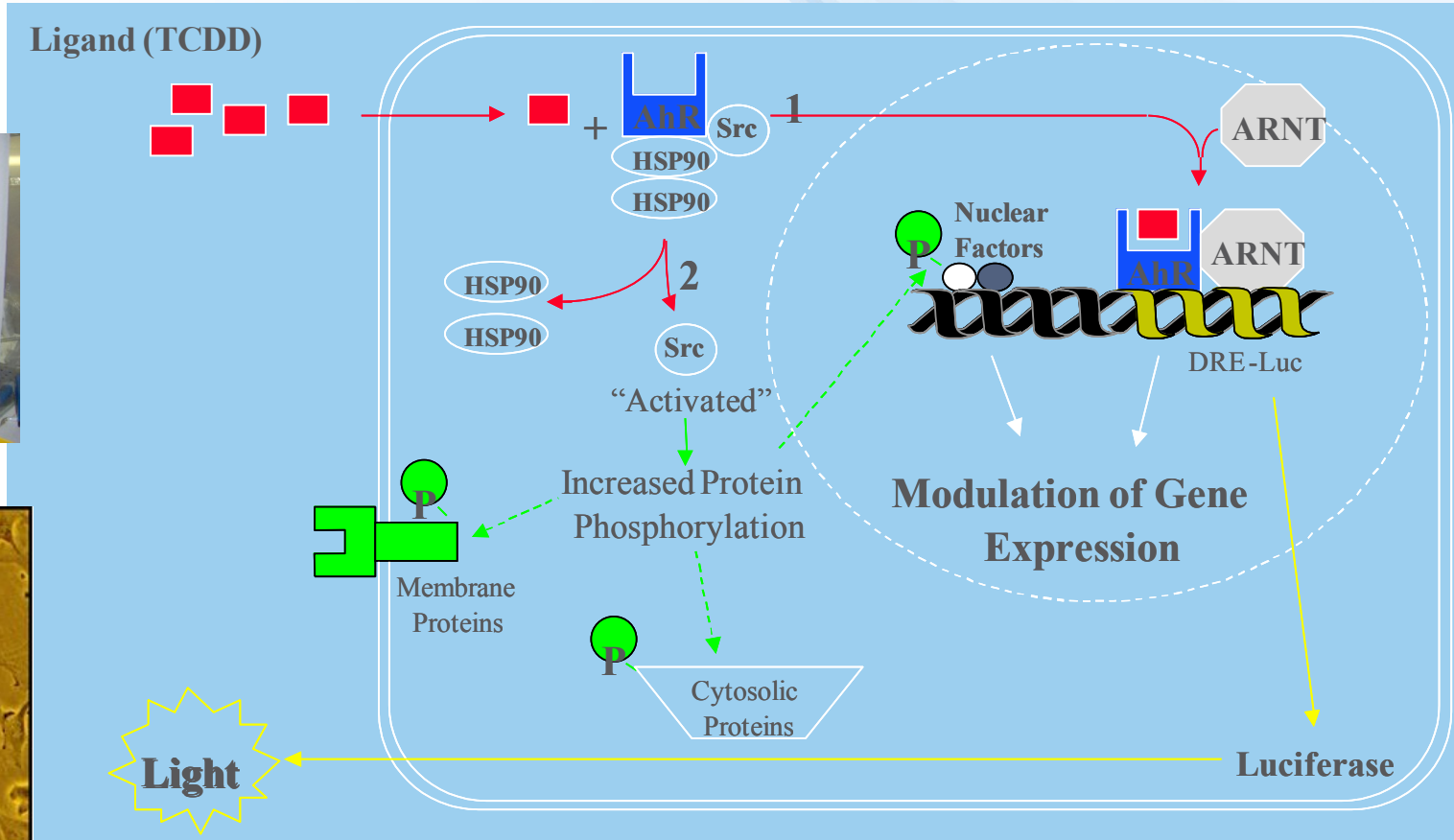
  ➔ flash test



Specific mechanisms of toxicity

- Cell lines (H4IIE.luc – diox.; MVLN, T47D.Luc – estrog.)
- Nuclear receptors (AhR, ER, AR, RAR/RXR)
- AhR/ER – luciferase reporter gene
- CALUX (Chemical Assisted LUciferase eXpression)





Adapted from Blankenship (1994)

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Soil ecotoxicology bioassays

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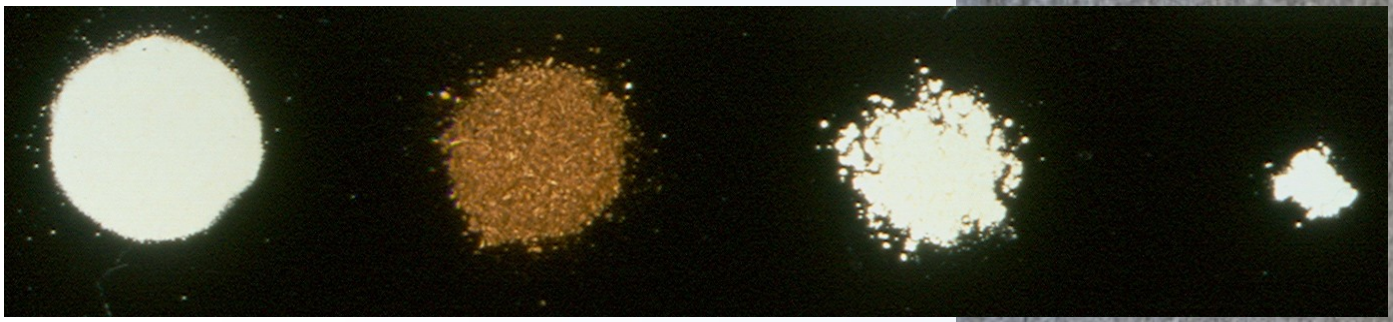
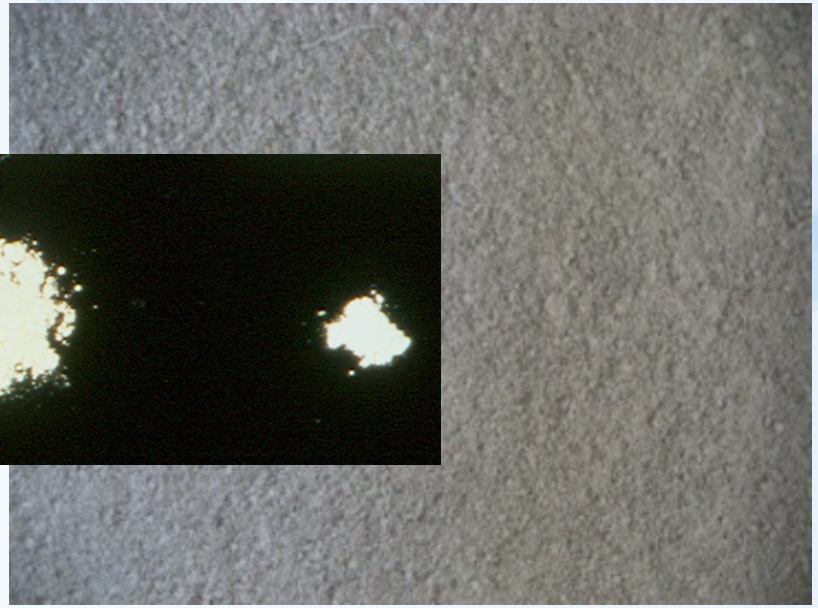
Exposure methods

- **Tested chemical mixed with soil**
  - **Artificial soil (OECD, ISO)**
  - **Real soil (LUFA 2.2 …)**





- Topic applications, injections, forced feeding … not so relevant

What is artificial soil ?

