MUNI | RECETOX

E5080 / E0323

Ecotoxicology

Ecotoxicological bioassays

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Content

- Introduction – what, why, how, concept
- Types of bioassays
- Ecotoxicological bioassays' design and results
- Aquatic bioassays - examples
- Soil bioassays – examples

Introduction – what, why, how, concept

Protection of environment / nature

- Is and must be primary aim of **sustainably developing society**
- ***why?***

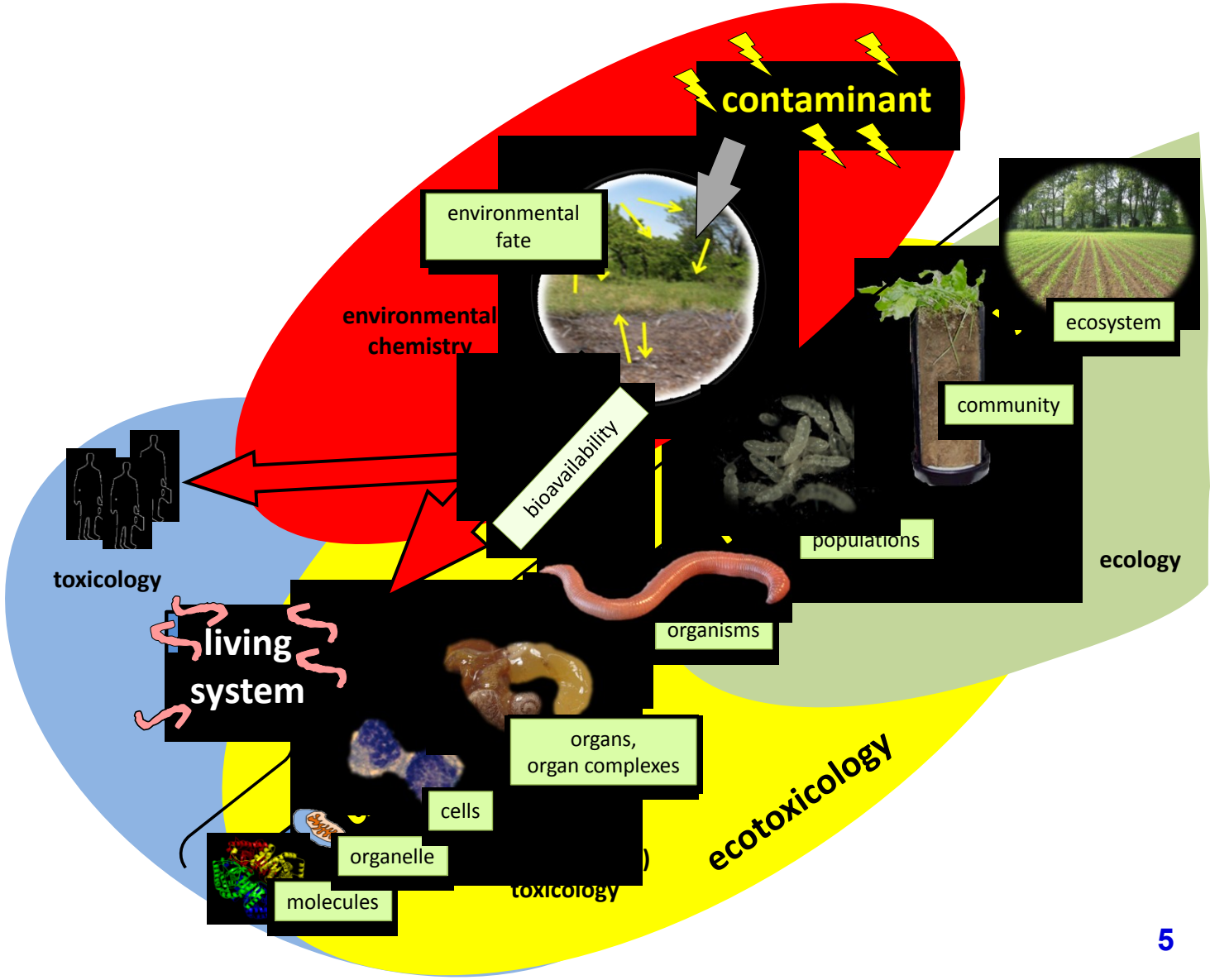
How to protect ?

- Policy
- Legislation
- Research
- Education

Ecotoxicology – offers knowledge and tools useful for the effective and reasonable environmental protection (these tools = ecotoxicological bioassays)

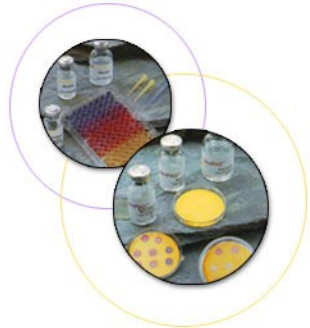
Ecotoxicology

Discipline on the border
of ecology and toxicology
studying and evaluating
direct and indirect effects
of man-made or natural harmful chemicals or other stressors
on animals (except human), plants and microorganisms
at all levels of biological organization



Ecotoxicity bioassay, ecotoxicity test

- **a tool (method, procedure ...) for ecotoxicological research and praxis – for environmental legislation and protection**
- **biota** (tissue, organism, population, ecosystem …) is exposed to **chemicals** (and/or other stress factors), in the lab (controlled conditions) or in the field (less controlled) and effects are evaluated and related to exposure **– its experiment (!)**
- **WHY?** To understand the cause-effects relationships (causality, dose-response …)
  - but sometimes also e.g. accumulation, biodegradation ...



Ecotoxicity bioassay, ecotoxicity test

toxicity test versus ecotoxicity test

→ see toxicology versus ecotoxicology

Why ecotoxicological tests ?

CHEMICAL ANALYSES ALONE CANNOT show real risk to living organisms:

(1) real exposure varies according to **bioavailability** of toxic substances

(2) in real environment, there is always **mixture** of toxicants that acts differently from individual compounds

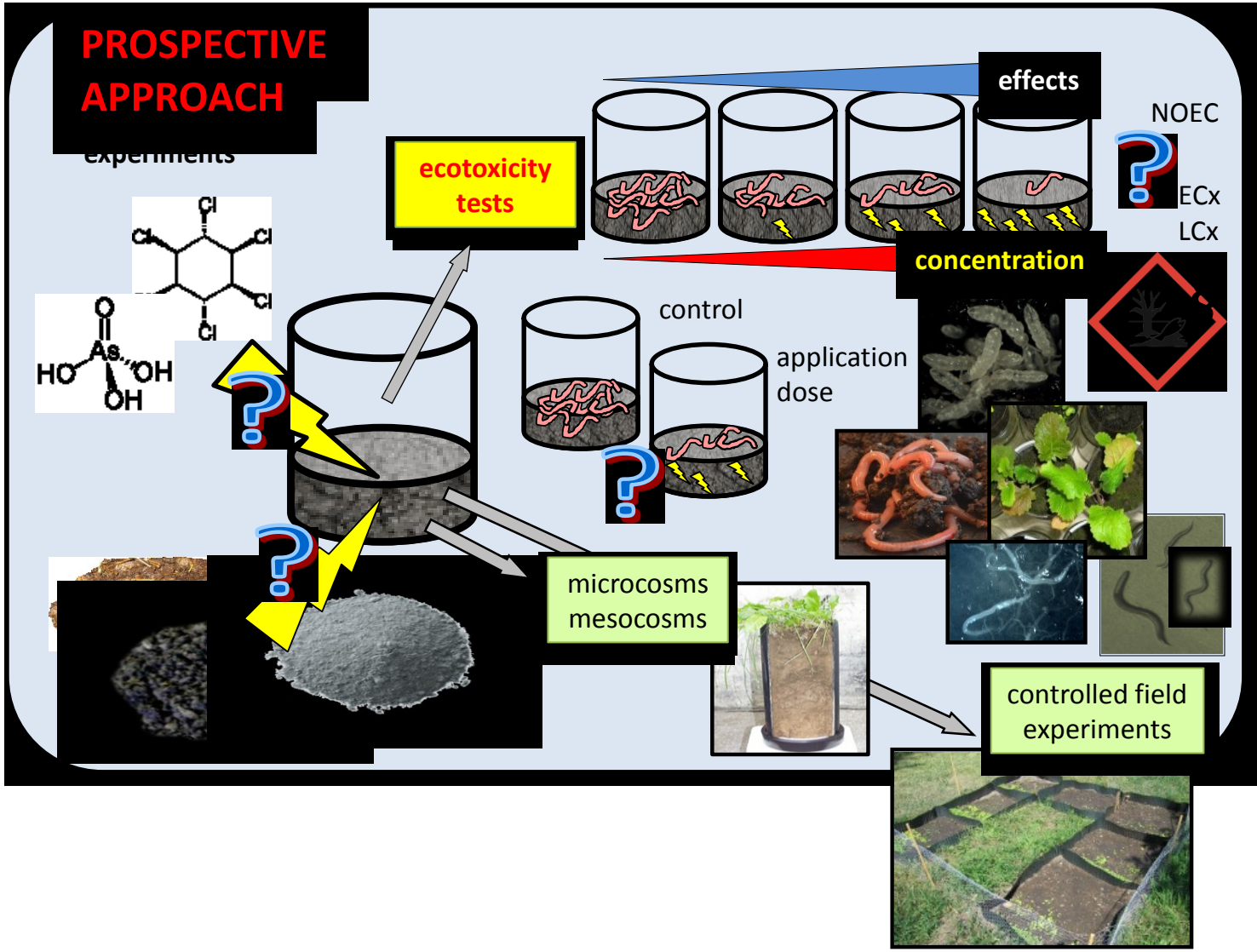
(3) negative effects of **matrix** itself, regardless of toxicant content, on organisms or interaction of matrix with effects of toxicants

(4) spectrum of **analytical methods** (i.e. limit values) is limited and **un-analysed** significantly toxic substances may be present in the sample

ADVANTAGES of chemical analyses
- Reproducibility, standard-ability
- Exact numerical outputs understandable to all people: use in the law

Bioassays useful for:

- **prospective ecological risk assessment**
  - using bioassays for chemical compounds, pesticides
  - using bioassays for materials, mixtures
  - before they enter the environment