Soil component	Content expressed on % dry mass basis
• Sphagnum peat (air dried), finely ground and with no visible plant remains	10
• Kaolinite clay (air dried), containing not less than 30 % kaolinite	20
• Industrial quartz sand (air dried), predominantly fine sand with more than 50 % by mass of particle size 0,05-0,2 mm (amount dependent on calcium carbonate required)	70
• Calcium carbonate ($CaCO_3$, pulverised, analytical grade) to obtain an initial pH of 6.0 ± 0.5	0.3-1.0

OECD 1984. Guideline for testing chemicals 207. Earthworm acute toxicity test.

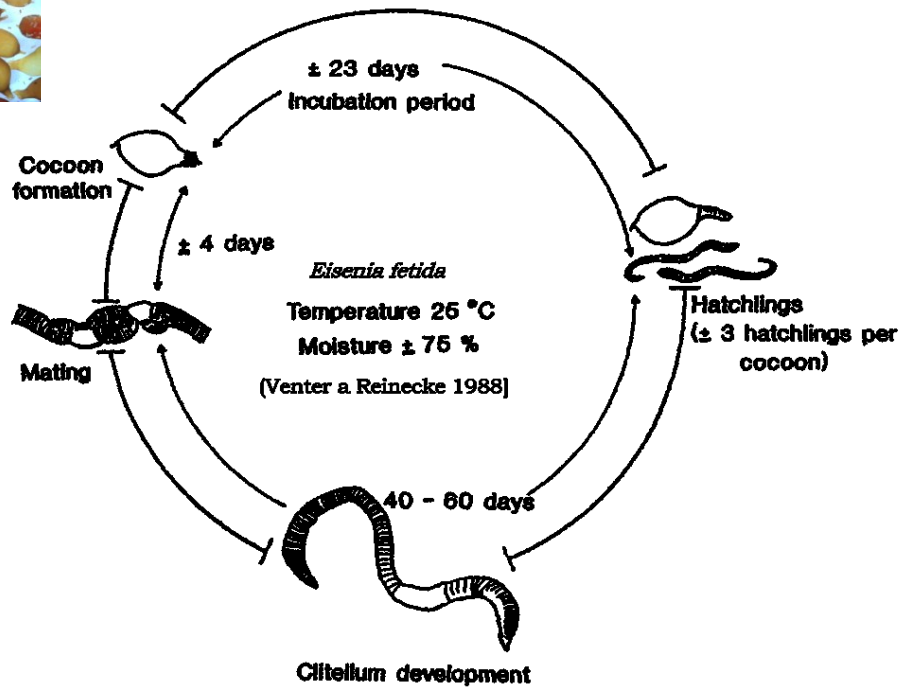
- **Is standard medium for many soil bioassays …**
- **Is much more relevant than solution, agar, filter paper …**
- **Should solve problem of high variability of natural soils …**
- **Should resemble natural loamy soil …**
- **Should enable the toxicity extrapolation to natural soils …**

LUFA soil (http://lufa-speyer.de/)

- Landwirtschaftliche Untersuchungs und Forschungsanstalt Speyer
- 4 EUR / 1 kg

	LUFA 2.1	LUFA 2.2	LUFA 2.3	LUFA 5M	LUFA 6S
organic carbon (%)	0.81 ± 0.21	2.16 ± 0.40	0.98 ± 0.05	1.29 ± 0.20	1.75 ± 0.11
particles < 0.02 mm (%)	8.2 ± 0.9	13.9 ± 1.1	22.7 ± 1.1	25.3 ± 1.8	65.1 ± 2.7
pH (0.01M $CaCl_2$)	5.1± 0.4	5.4 ± 0.1	6.4 ± 0.6	7.2 ± 0.1	7.2 ± 0.1
cation exchange capacity (meq/100g)	4± 1	10 ± 1	8 ± 2	15 ± 3	22 ± 6
water holding capacity (g/100g)	33.2 ± 1	48.2 ± 5	34.4 ± 2	42.1 ± 4	40.7 ± 5
weight per volume (g/1000ml)	1404 ± 46	1197 ± 60	1291 ± 30	1212 ± 56	1264 ± 90
Particle size (mm) distribution according to German DIN (in %):					
<0.002	3.0 ± 0.9	6.4 ± 0.9	9.4 ± 0.9	10.8 ± 1.3	42.1 ± 1.8
0.002 - 0.006	2.2 ± 0.7	3.5 ± 0.7	4.2 ± 0.8	5.4 ± 0.3	10.8 ± 0.7
0.006 - 0.02	2.9 ± 0.7	3.8 ± 0.7	9.1 ± 0.5	9.1 ± 0.5	12.1 ± 1.3
0.02 - 0.063	5.3 ± 1.8	5.4 ± 1.2	18.6 ± 2.3	19.5 ± 1.3	14.1 ± 2.5
0.063 - 0.2	27.0 ± 3.1	35.4 ± 2.3	29.3 ± 3.4	38.9 ± 1.0	8.7 ± 0.9
0.2 - 0.63	57.2 ± 4.3	44.8 ± 2.7	26.9 ± 0.7	14.9 ± 1.0	9.0 ± 0.3
0.63 - 2.0	2.4 ± 0.6	0.7 ± 0.1	2.5 ± 0.8	1.4 ± 0.1	3.2 ± 0.7
soil type	sand (S)	loamy sand (lS)	loamy sand (lS)	silty sand (uS)	clayey loam (tL)
Particle size (mm) distribution according to USDA (in %)					
<0.002	3.0 ± 0.9	6.4 ± 0.9	9.4 ± 0.9	10.8 ± 1.3	42.1 ± 1.8
0.002 - 0.05	8.8 ± 1.8	12.2 ± 0.6	29.8 ± 3.0	27.5 ± 2.2	36.0 ±2.3
0.05 - 2.0	88.2 ± 1.2	81.4 ± 1.2	60.8 ± 2.6	61.7 ± 3.2	21.9 ± 1.6
soil type	sand	loamy sand	sandy loam	sandy loam	clay

Earthworm bioassays



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Earthworm acute toxicity test

- 500 g soil + 10 adult *Eisenia fetida*
- 14 days
- mortality and weight

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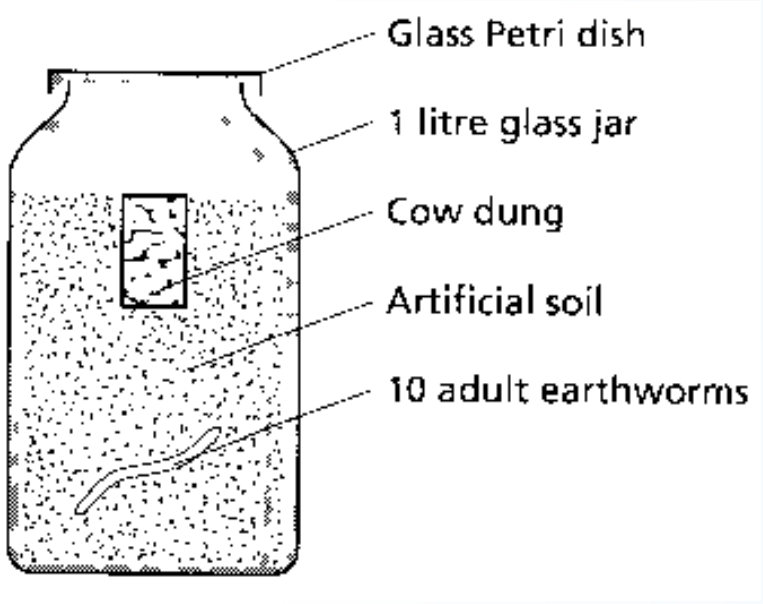
Earthworm reproduction test ISO 11268-1

- 56 days
- 500 g soil + 10 adult *Eisenia fetida*
- horse manure as food
- juveniles extracted using water bath



Eisenia fetida reproduction test ISO 11268-2

Soil preparation

WHC measurement

Water added

Soil weighted to jars

10 adults from culture

Washed

Weighting worms

10 adults to 1 jar

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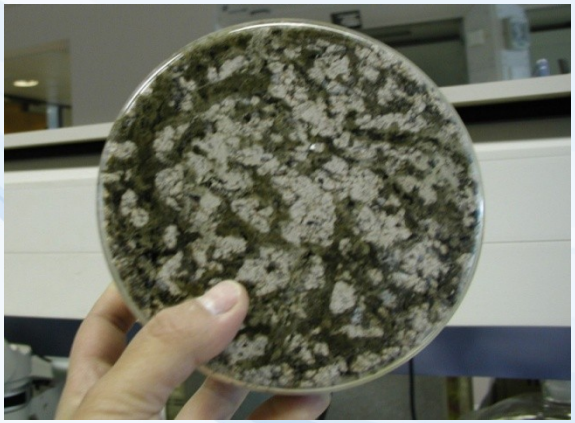
E. fetida test – after 28 days ISO 11268-2

Temperated room



Control of the jars, activity markers

Mortality assessment

Weighting the worms

E. fetida – 8 weeks

ISO 11268-2

Water bath, increasing temperature 40ºC - 60ºC

After 20 min juveniles appear

Collecting and counting juveniles

Sieving the soil

Hand sorting of cocoons

Counting

Avoidance test

ISO 17512-1

Guideline: ISO/DIS 17512 (draft)

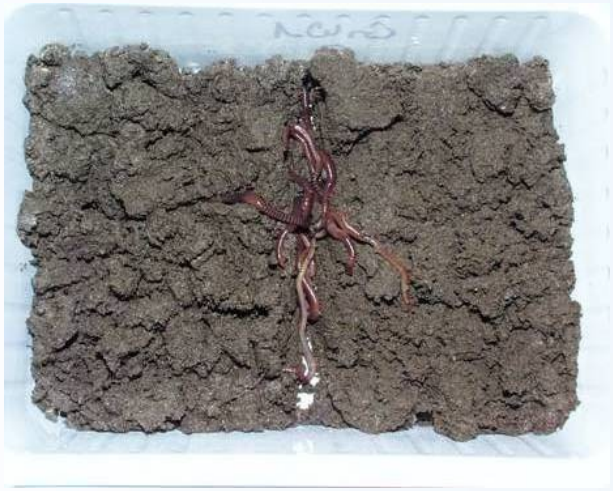
Species: ***E. andrei***

Substrate: LUFA St. 2.2 standard soil

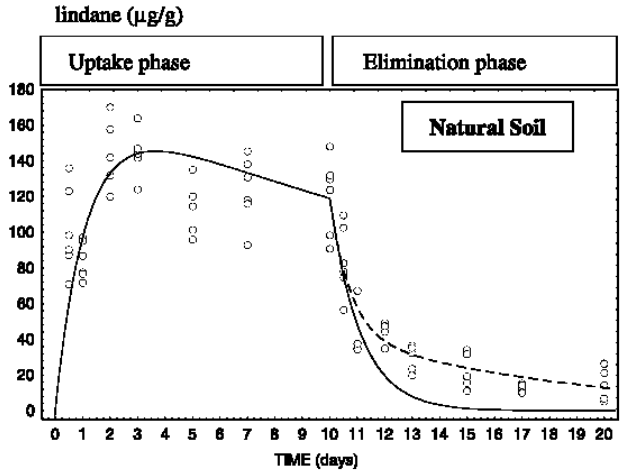
Duration: 1 - 2 days

Parameter: Behaviour of the worms

Test vessels: Dual chamber



Bioaccumulation test



Test organism	*Eisenia fetida/andrei*, or *Enchytraeus albidus*, adult worms of similar size
Test substrate	spiked artificial soil based on OECD guideline No. 207 (OECD 1984)
Control substrate	uncontaminated artificial soil based on OECD guideline No. 207 (OECD 1984)
Biological parameters	concentration of test item in worms during uptake and elimination period
Endpoints	bioaccumulation factor, uptake rate coefficient, elimination rate constant
Test duration	uptake period: until steady state or 28 d; plus 10 day elimination period
Temperature	20 ± 2°C
Light regime & light intensity	constant light; 400 to 800 lx
Test chambers	e.g. 250 mL/50 mL glass tubes; additional chambers of appropriate size for chemical analyses
Feeding during exposure	food added to soil directly after spiking; additional feeding once per week during exposure
Water	periodic addition of deionised water
Equilibration	4 days; spiked soil under test conditions
Test item	^{14}C-labelled/metal
Spiking of soil	if possible, test item dissolved in water mixed with dry soil; optional: coating of sand
Analysis of test item	in worms and soil
Number of test concentrations	1 plus control(s)
Test concentrations	to be fixed (expressed in Bq or mg kg^{-1} soil dry weight)
Number of replicates per test concentration/sampling date	at least 3 per sampling date
Number of organisms per test chamber	*Eisenia*: 1; *Enchytraeus*: 20
Determination of soil dry weight and organic carbon content in soil	4 samples after soil preparation
Determination of lipid content in biota	4 samples at end of uptake phase*
Validity of test	mortality during test period ≤ 10% (earthworms), ≤ 20% (enchytraeids)
Evaluation	Use of appropriate methods (e.g. nonlinear regression analysis, ANOVA, Dunnett´s t-test)

Enchytraeidae

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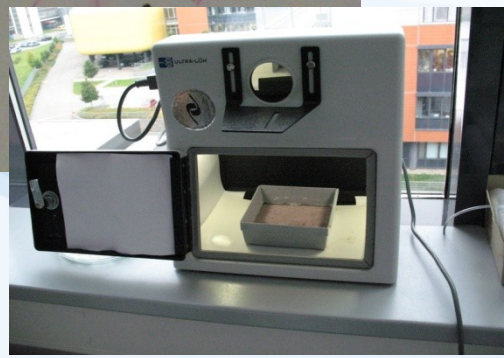
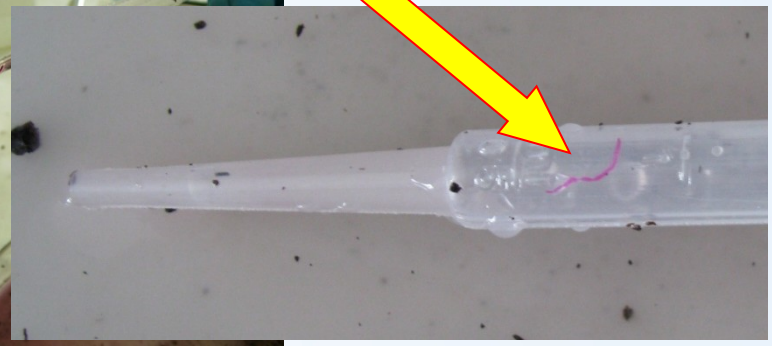
Enchytraeidae ISO 16387