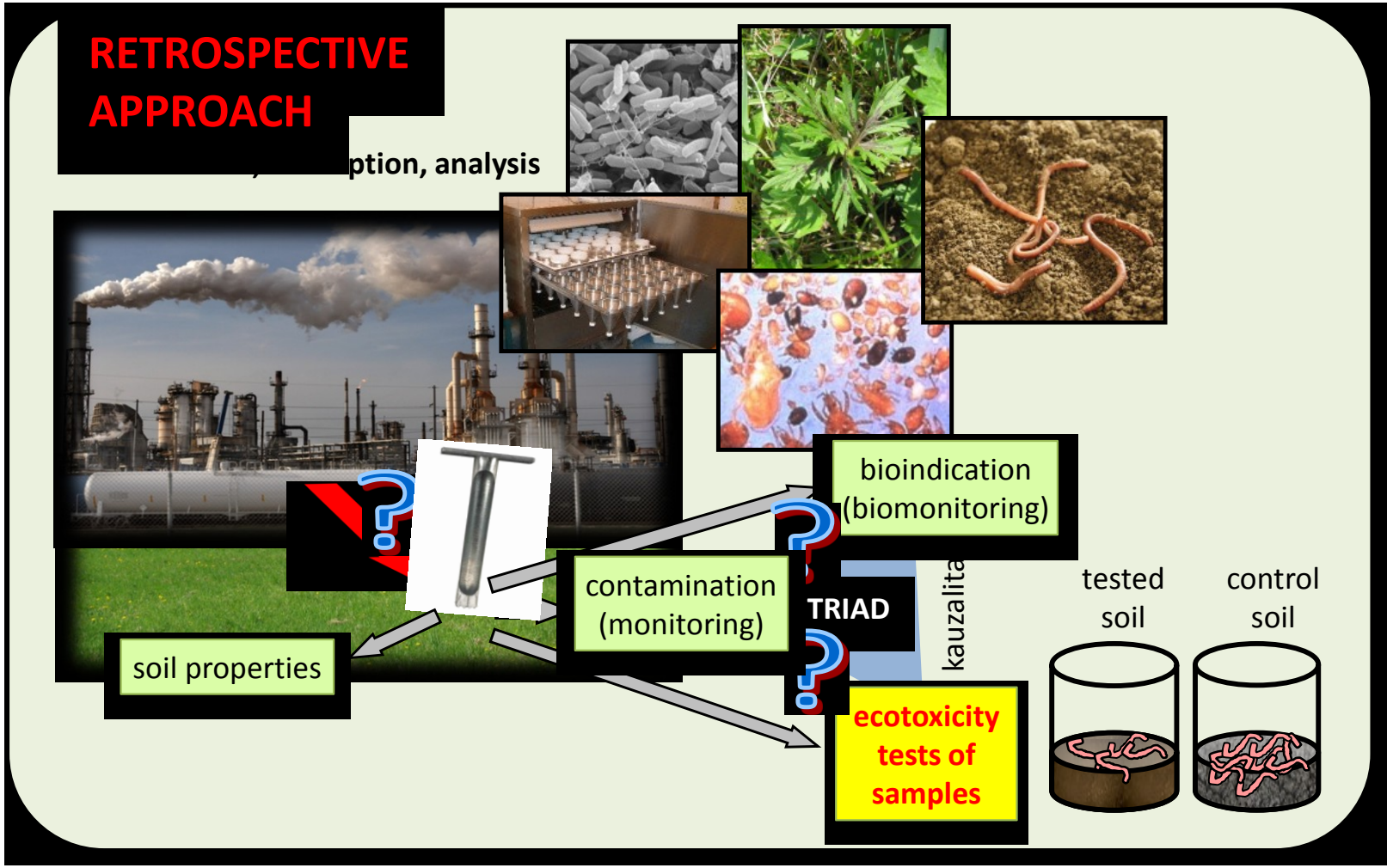
Bioassays useful for:

Objectives of the prospective approach:

- evaluation of **hazards** of contaminants (individual and mixtures) and other stressors
- analysis of relationships between concentration and effect ("dose-response relationship")
- **hazard** quantification, **risk** assessment (including legislatively required assessment) and prediction of negative effects of real environmental samples
- setting limit values for (legislative) regulation of chemical substances, pesticides and materials that may come in contact with the environment (waste, sludge, fertilizers…)
- knowledge of processes and mechanisms related to the effects of contaminants (or other stressors) on biota, fate and bioavailability of contaminants in the environment and exposure of organisms
- understanding causes of harmful effects of contaminants on organisms

Bioassays useful for:

- **retrospective ecological risk assessment**
  - using bioassays for real environmental samples
  - searching the causalities between pollution and effects



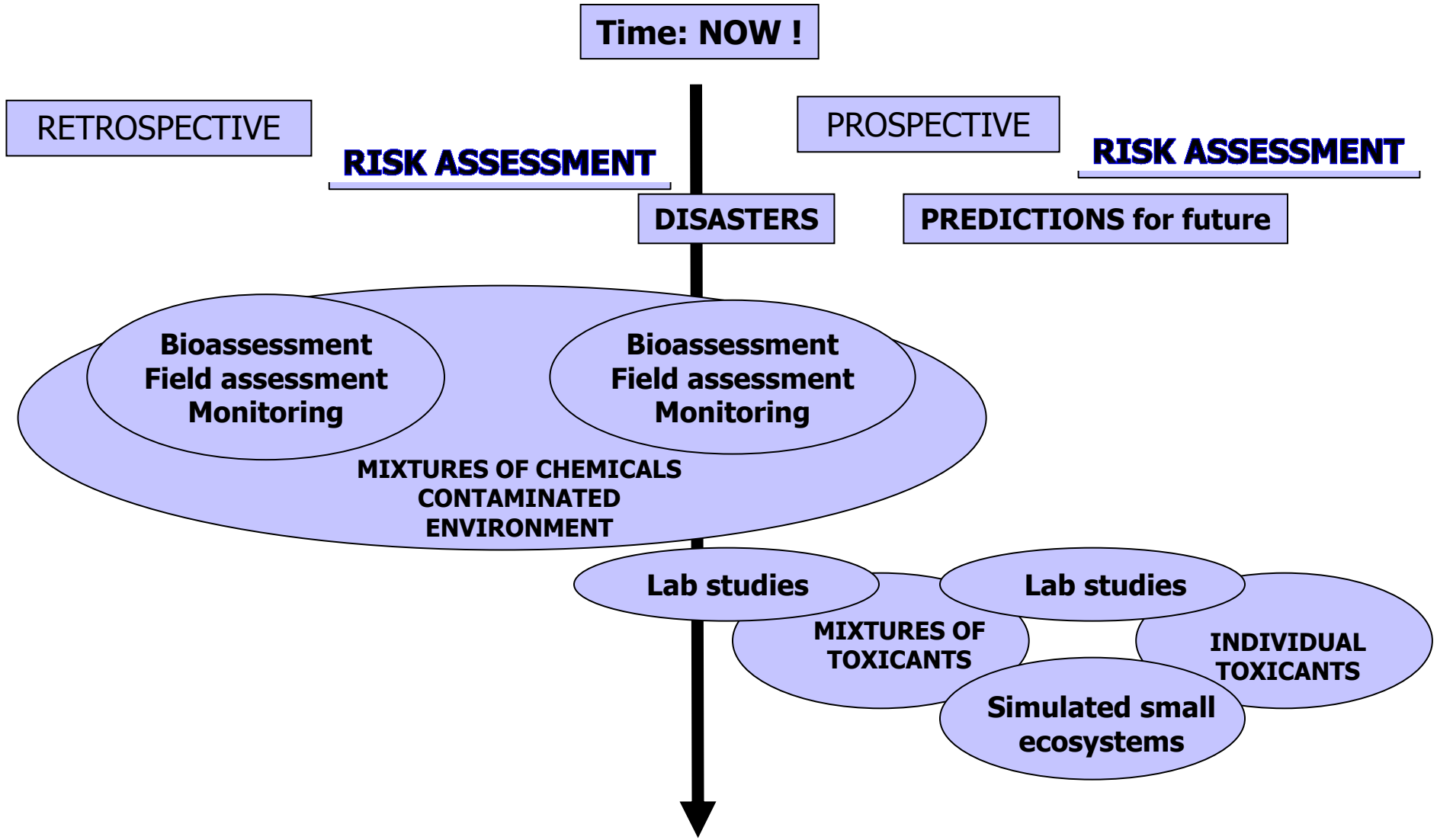
ISO 19204:2017 Soil quality — Procedure for site-specific ecological risk assessment of soil contamination (soil quality TRIAD approach)

Bioassays useful for:

Objectives of the retrospective approach:

- knowledge of the links (causality) between the occurrence and fate of contaminants (stressors) and the state of the biota
- knowledge of past events and their regularities allows to estimate the development for the future in similar situations (prediction)
- evaluation of interventions on real components of the environment in real ecosystems (evaluation of fertilization, remediation, assessment of contaminated sites…)
- knowledge of processes and mechanisms related to the effects of contaminants (or other stressors) on the biota, the fate and bioavailability of contaminants in the environment and the exposure of organisms
- understanding the consequences of the harmful effects of contaminants, especially at higher levels of the biological organization

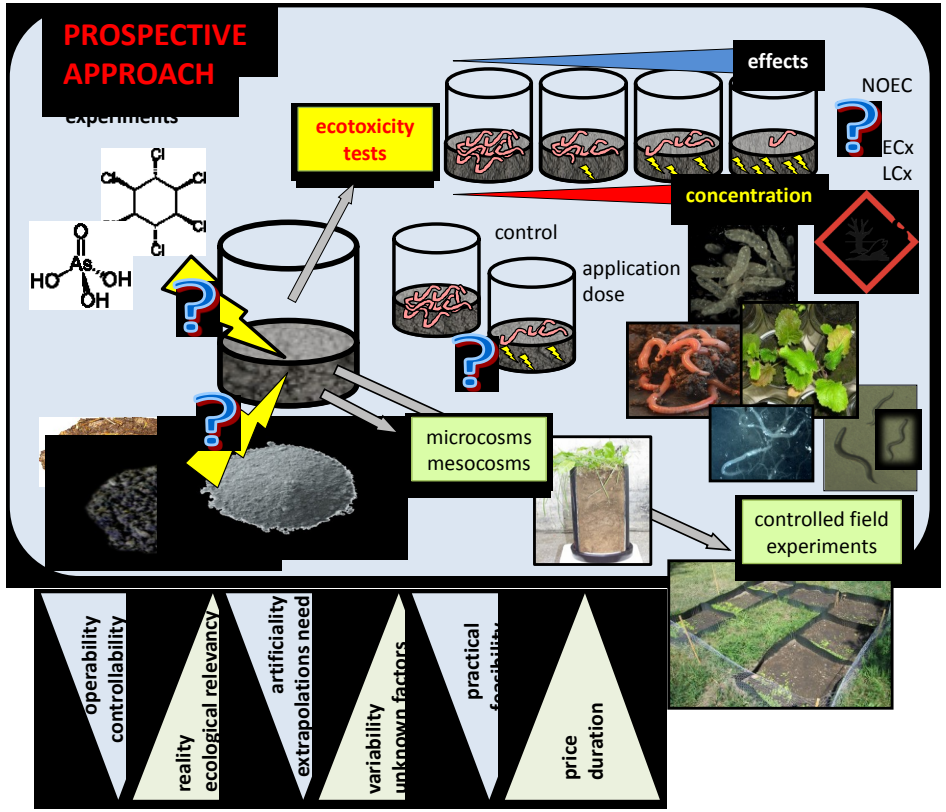
Bioassays useful for:

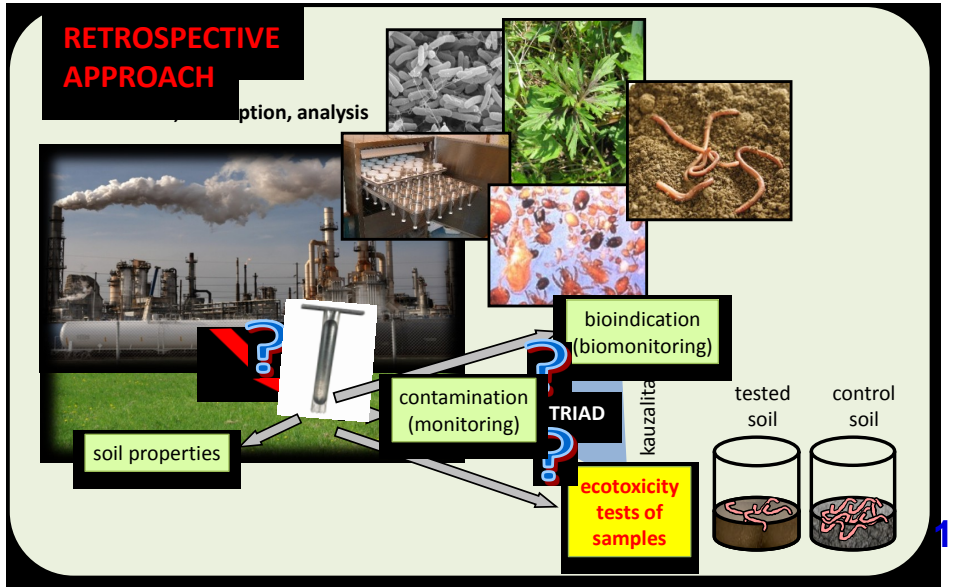


Bioassays useful for:

- Each methodological approach has its limitations and can be interpreted only with regard to its information content and focus

- **It is optimal to combine both approaches !!!**





Types of bioassays

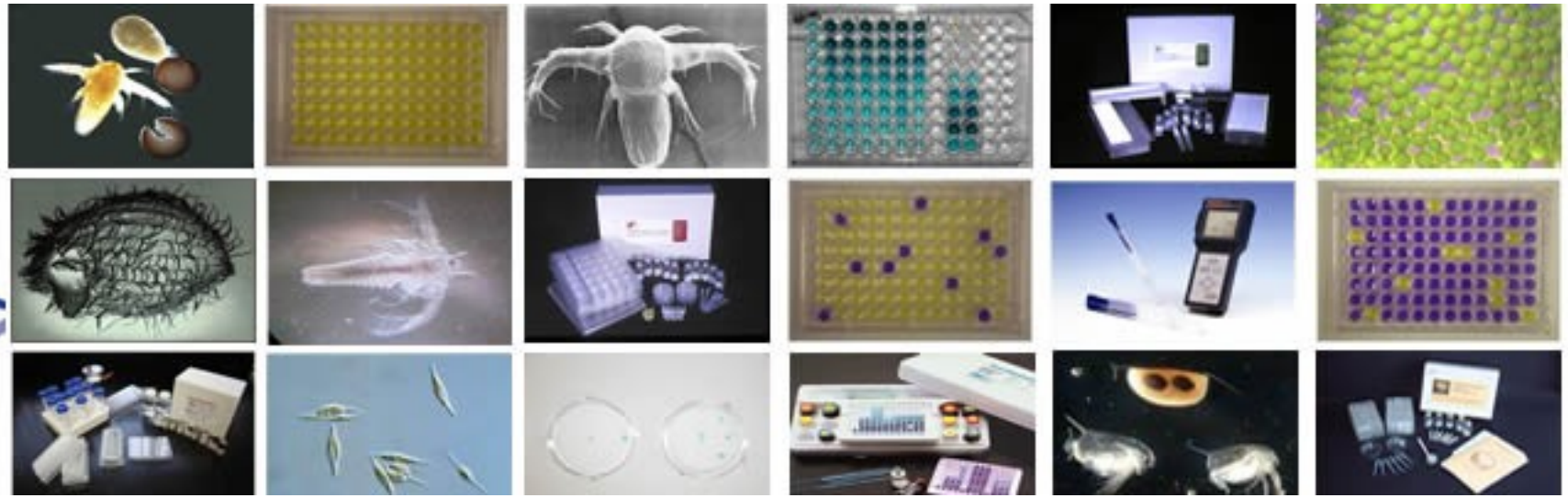
Bioassay development

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

Microbiotests

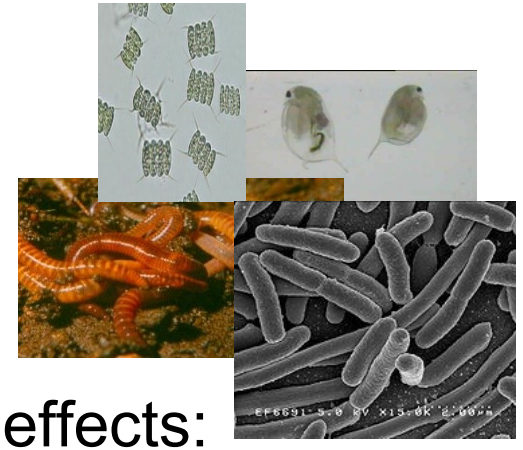
- they use some dormant stages of testing organisms
- practical = everything in one box
- cheap and easy, however, often not very relevant for real situations
- http://ebpi.ca/index.php?option=com_content&view=article&id=20&Itemid=50

ebpi environmental bio-detection products inc.



- https://www.microbiotests.com/

Differenciation of bioassays



- According to the trophic level of test organisms:
  - tests with producers, consumers, destruents
- Depending on the duration of exposure and the nature of the effects:
  - acute, semiacute (semichronic), sub-acute, chronic
    - the specific length depends on the generation time of the organism (bacteria <<< trout), the classification is not completely uniform; Division usually into:

      acute = 24, 48 to 96 hours, usually assessment of lethality

      chronic - days, weeks to months, evaluation of non-lethal effects
- According to the number of species involved:
  - single species, two species, multi-species

Differenciation of bioassays

- According to the level of the biological system (and complexity):
  - enzymes, bioprobes, in vitro cell and tissue cultures, intact living organism, population, micro / mesocosm, field experiments

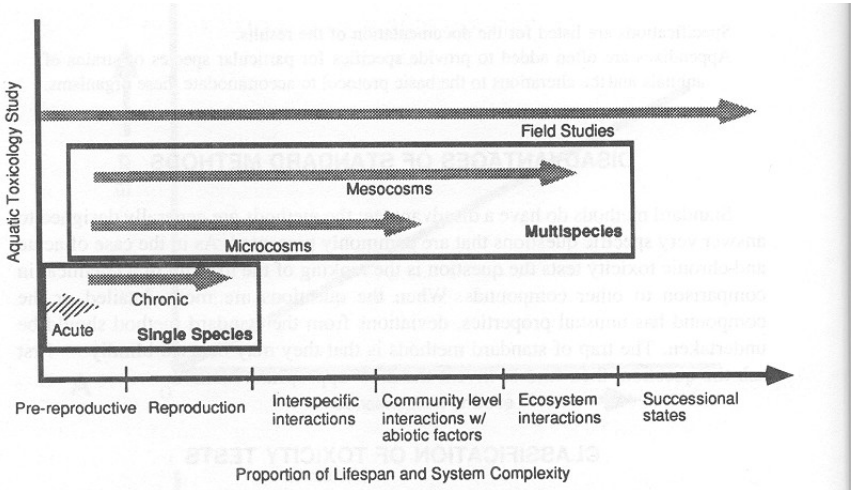
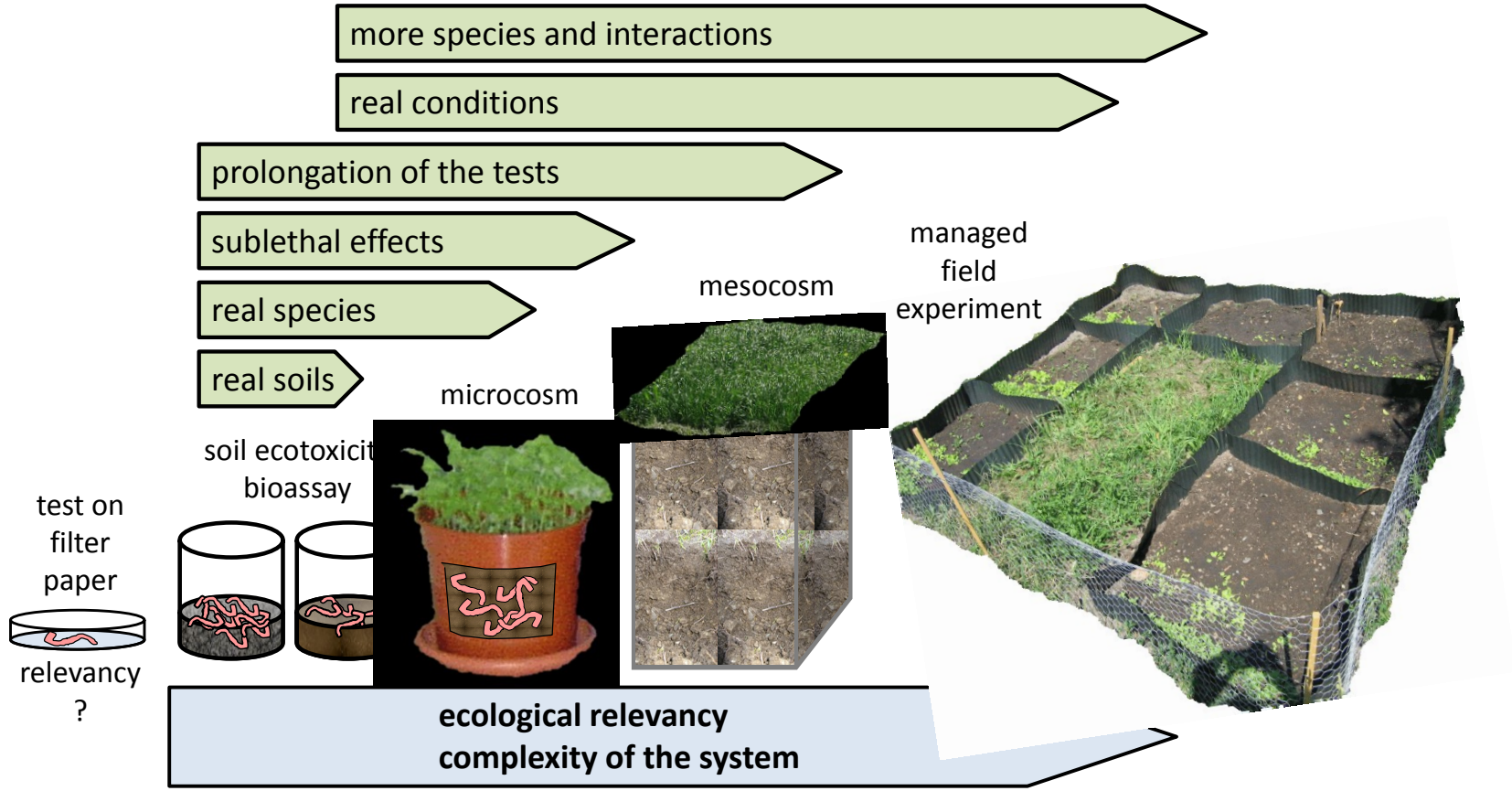


Figure 3.9 Classification of toxicity tests in environmental toxicology. Generally, the two parameters that are involved are the length of the test relative to the test organism and the species composition of the test system.