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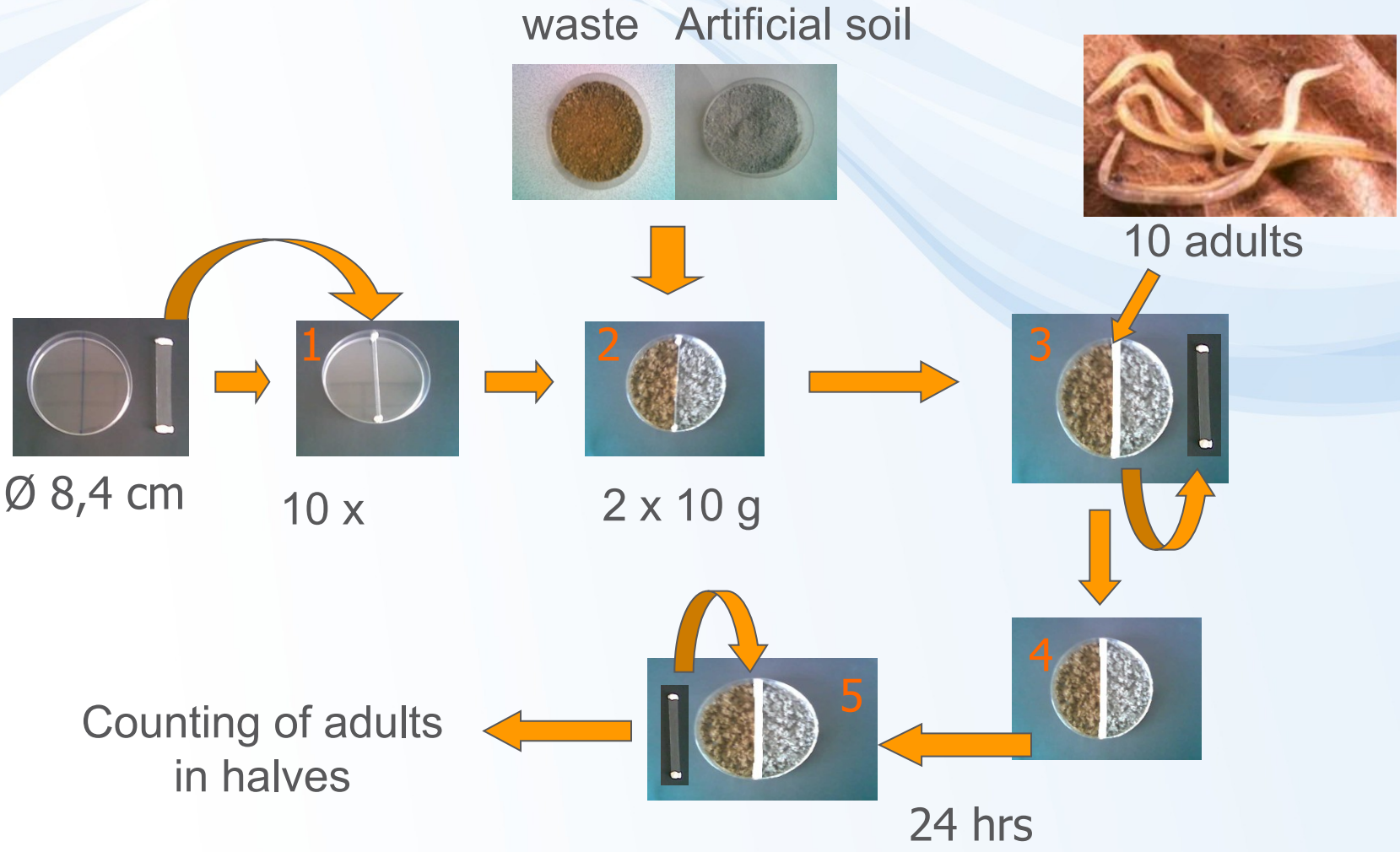
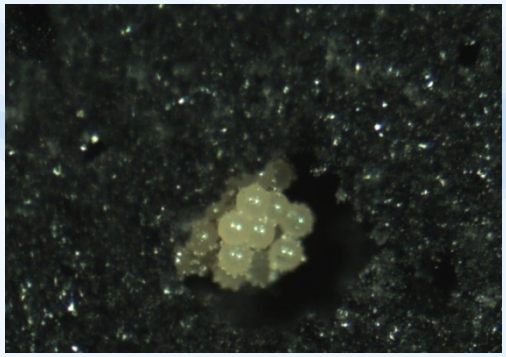
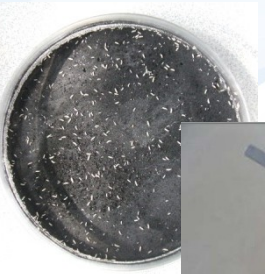
Avoidance test with *E. albidus*



Folsomia candida

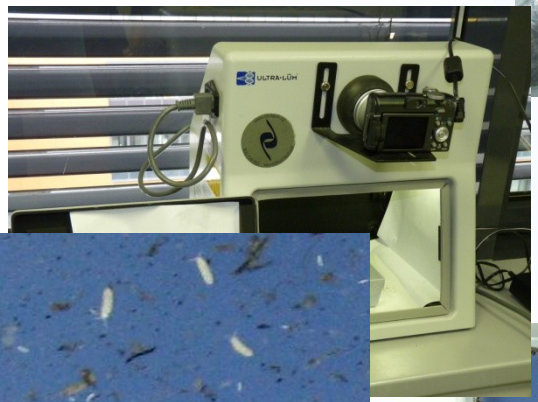
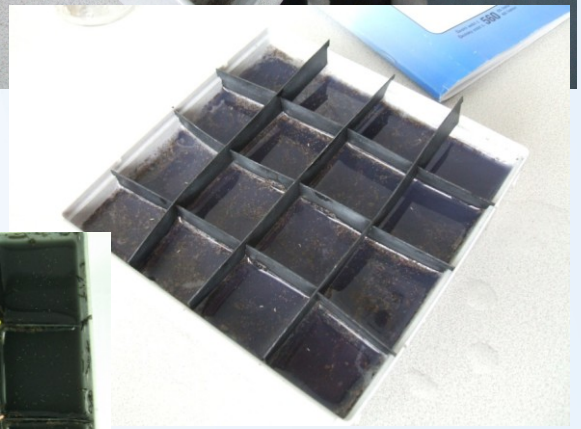
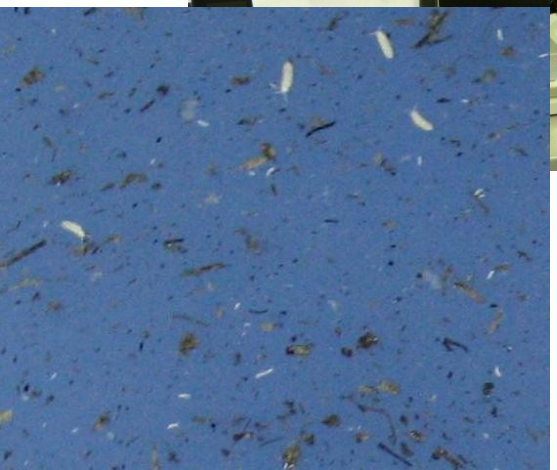
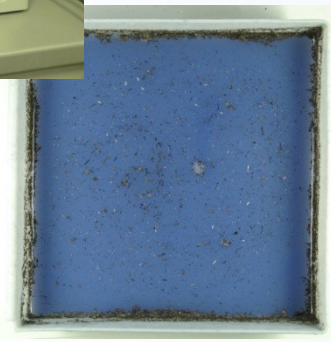
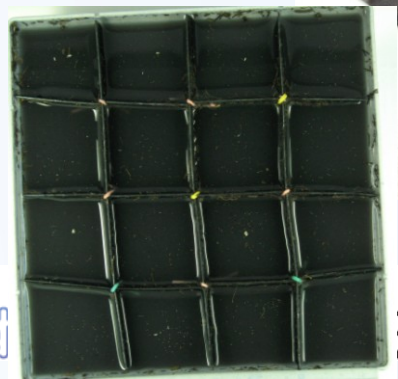


Folsomia candida ISO 11267







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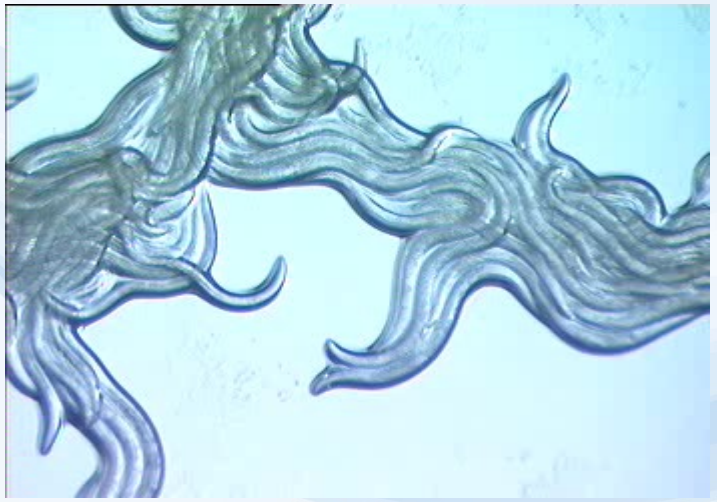
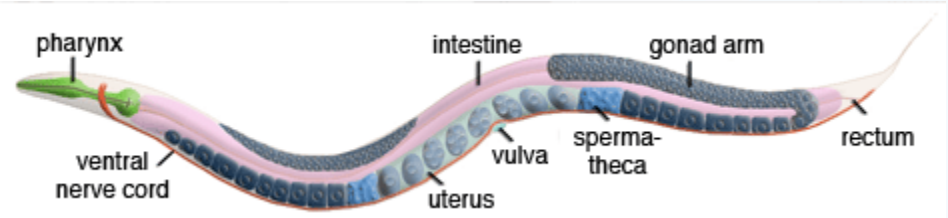
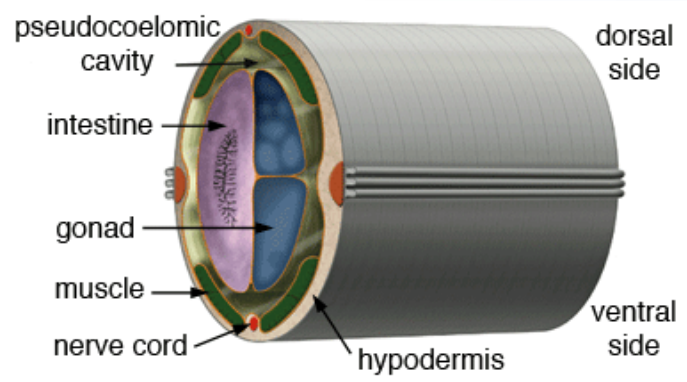
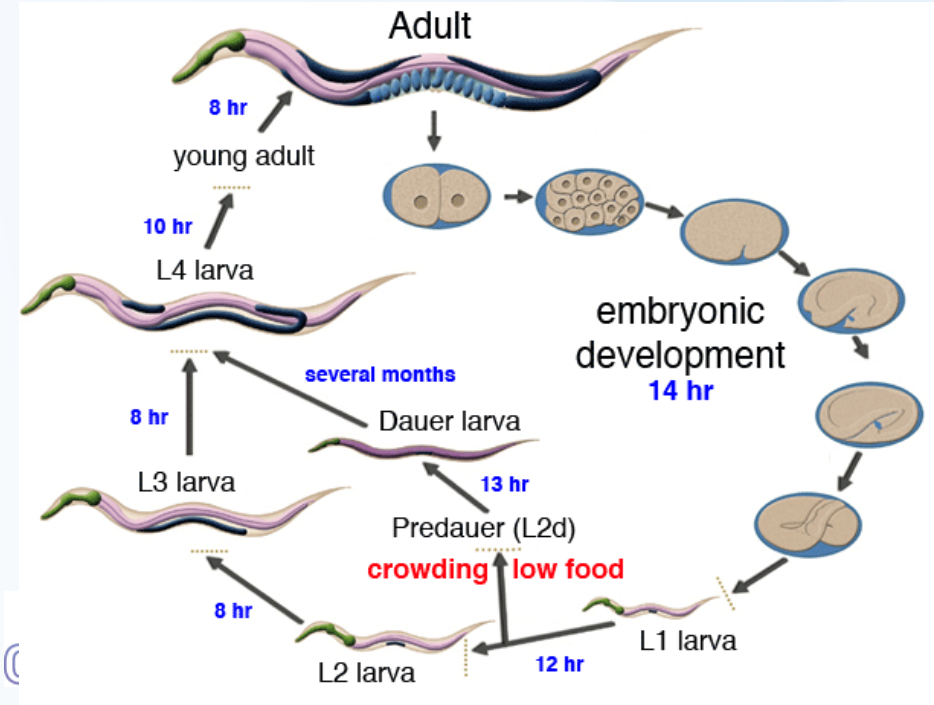
Caenorhabditis elegans test





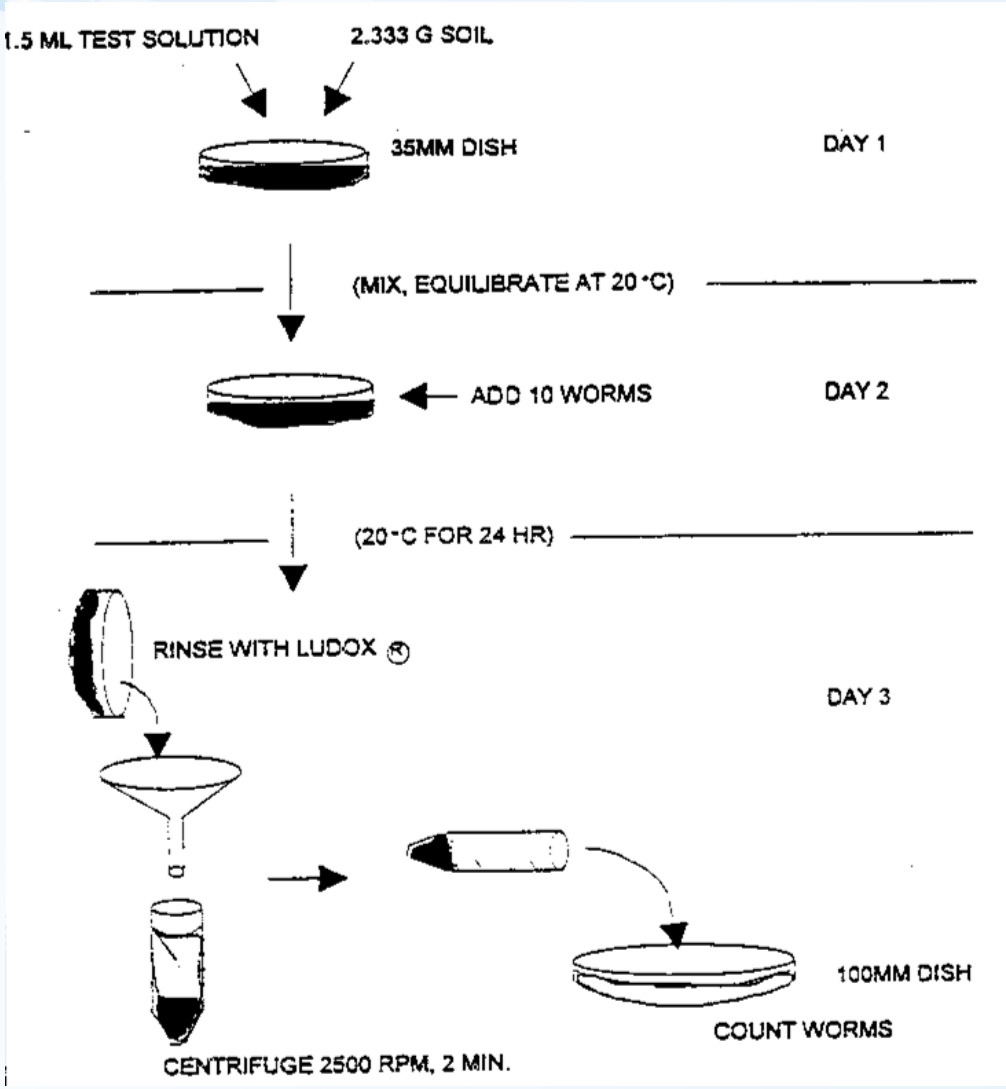




Caenorhabditis elegans test

ASTM: E2172-01 Standard Guide for Conducting Laboratory Soil Toxicity Tests with the Nematode *Caenorhabditis elegans*