Differenciation of bioassays

- According to the type of sample:
  - chemical substance, mixture of substances, natural sample from the environment
- According to the tested matrix:
  - water, soil, air, sediment, waste, chemical
- According to the sample modification:
  - leachate (organic solvent, DMSO, water…), contact (Solid Phase Tests), direct (Direct tests, Whole effluent test), TIE - toxicity identification evaluation
- According to the evaluated effect:
  - mortality tests, reproduction tests, escape tests, growth tests, teratogenicity tests, carcinogenicity, xenoestrogenicity, etc.
- According to implementation:
  - in situ and in vitro
- + process bioassays: bioaccumulation, bioconcentration, biodegration

Ecological relevance of the bioassays

- the tested species should represent the relevant functional group
- the test should respect the ecology of the organism
- monitored responses should be ecologically relevant and indicate the state and function of the organism (survival, growth, reproduction, food intake and mobility)
- when monitoring reproduction, the exposure should cover most of the life cycle
- abiotic and biotic factors in the test should be similar to those in the habitat
- exposure paths should mimic real exposures
- the bioavailability of the contaminant should be similar to that in reality
- concentrations should be environmentally realistic

Ecological relevance of test species

- play a key role in the functioning of the ecosystem
- they occur in a number of ecosystems in higher abundance
- easy to use in field and laboratory conditions
- they come into contact with pollutants
- they are sensitive enough to stress

The problem of ecotoxicology in general:

I will use organisms A in the tests (for a number of reasons), but the target organisms in the system are B → what is the relationship of the results for A and B?

Example: *Eisenia fetida* - the most famous soil test

Earthworm species

žížala hnojní
Eisenia fetida

žížala kalifornská
Eisenia andrei

žížala polní
Aporrectodea caliginosa

žížala obecná
Lumbricus terrestris

žížala růžová
Aporrectodea rosea

žížala zelená
Allolobophora chlorotica

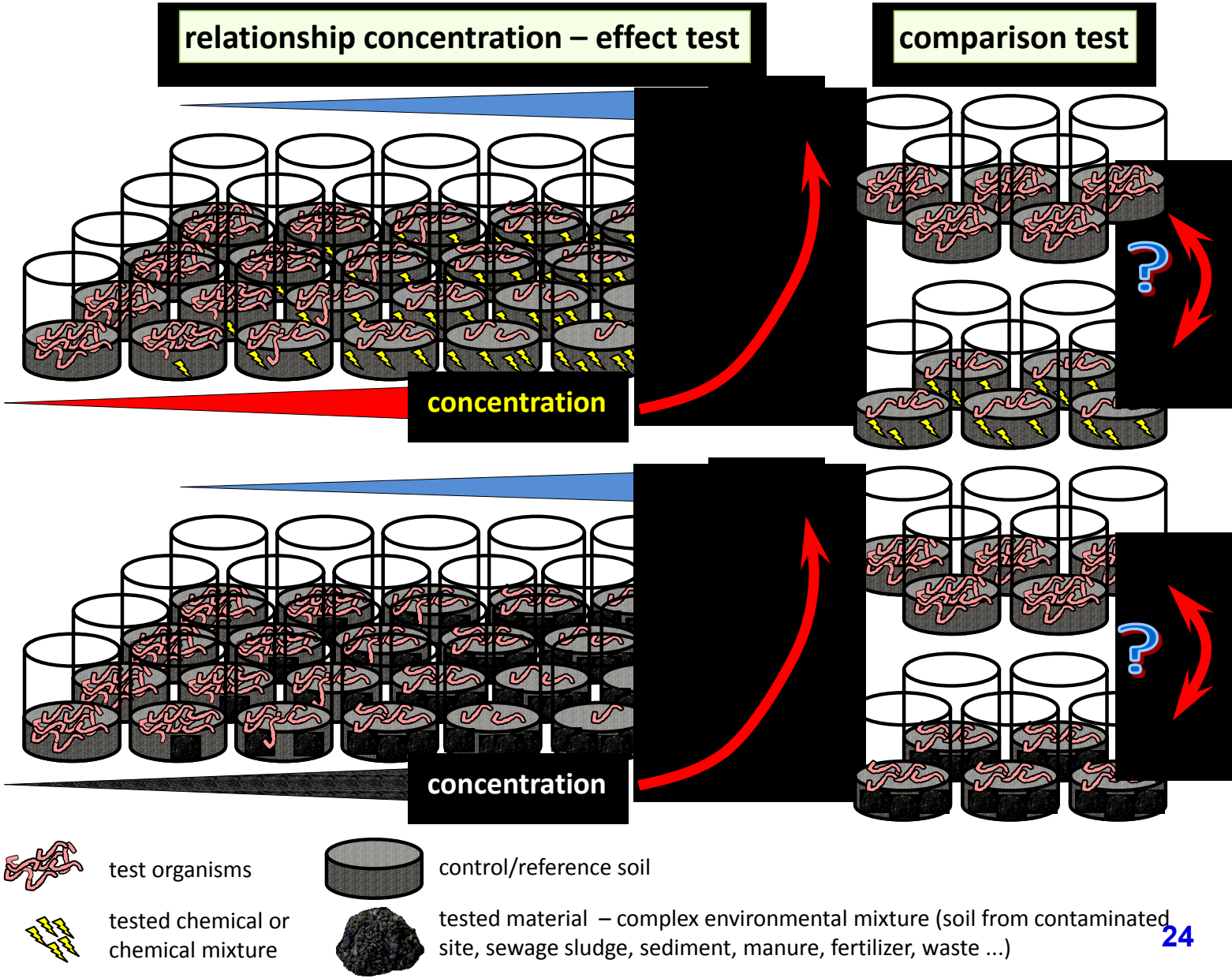
žížala dlouhá
Aporrectodea longa

žížala mléčná
Octolasion cyaneum

žížala červená
Lumbricus rubellus

Differenciation of bioassays

- limit test / comparison test
- concentration – response tests – preliminary, final



Standard-ability, legislation …

- Law given tests
  - Very little, especially for new chemicals, pesticides, waste
  - The big boom in the use of bioassays in recent years - ecological criteria for environmental quality
- Standardized, standardized
  - Many tests
  - Standardization ≠ duty, binding
  - Economic reasons - accreditation of laboratories
- Experimental
  - A series of tests
  - Space for efforts to achieve ecological realism
  - Application of new knowledge about mechanisms and effects
  - Ecological studies

Norms, standards, guidelines

Objective: to reduce interlaboratory variability

Over time, standard procedures have been developed for evaluating effects in laboratory tests up to in situ bioindication methods

Advantages:

- guaranteeing uniformity and repeatability of results
- comparability of results from different laboratories following the procedure
- validated results suitable for decision making
- little need for optimization

Disadvantages:

- very specific and limited informative value ("acute lethality for Daphnia crustaceans")
- usually suitable only for classification of substances (more - moderately - less toxic ...)
- limited number of standardized procedures, usually simple (acute) effects
- difficult to apply to other situations or to answer other questions
- only on a few model species - the question of transferability of results
- used in inappropriate situations (research, evaluation of cause and effect)
- it may not be applicable to a real environment

OECD guidelines – water 1

Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test	2011
Test No. 221: Lemna sp. Growth Inhibition Test	2006
Test No. 238: Sediment-Free Myriophyllum Spicatum Toxicity Test	2014
Test No. 239: Water-Sediment Myriophyllum Spicatum Toxicity Test	2014
Test No. 202: Daphnia sp. Acute Immobilisation Test	2004
Test No. 211: Daphnia magna Reproduction Test	2012
Test No. 231: Amphibian Metamorphosis Assay	2009
Test No. 242: Potamopyrgus antipodarum Reproduction Test	2016
Test No. 243: Lymnaea stagnalis Reproduction Test	2016
Test No. 235: Chironomus sp., Acute Immobilisation Test	2011
Test No. 218: Sediment-Water Chironomid Toxicity Using Spiked Sediment	2004
Test No. 219: Sediment-Water Chironomid Toxicity Using Spiked Water	2004
Test No. 233: Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment	2010
Test No. 225: Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment	2007
Test No. 224: Determination of the Inhibition of the Activity of Anaerobic Bacteria	2007
Test No. 209: Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation)	2010
Test No. 244: Protozoan Activated Sludge Inhibition Test	2017

https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761

OECD guidelines – water 2

Test No. 210: Fish, Early-life Stage Toxicity Test	2013
Test No. 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages	1998
Test No. 215: Fish, Juvenile Growth Test	2000
Test No. 234: Fish Sexual Development Test	2011
Test No. 236: Fish Embryo Acute Toxicity (FET) Test	2013
Test No. 203: Fish, Acute Toxicity Test	2019
Test No. 229: Fish Short Term Reproduction Assay	2012
Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study	1984
Test No. 230: 21-day Fish Assay	2009
Test No. 240: Medaka Extended One Generation Reproduction Test (MEOGRT)	2015
Test No. 241: The Larval Amphibian Growth and Development Assay (LAGDA)	2015
Test No. 248: Xenopus Eleutheroembryonic Thyroid Assay (XETA)	2019

https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761

OECD guidelines - soil

Test No. 216: Soil Microorganisms: Nitrogen Transformation Test	2000
Test No. 217: Soil Microorganisms: Carbon Transformation Test	2000
Test No. 207: Earthworm, Acute Toxicity Tests	1984
Test No. 222: Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)	2016
Test No. 232: Collembolan Reproduction Test in Soil	2016
Test No. 220: Enchytraeid Reproduction Test	2016
Test No. 226: Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil	2016
Test No. 228: Determination of Developmental Toxicity to Dipteran Dung Flies(Scathophaga stercoraria L. (Scathophagidae), Musca autumnalis De Geer (Muscidae))	2016
Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test	2006
Test No. 227: Terrestrial Plant Test: Vegetative Vigour Test	2006






https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761

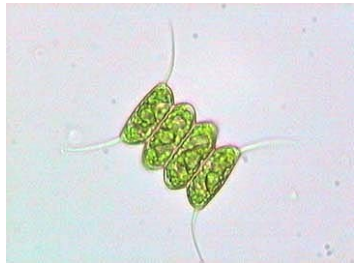
OECD guidelines - other

Test No. 237: Honey Bee (Apis Mellifera) Larval Toxicity Test, Single Exposure	2013
Test No. 213: Honeybees, Acute Oral Toxicity Test	1998
Test No. 214: Honeybees, Acute Contact Toxicity Test	1998
Test No. 245: Honey Bee (Apis Mellifera L.), Chronic Oral Toxicity Test (10-Day Feeding)	2017
Test No. 246: Bumblebee, Acute Contact Toxicity Test	2017
Test No. 247: Bumblebee, Acute Oral Toxicity Test	2017
Test No. 228: Determination of Developmental Toxicity to Dipteran Dung Flies(Scathophaga stercoraria L. (Scathophagidae), Musca autumnalis De Geer (Muscidae))	2016
Test No. 223: Avian Acute Oral Toxicity Test	2016
Test No. 205: Avian Dietary Toxicity Test	1984
Test No. 206: Avian Reproduction Test	1984

https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761

ISO standards – aquatic plants

ISO 8692:2012	Water quality — Fresh water algal growth inhibition test with unicellular green algae
ISO 14442:2006	Water quality — Guidelines for algal growth inhibition tests with poorly soluble materials, volatile compounds, metals and waste water
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 20227:2017	Water quality — Determination of the growth inhibition effects of waste waters, natural waters and chemicals on the duckweed Spirodela polyrhiza — Method using a stock culture independent microbiotest
ISO 16191:2013	Water quality — Determination of the toxic effect of sediment on the growth behaviour of Myriophyllum aquaticum
ISO 10253:2016	Water quality — Marine algal growth inhibition test with Skeletonema sp. and Phaeodactylum tricornutum
ISO 10710:2010	Water quality — Growth inhibition test with the marine and brackish water macroalga Ceramium tenuicorne



ISO standards – aquatic invertebrates

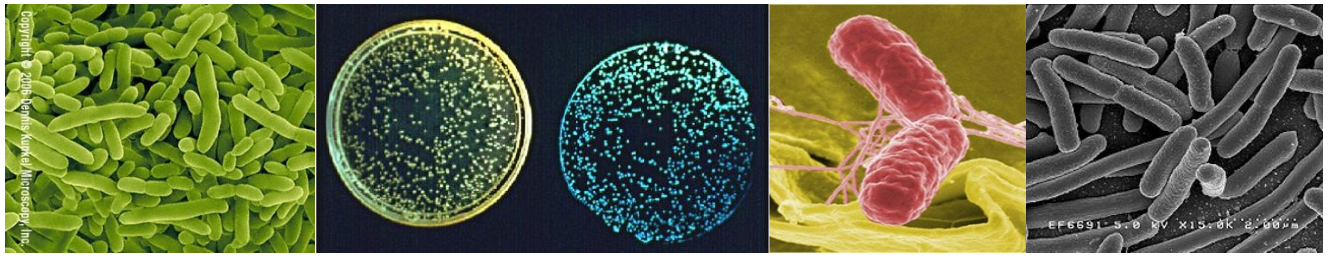
ISO 6341:2012	Water quality — Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) — Acute toxicity test
ISO 10706:2000	Water quality — Determination of long term toxicity of substances to Daphnia magna Straus (Cladocera, Crustacea)
ISO 10872:2020	Water and soil quality — Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of Caenorhabditis elegans (Nematoda)
ISO 14371:2012	Water quality — Determination of fresh water sediment toxicity to Heterocypris incongruens (Crustacea, Ostracoda)
ISO 14380:2011	Water quality — Determination of the acute toxicity to Thamnocephalus platyurus (Crustacea, Anostraca)
ISO 14669:1999	Water quality — Determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea)
ISO 20665:2008	Water quality — Determination of chronic toxicity to Ceriodaphnia dubia
ISO/TS 18220:2016	Water quality — Larval development test with the harpacticoid copepod Nitocra spinipes
ISO 16303:2013	Water quality — Determination of toxicity of fresh water sediments using Hyalella azteca
ISO 16778:2015	Water quality — Calanoid copepod early-life stage test with Acartia tonsa
ISO 17244:2015	Water quality — Determination of the toxicity of water samples on the embryo-larval development of Japanese oyster (Crassostrea gigas) and mussel (Mytilus edulis or Mytilus galloprovincialis)
ISO 20666:2008	Water quality — Determination of the chronic toxicity to Brachionus calyciflorus in 48 h
ISO 19820:2016	Water quality — Determination of the acute toxicity to the marine rotifer Brachionus plicatilis
ISO 19827:2016	Water quality — Determination of the acute toxicity to the freshwater rotifer Brachionus calyciflorus

ISO standards – aquatic vertebrates

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 12890:1999	Water quality — Determination of toxicity to embryos and larvae of freshwater fish — Semi-static 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 15088:2007	Water quality — Determination of the acute toxicity of waste water to zebrafish eggs (Danio rerio)
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 23893-3:2013	Water quality — Biochemical and physiological measurements on fish — Part 3: Determination of vitellogenin
ISO 21115:2019	Water quality — Determination of acute toxicity of water samples and chemicals to a fish gill cell line (RTgill-W1)

ISO standards – aquatic microorganisms

ISO 11348-1:2007/Amd 1:2018	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 — Amendment 1
ISO 11348-2:2007/Amd 1:2018	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 — Amendment 1
ISO 11348-3:2007/Amd 1:2018	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 — Amendment 1
ISO 10712:1995	Water quality — Pseudomonas putida growth inhibition test (Pseudomonas cell multiplication inhibition test)
ISO 15522:1999	Water quality — Determination of the inhibitory effect of water constituents on the growth of activated sludge microorganisms
ISO 11350:2012	Water quality — Determination of the genotoxicity of water and waste water — Salmonella/microsome fluctuation test (Ames fluctuation test)
ISO 16240:2005	Water quality — Determination of the genotoxicity of water and waste water — Salmonella/microsome test (Ames test)
ISO 13829:2000	Water quality — Determination of the genotoxicity of water and waste water using the umu-test
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 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



ISO standards – in vitro tests

ISO 19040-1:2018	Water quality — Determination of the estrogenic potential of water and waste water — Part 1: Yeast estrogen screen (Saccharomyces cerevisiae)
ISO 19040-2:2018	Water quality — Determination of the estrogenic potential of water and waste water — Part 2: Yeast estrogen screen (A-YES, Arxula adeninivorans)
ISO 19040-3:2018	Water quality — Determination of the estrogenic potential of water and waste water — Part 3: In vitro human cell-based reporter gene assay
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/Cor 1:2009	Water quality — Evaluation of genotoxicity by measurement of the induction of micronuclei — Part 2: Mixed population method using the cell line V79 — Technical Corrigendum 1
ISO/CD 24295	Water quality — Determination of the dioxin-like potential of water and wastewater — Method using in vitro mammalian cell-based reporter gene assay

ISO standards – biodegradation

ISO 11733:2004	Water quality — Determination of the elimination and biodegradability of organic compounds in an aqueous medium — Activated sludge simulation test
ISO 10707:1994	Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by analysis of biochemical oxygen demand (closed bottle test)
ISO 7827:2010	Water quality — Evaluation of the "ready", "ultimate" aerobic biodegradability of organic compounds in an aqueous medium — Method by analysis of dissolved organic carbon (DOC)
ISO 10708:1997	Water quality — Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds — Determination of biochemical oxygen demand in a two-phase closed bottle test
ISO 11734:1995	Water quality — Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge — Method by measurement of the biogas production
ISO 14592-1:2002	Water quality — Evaluation of the aerobic biodegradability of organic compounds at low concentrations — Part 1: Shake-flask batch test with surface water or surface water/sediment suspensions
ISO 14592-2:2002	Water quality — Evaluation of the aerobic biodegradability of organic compounds at low concentrations — Part 2: Continuous flow river model with attached biomass
ISO 14593:1999	Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Method by analysis of inorganic carbon in sealed vessels (CO2 headspace test)
ISO 16221:2001	Water quality — Guidance for determination of biodegradability in the marine environment
ISO 9408:1999	Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium by determination of oxygen demand in a closed respirometer
ISO 9439:1999	Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Carbon dioxide evolution test
ISO 9887:1992	Water quality — Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium — Semi-continuous activated sludge method (SCAS)
ISO 9888:1999	Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Static test (Zahn-Wellens method)

ISO standards – terrestrial plants

ISO 11269-1:2012	Soil quality — Determination of the effects of pollutants on soil flora — Part 1: Method for the measurement of inhibition of root growth
ISO 11269-2:2012	Soil quality — Determination of the effects of pollutants on soil flora — Part 2: Effects of contaminated soil on the emergence and early 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 18763:2016	Soil quality — Determination of the toxic effects of pollutants on germination and early growth of higher plants
ISO 22030:2005	Soil quality — Biological methods — Chronic toxicity in higher plants
ISO 29200:2013	Soil quality — Assessment of genotoxic effects on higher plants — Vicia faba micronucleus test
ISO 21479:2019	Soil quality — Determination of the effects of pollutants on soil flora — Leaf fatty acid composition of plants used to assess soil quality

ISO standards – soil invertebrates

ISO 11268-1:2012	Soil quality — Effects of pollutants on earthworms — Part 1: Determination of acute toxicity to Eisenia fetida/Eisenia andrei
ISO 11268-2:2012	Soil quality — Effects of pollutants on earthworms — Part 2: Determination of effects on reproduction of Eisenia fetida/Eisenia andrei
ISO 11268-3:2014	Soil quality — Effects of pollutants on earthworms — Part 3: Guidance on the determination of effects in field situations
ISO 11267:2014	Soil quality — Inhibition of reproduction of Collembola (Folsomia candida) by soil contaminants
ISO 16387:2014	Soil quality — Effects of contaminants on Enchytraeidae (Enchytraeus sp.) — Determination of effects on reproduction
ISO 21285:2019	Soil quality — Inhibition of reproduction of the soil mite (Hypoaspis aculeifer) by soil contaminants
ISO 23266:2020	Soil quality — Test for measuring the inhibition of reproduction in oribatid mites (Oppia nitens) exposed to contaminants in soil
ISO 15952:2018	Soil quality — Effects of pollutants on juvenile land snails (Helicidae) — Determination of the effects on growth by soil contamination
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 E. andrei)
ISO 17512-2:2011	Soil quality — Avoidance test for determining the quality of soils and effects of chemicals on behaviour — Part 2: Test with collembolans (Folsomia candida)
ISO 20963:2005	Soil quality — Effects of pollutants on insect larvae (Oxythyrea funesta) — Determination of acute toxicity
ISO 18311:2016	Soil quality — Method for testing effects of soil contaminants on the feeding activity of soil dwelling organisms — Bait-lamina test
ISO/DIS 24032	Soil quality — In situ caging of snails to assess bioaccumulation of contaminants