ISO 10872



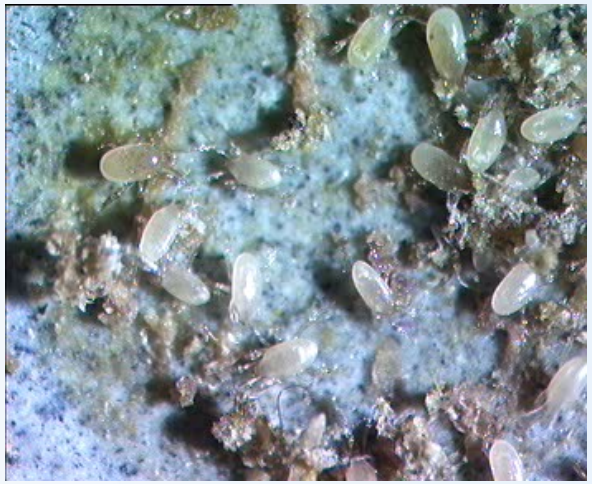
Mites

Hypoaspis aculeifer

predator

cont. soil

prey



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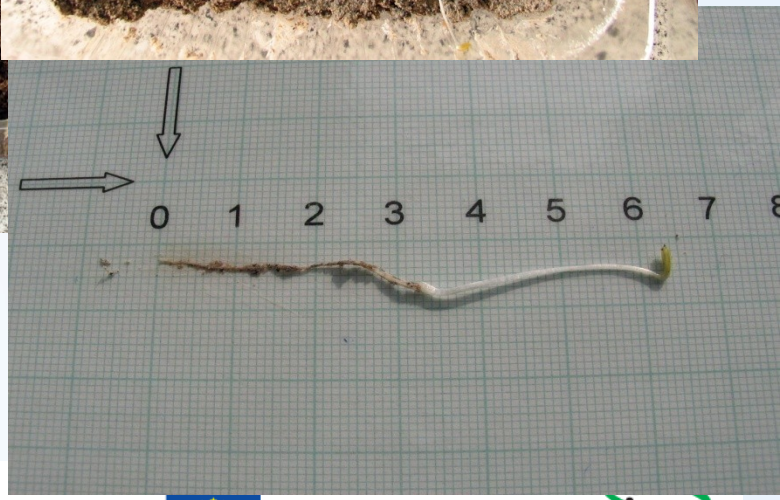
Lactuca sativa root growth ISO 11269-1

Germination tests with plants – testing toxicity in the aquatic media (*Sinapis alba*) or in soil (*Lactuca sativa*)



Higher plants chronic toxicity ISO 22030

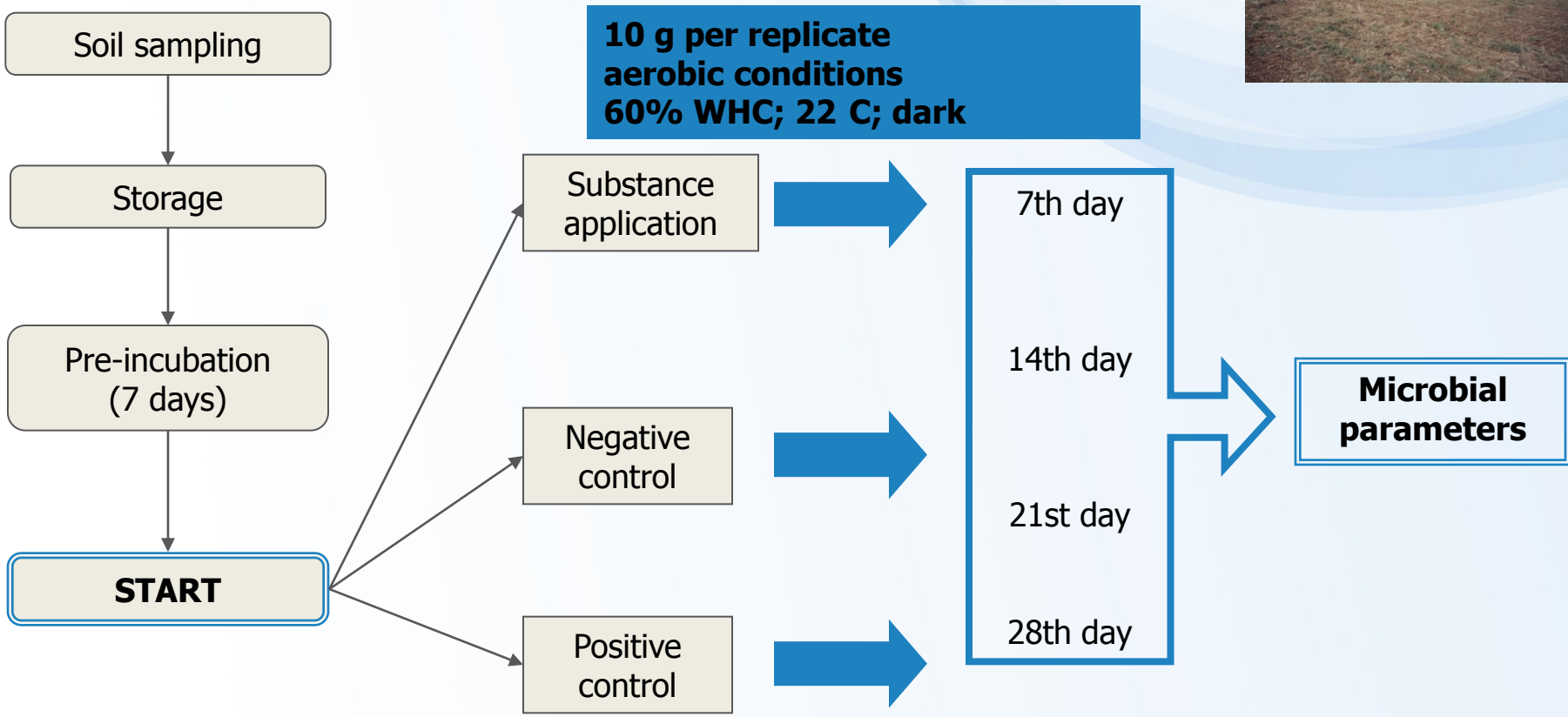
Soil microbial assay according to OECD, ISO

Real uncontaminated agricultural soil with indigenous microflora:

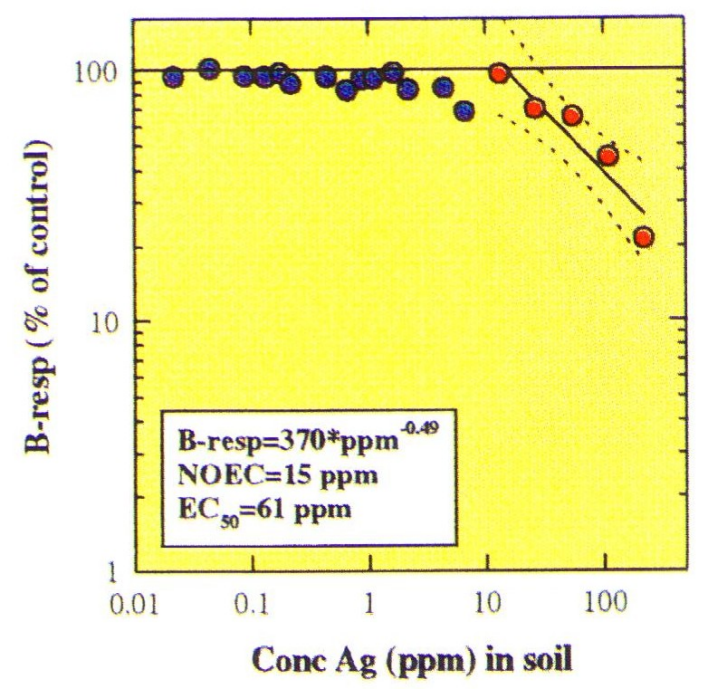
pH_{KCl} = 7 – 7.5 C_{bio} = 400 – 700 µg.$g_{d.w.}^{-1}$

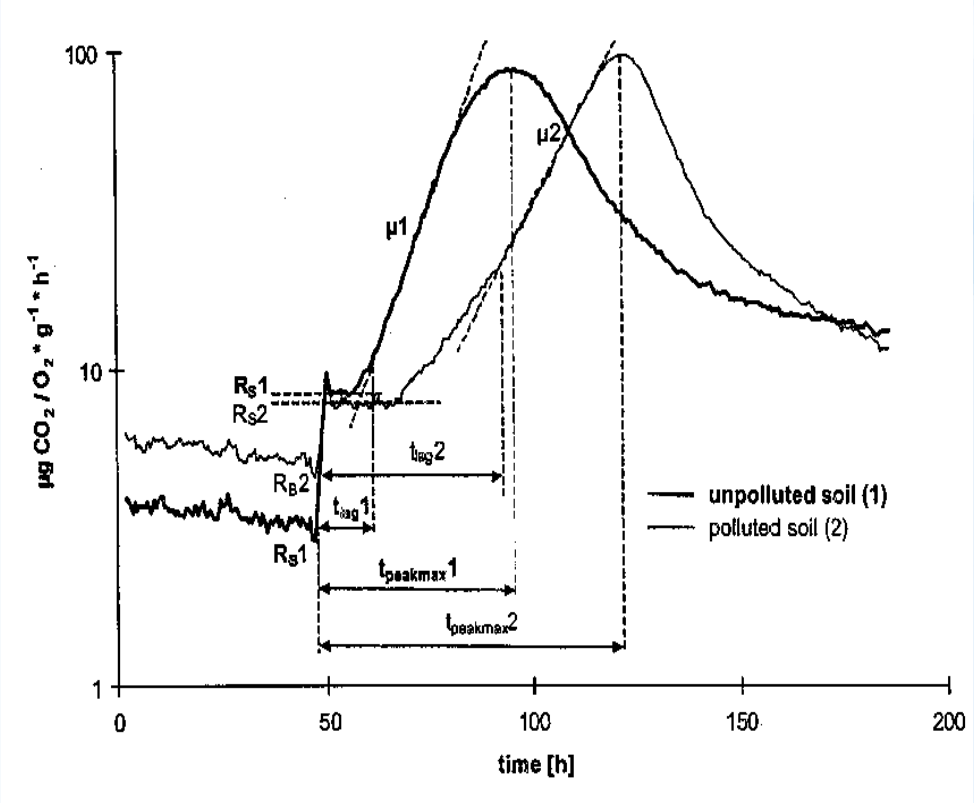
C_{org} = 1.5% BR = 0.5 – 0.7 µg CO_2-C.h^{-1}.$g_{d.w.}^{-1}$

sand = 70%



Effects on microbial respiration





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Results of the bioassays and their use

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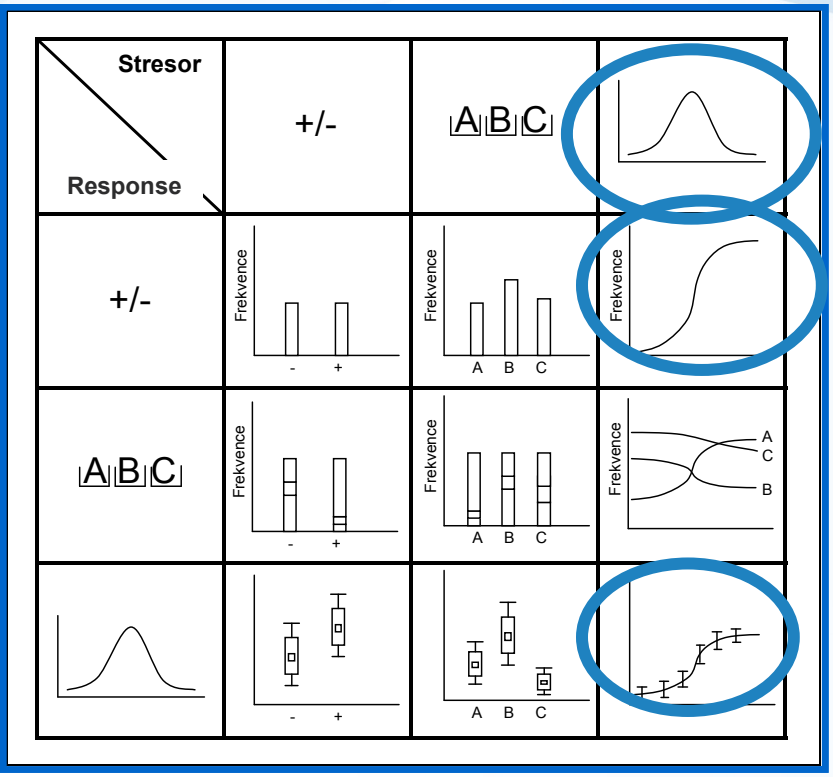
Data from ecotoxicity tests



Quantal data

Quantal data arise when a particular property is recorded to be present or absent in each individual (e.g. an individual shows an effect or it does not show an effect). Therefore, these data can exhibit only two states. Typically, quantal data are presented as the number of individuals showing the property (e.g., mortality) out of a total number of individuals observed in each experimental unit. Although this can be expressed as a fraction, it should be noted that the total number of individuals cannot be omitted.

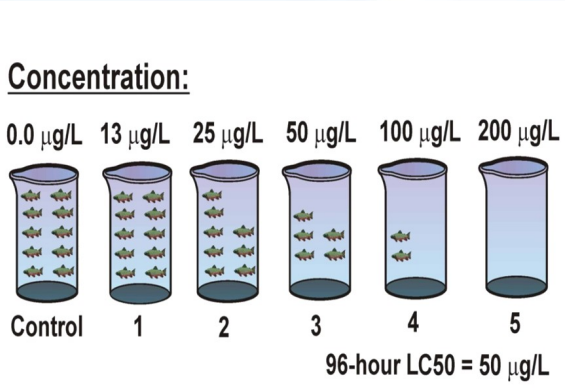
Continuous data

Data are continuous when they can (theoretically) take any value in an open interval, for instance any positive number. Examples include measurements of length, body weight, etc. Due to practical reasons the measured resolution depends on the quality of the measurement device. For example, if test units are observed once per day then 'time to hatch' can only be recorded in whole days; however, the underlying distribution of 'time to hatch' is continuous. Typically, continuous data have a dimension (e.g. grams, moles/litre).