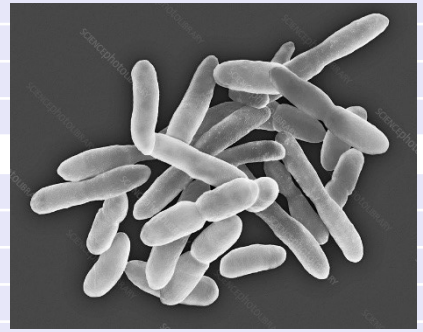
ISO 23611-1:2018	Soil quality — Sampling of soil invertebrates — Part 1: Hand-sorting and extraction of earthworms
ISO 23611-2:2006	Soil quality — Sampling of soil invertebrates — Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina)
ISO 23611-3:2019	Soil quality — Sampling of soil invertebrates — Part 3: Sampling and extraction of enchytraeids
ISO 23611-4:2007	Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes
ISO 23611-5:2011	Soil quality — Sampling of soil invertebrates — Part 5: Sampling and extraction of soil macro-invertebrates
ISO 23611-6:2012	Soil quality — Sampling of soil invertebrates — Part 6: Guidance for the design of sampling programmes with soil invertebrates






ISO standards – soil microorganisms

ISO 14238:2012	Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes
ISO 15685:2012	Soil quality — Determination of potential nitrification and inhibition of nitrification — Rapid test by ammonium oxidation
ISO 18187:2016	Soil quality — Contact test for solid samples using the dehydrogenase activity of Arthrobacter globiformis
ISO 17155:2012	Soil quality — Determination of abundance and activity of soil microflora using respiration curves
ISO/TS 10832:2009	Soil quality — Effects of pollutants on mycorrhizal fungi — Spore germination test
ISO/CD 23265	Soil quality — Test for estimating organic matter decomposition in contaminated soil
ISO 16072:2002	Soil quality — Laboratory methods for determination of microbial soil respiration
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 23753-1:2019	Soil quality — Determination of dehydrogenases activity in soils — Part 1: Method using triphenyltetrazolium chloride (TTC)
ISO 23753-2:2019	Soil quality — Determination of dehydrogenases activity in soils — Part 2: Method using iodotetrazolium chloride (INT)
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/TS 29843-2:2011	Soil quality — Determination of soil microbial diversity — Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method
ISO 11063:2020	Soil quality — Direct extraction of soil DNA
ISO 17601:2016	Soil quality — Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil
ISO 20130:2018	Soil quality — Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates
ISO/TS 20131-1:2018	Soil quality — Easy laboratory assessments of soil denitrification, a process source of N2O emissions — Part 1: Soil denitrifying enzymes activities
ISO/TS 20131-2:2018	Soil quality — Easy laboratory assessments of soil denitrification, a process source of N2O emissions — Part 2: Assessment of the capacity of soils to reduce N2O
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 14239:2017	Soil quality — Laboratory incubation systems for measuring the mineralization of organic chemicals in soil under aerobic conditions



US EPA - US Environmental Protection Agency

OPPTS - Office of Prevention, Pesticides & Toxic Substances

Group A - Aquatic and Sediment-dwelling Fauna and Aquatic Microcosms

850.1010 - Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (December 2016)
850.1300 - Daphnid Chronic Toxicity Test (December 2016)
850.1020 - Gammarid Amphipod Acute Toxicity Test (December 2016)
850.1025 - Oyster Acute Toxicity Test (Shell Deposition) (December 2016)
850.1035 - Mysid Acute Toxicity Test (December 2016)
850.1045 - Penaeid Acute Toxicity Test (December 2016)
850.1055 - Bivalve Acute Toxicity Test (Embryo-Larval) (December 2016)
850.1710 - Oyster Bioconcentration Factor (BCF) (December 2016)

850.1075 - Freshwater and Saltwater Fish Acute Toxicity Test (December 2016)
850.1400 - Fish Early Life Stage Toxicity Test (December 2016)
850.1730 - Fish Bioconcentration Factor (BCF) (December 2016)

850.1735 - Spiked Whole Sediment 10-Day Toxicity Test , Freshwater Invertebrates (December 2016)
850.1740 - Spiked Whole Sediment 10-Day Toxicity Test, Saltwater Invertebrates (December 2016)

https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines

US EPA - US Environmental Protection Agency

OPPTS - Office of Prevention, Pesticides & Toxic Substances

Group B – Terrestrial Wildlife

850.2100 - Avian Acute Oral Toxicity Test (June 2012)
850.2200 - Avian Dietary Toxicity Test (June 2012)
850.2300 - Avian Reproduction Test (June 2012)

850.2400 - Wild Mammal Toxicity Testing (June 2012)
850.2500 - Field Testing for Terrestrial Wildlife (June 2012)

https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines

US EPA - US Environmental Protection Agency

OPPTS - Office of Prevention, Pesticides & Toxic Substances

Group C – Terrestrial Beneficial Insects, Invertebrates, and Soil and Wastewater Microorganisms

850.3020 - Honey Bee Acute Contact Toxicity Test (June 2012)
850.3030 - Honey Bee Toxicity of Residues on Foliage (June 2012)
850.3040 - Field Testing for Pollinators (June 2012)

850.3100 - Earthworm Subchronic Toxicity Test (June 2012)

850.3200 - Soil Microbial Community Toxicity Test (June 2012)

850.3300 - Modified Activated Sludge, Respiration Inhibition Test (June 2012)

https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines

US EPA - US Environmental Protection Agency

OPPTS - Office of Prevention, Pesticides & Toxic Substances

Group D – Terrestrial and Aquatic Plants, Cyanobacteria, and Terrestrial Soil Core Microcosm

850.4230 - Early Seedling Growth Toxicity Test (June 2012)
850.4100 - Seedling Emergence and Seedling Growth (June 2012)
850.4150 - Vegetative Vigor (June 2012)
850.4800 - Plant Uptake and Translocation Test (June 2012)
850.4300 - Terrestrial Plants Field Study (June 2012)

850.4500 - Algal Toxicity (June 2012)
850.4550 - Cyanobacteria (Anabaena flos-aquae) Toxicity (June 2012)
850.4400 - Aquatic Plant Toxicity Test Using Lemna spp. (June 2012)
850.4450 - Aquatic Plants Field Study (June 2012)

850.4600 - Rhizobium-Legume Toxicity (June 2012)
850.4900 - Terrestrial Soil-Core Microcosm Test (June 2012)

https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines

Bioassays' general design

General scheme of bioassay

1) Prepare the organism

Culture media, standardized numbers, age, etc.

0) culture of organisms

2) Prepare the sample

Dilution series

water/culture media – direct organism exposure

Include BLANK (medium only)

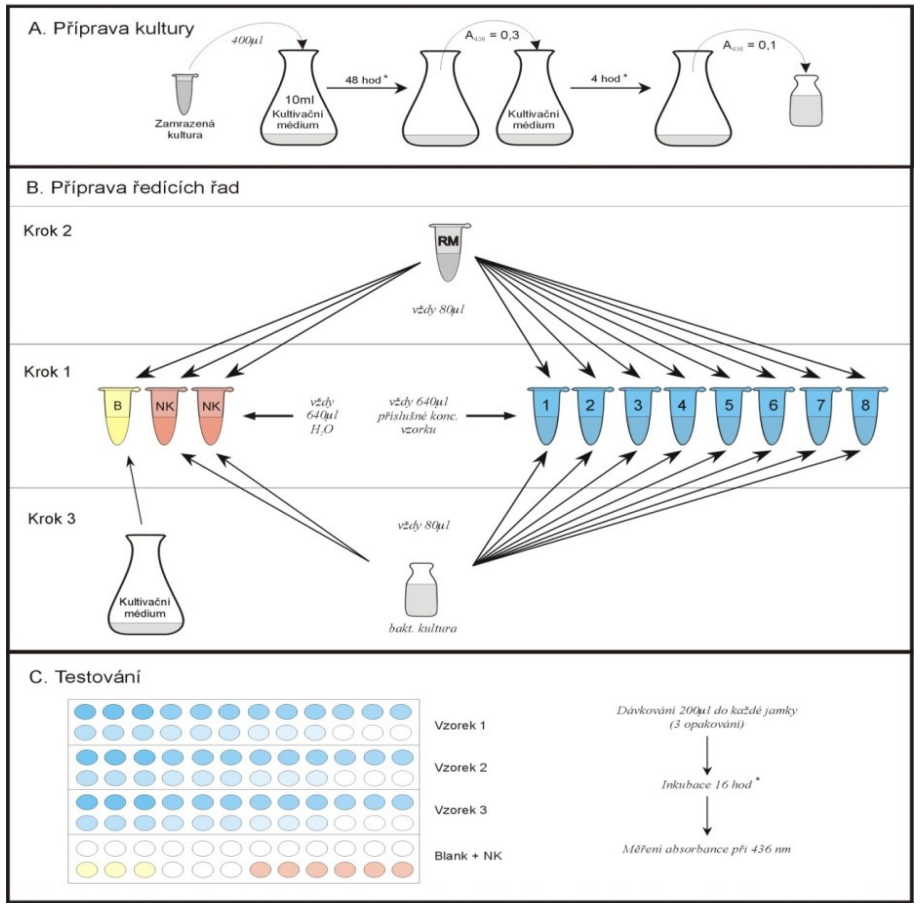
solvent for organic compounds – minimum to be added

Include SOLVENT CONTROL

specifics for the SOLID MATRICES

3) Expose of 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 …)

Organisms

1) Prepare the organism
Culture media, standardized numbers, age, etc.

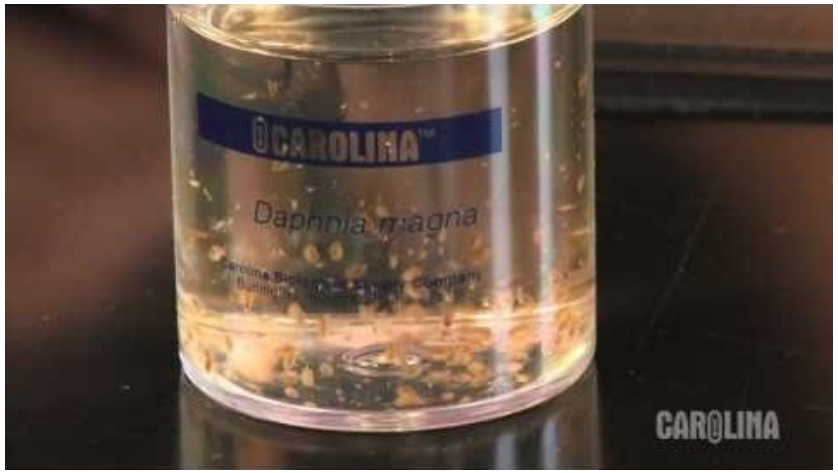
← **0) culture of organisms**

Ideally

- good availability (laboratory cultures, commercial availability ...)
- easy storage and breeding in laboratory conditions in sufficient quantities for experiments
- the biology of the species and the genetics of the respective culture are characterized
- the relative sensitivities of the species / culture to different classes of toxic substances are studied
- the susceptibility of the species should be a good representative of the relevant group of organisms (Daphnia - crustaceans, Danio rerio - freshwater fish)

Organisms

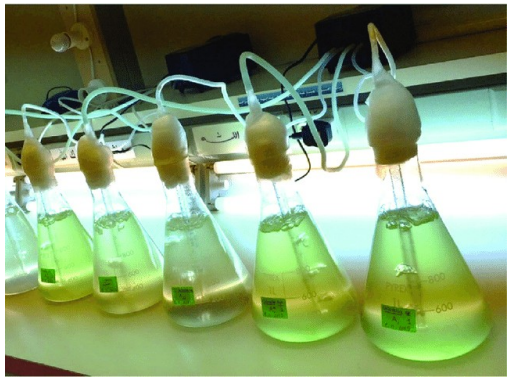
Cultures !!!



1) Prepare the organism

Culture media, standardized numbers, age, etc.

0) culture of organisms



Organisms

- the result of toxicity determination and interpretation is influenced by a number of other biological parameters
  - genetically determined sensitivity of the respective culture / clone / variety ...
  - size and age of individuals
  - sex
  - developmental stage (eggs, embryos, larvae, adults ...)
  - physiological conditions - optimum (diseases, food - antioxidants ...)

- in general, the organisms must be in optimal status before the test ➔ this is checked by the **test validity criteria** and testing **reference substances**

VALIDITY OF THE TEST

8. For a test to be valid, the following performance criteria should be met in the control(s):

- the mortality of the parent animals (female *Daphnia*) does not exceed 20% at the end of the test;
- the mean number of living offspring produced per parent animal surviving at the end of the test is ≥ 60.

REFERENCE SUBSTANCES

5. A reference substance may be tested for EC_{50} as a means of assuring that the test conditions are reliable. Toxicants used in international ring-tests (1)(5) are recommended for this purpose[1]. Test(s) with a reference substance should be done preferably every month and at least twice a year.

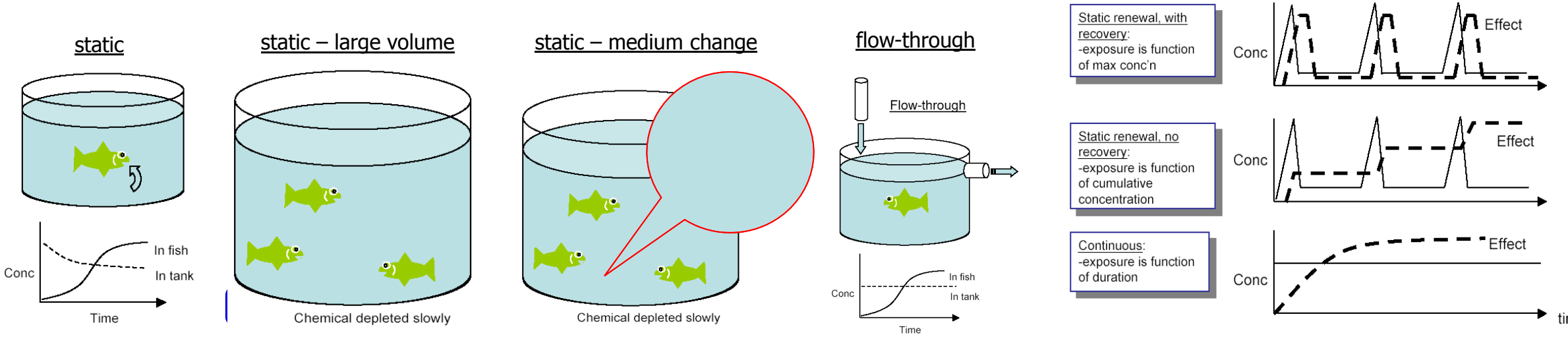
[1] The results of these inter laboratory tests and a Technical Corrigendum to ISO 6341 give an EC_{50}-24 h of the potassium dichromate ($K_2Cr_2O_7$) within the range 0.6 mg/l to 2.1 mg/l

Exposure in aquatic bioassays

- Usually exposure of whole organisms (intake by body surface area, respiratory system, food) less often: single injections (fish, input and dose are not affected by the environment)

Distribution according to the arrangement of the exposure

- static (without exchange of solutions - possible changes of concentrations, oxygen)
- static with medium change/renewal (change at defined times, ~24 h)
- recirculation (medium recirculation, more technically demanding ...)
- flow-through (continuous maintenance of concentrations, technically demanding ...)



Exposure of vertebrate animals in bioassays

- vertebrates - laboratory rodents, birds

- like in "classical" toxicology:
  - injection intramuscular (IM), intraperitoneal (IP), intravenous (IV), subcutaneous (SC)
  - oral - dosing in food, application of gauze (tube directly into the stomach) ...
  - respiration - air contamination - closed containers / cells, inhalation ...

Exposure in soil bioassays

- soil, sediments - bacteria, invertebrates - contact with the whole surface (direct contact tests - solid phase tests)
  - real soil / sediment
  - artificial soil / sediment

- plants - roots - contact with solid or liquid medium, exposure to gaseous pollutants from the air

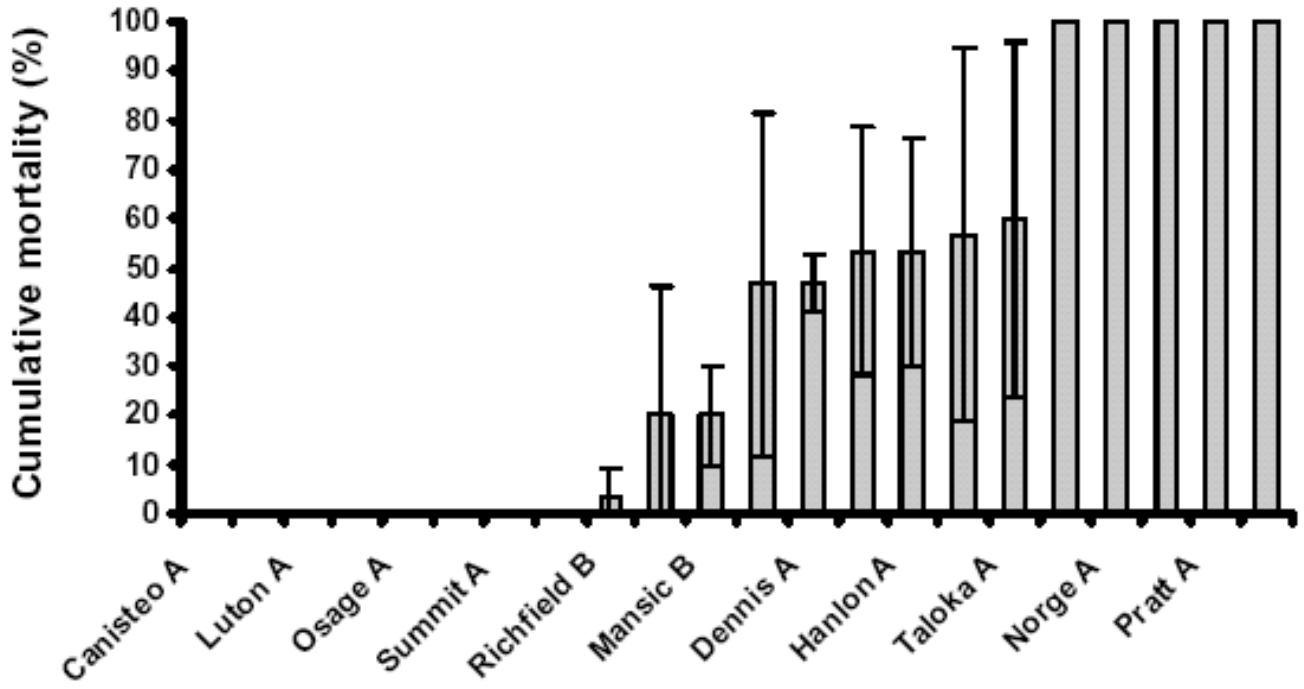
- often several exposure routes can be realistically assumed at the same time

Exposure in soil bioassays

it is specific:

- the fate of the contaminant in the soil environment, the influence on the real **bioavailability** for soil organisms comes into play significantly