Discrete data

Discrete data are data that have a finite or countable number of values. There are three classes of discrete data: nominal, ordinal and interval. *Nominal data* express qualitative attributes that do not form a natural order (e.g. colours). *Ordinal data* reflect the relative magnitude from low to high (e.g. an individual shows no effect, minimal effect, moderate effect or high effect)These data cannot be interpreted with regard to relative scale (i.e., an ordinal variable with a value of '4' can be interpreted as being higher then the value of '2', but not twice as high). *Interval data* (e.g., number of eggs or offspring per parent) allows the ranking of the items that are measured, and the differences between individuals and groups can be quantified. Often, interval data can be analysed as if the data were continuous. The analyses for interval discrete data are presented in this document; analyses of nominal and ordinal data are not included but will be addressed in a future revision. Ordinal data can often be reduced to quantal data.

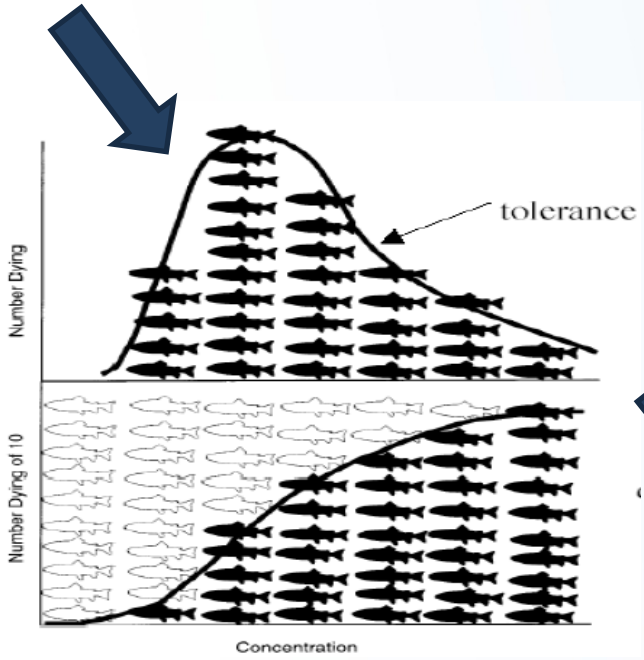
Dose(concentration) - response relationship

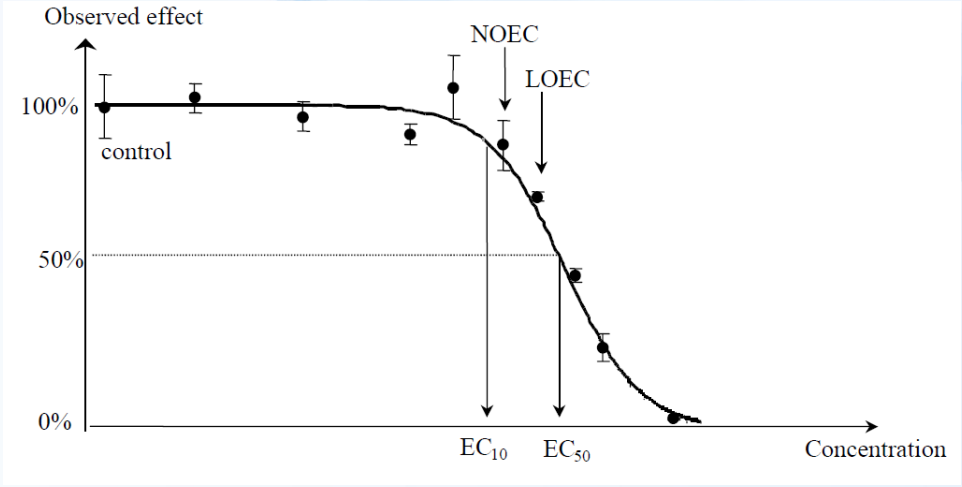


Note

Concentration and Dose

Concentration and dose both refer to the amount of test material to which the test organism is subjected. Concentrations are used to describe the amount of test material in the testing environment (e.g., mg/L in water, mg/kg in soil or mg/kg in food). Doses are used to describe the amount of test material administered to a subject (e.g., mg/kg-bodyweight in an avian bolus study). Statistical methods for both types of studies are identical; however, interpretations are different. Although "concentration" is used throughout this document, all the statistical methods presented here also apply to studies in which a dose is used.





No Observed Effect Concentration (NOEC)
Lowest Observed Effect Concentration (LOEC)
ECx (x % effects concentration)
LCx (x % lethal concentration)

Bioassay vs real ecosystem

- Bioassay is only simplified ecosystem model
- species differs, matrix differs, single species vs multispecies, individuals vs populations

Aporrectodea caliginosa

Lumbricus terrestris

vs

Eisenia fetida – compost worm !!

Bioassay vs reality

Objective = to protect real ecosystems, safe concentration

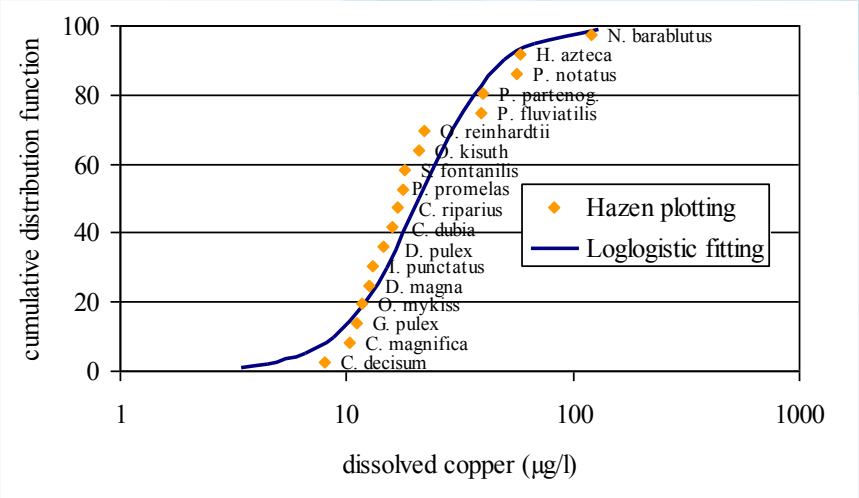
➔ need to extrapolate bioassay results to be valid for ecosystems

- how much information we have?
- uncertainty factors, 1, 10, 100, 1000

Extrapolace

Data	Assessment factor
L(E)C50 short-term toxicity tests	1000
NOEC for 1 long-term toxicity test	100
NOEC for additional long-term toxicity tests of 2 trophic levels	50
NOEC for additional long-term toxicity tests of 3 species of 3 trophic levels	10

Species sensitivity distribution (SSD)



HC5 = 95% protection level

PNEC (limits, EQS)

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Risk assessment with earthworms

Prüfung der Auswirkungen auf Regenwürmer

Labortest mit Kompostwurm

1. Akute Toxizität (2 Wochen)

Bewertung: Mortalität, Körpergewicht

$$TER = \frac{LC50}{PEC} < 10$$

Kokons des Kompostwurms

2. Einfluss auf die Fortpflanzung (8 Wochen)

Bewertung: Anzahl der Jungtiere, Körpergewicht

$$TER = \frac{NOEC}{PEC} < 5$$

einheimische Regenwurmart

3. Auswirkungen im Freiland (1 Jahr)

Bewertung: Individuenzahlen, Risiken für Populationen und Lebensgemeinschaften

Summary - Take home message

- Bioassays are necessary addition to chemical analyses
- Lot of standardized/experimental bioassays to choose from
- Necessary know-how (lab tricks, weakpoints, when to use, interpretation, data evaluation …)
- No single test gives all information - battery of bioassays

Thanks for your attention!!!!

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