Pb: 2 g/kg in soil
mortality of earthworms

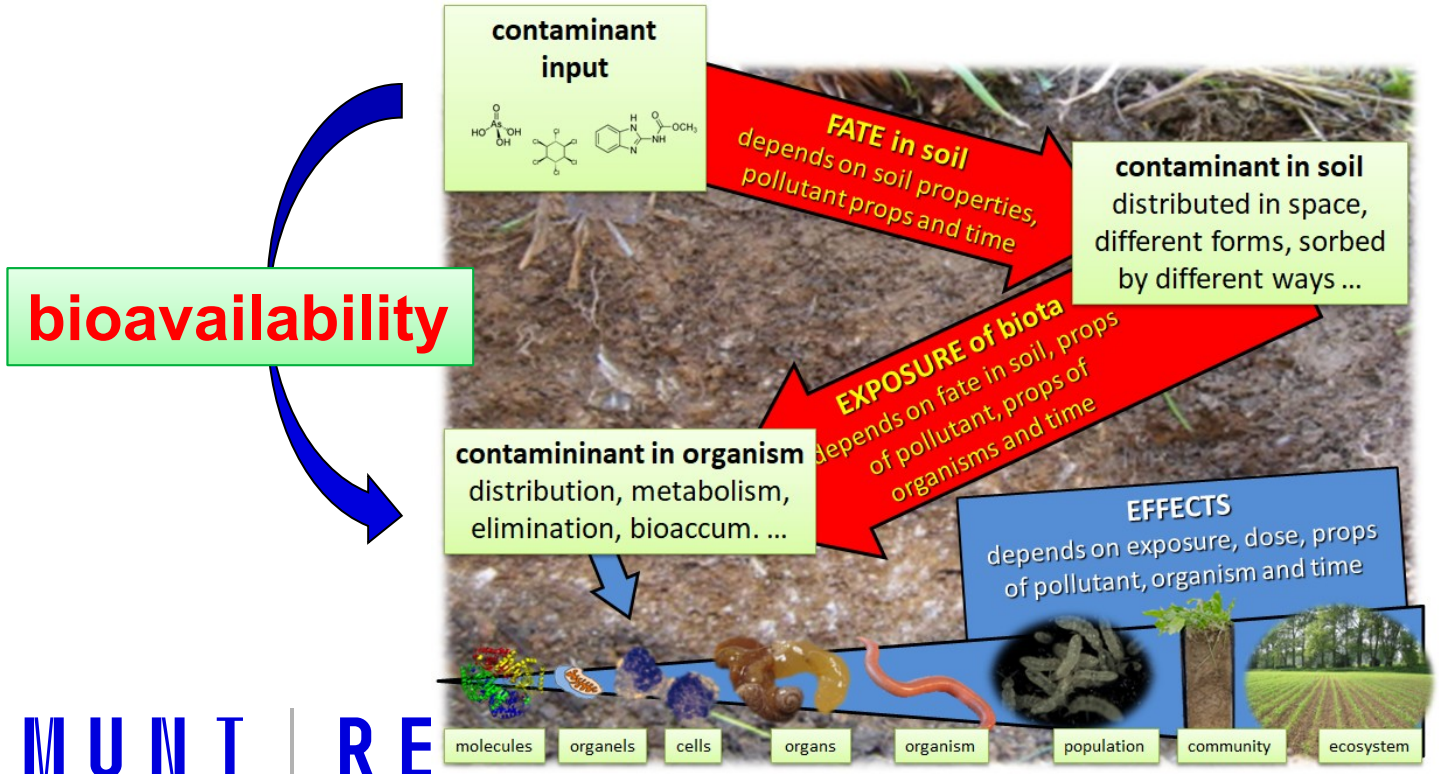


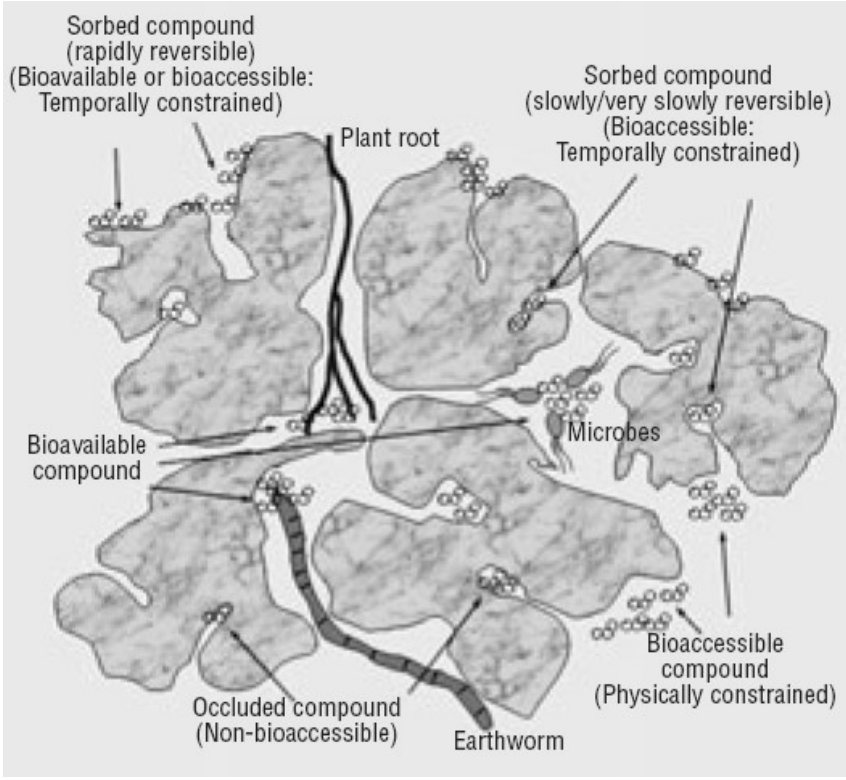
Lanno et al. (2004) Ecotoxicology and Environmental Safety 57, 39-47

Exposure in soil bioassays

Bioavailability

- soil is heterogenous and there is lot of places available for **sorption** or **sequestration** of the pollutants ➔ **fate, beahviour, distribution affected** ➔ **exposure, toxicity, risks affected**





Exposure in soil bioassays

Ingestion and oral

- food and soil particles - organisms consume mineral and organic matter - an important route of exposure for sorbed chemicals
- contaminants can be biomagnified - for example in fungi that are consumed by sprintails
- important path for arthropods

Dermal

- from the soil or soil solution - especially organisms drilling in the soil (earthworms and enchytraeids), which have a thin cuticle and are in contact with the soil and pore water
- it is also possible to model the results of tests in an aquatic environment by supplementing the model of the distribution of the substance between the soil solution and the sorption on particles = the so-called **Equilibrium partitioning theory (EqP)**

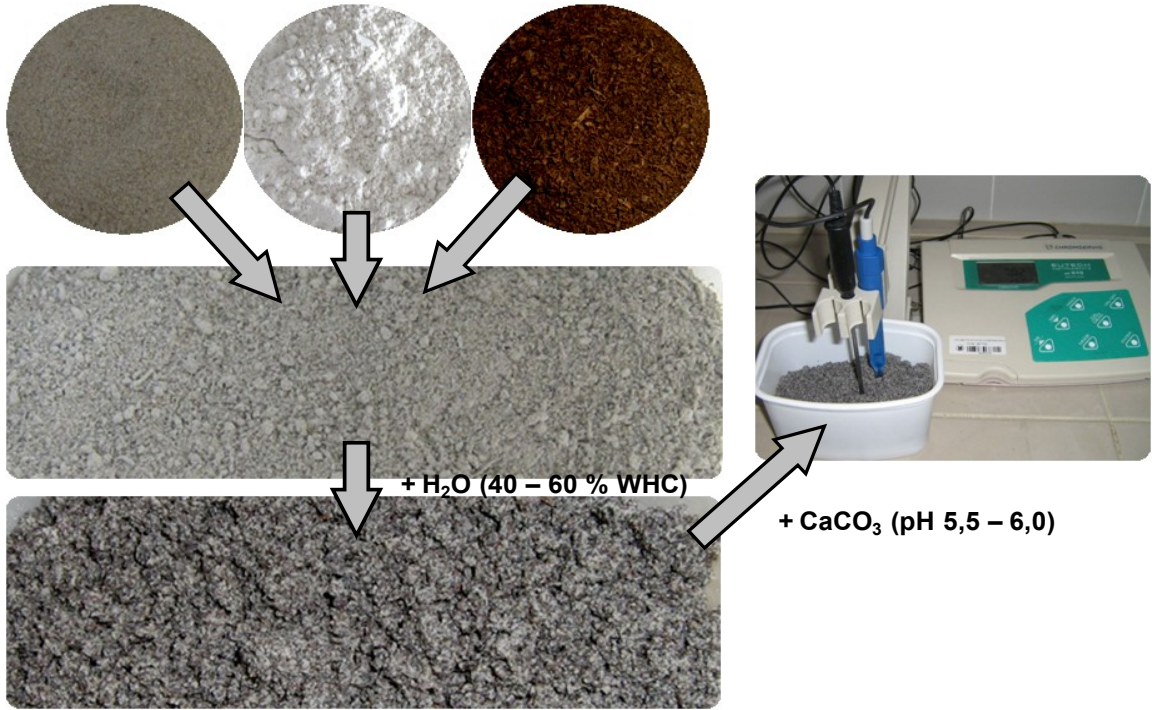
Breathing

almost no data

Exposure in soil bioassays

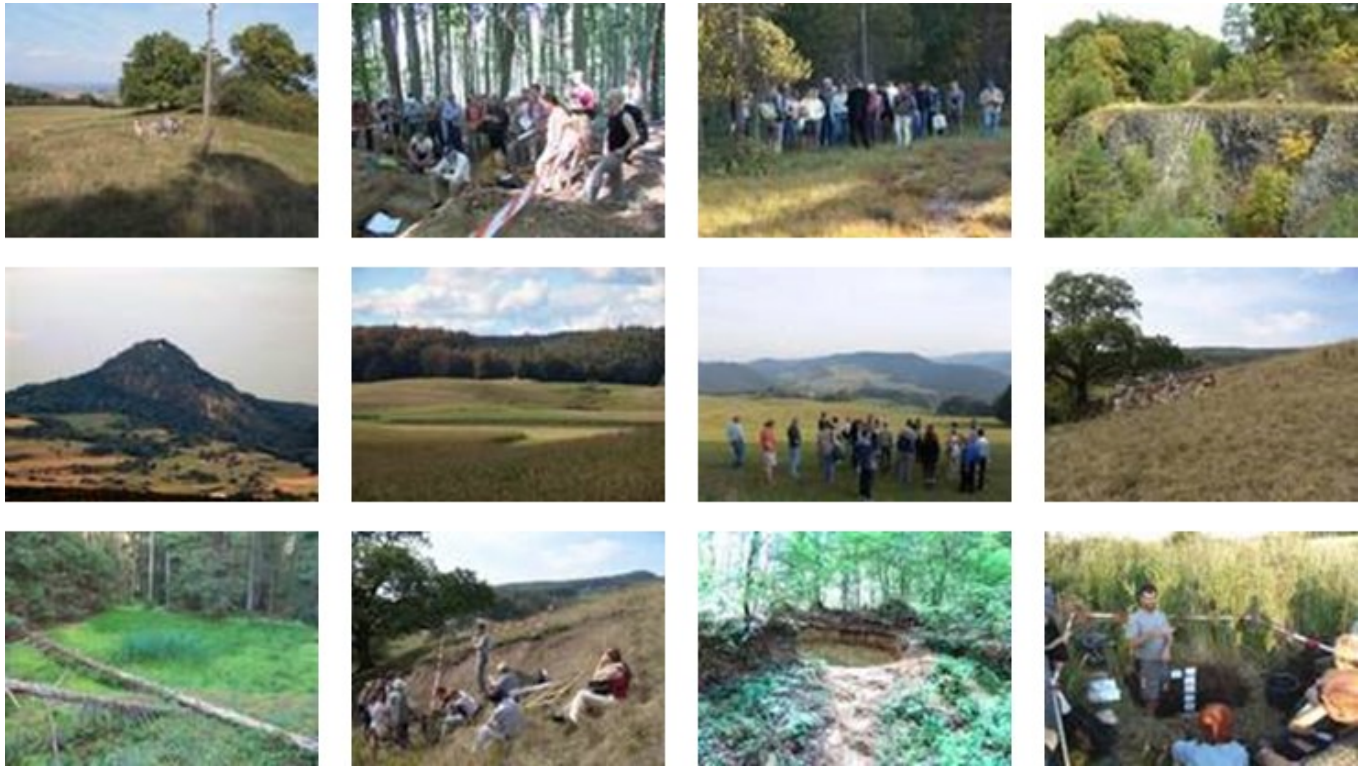
ARTIFICIAL SOIL

- 10% dry fine peat
- 20% caoline clay, min 30% calolinite
- 70% quartz sand fine min. 50% of size 0.05 – 0.2 mm
- 0.3-1% calcium carbonate → pH of 6 ± 0.5



Exposure in soil bioassays

- artificial soil is NOT real soil



Exposure in soil bioassays

- artificial soil is NOT real soil

http://lufa-speyer.de

	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

Exposure in soil bioassays

- the goal is **HOMOGENITY of exposure** to the test substance

water soluble chemicals

- in water which is also used to adjust soil moisture

insoluble in water

- using carrier - non-toxic, water soluble/miscible (acetone, ethanol)
- using carrier - non-toxic volatile organic solvent and evaporated rapidly
- in both cases, solution can be added to:
  - small amount (1-10%) of fine quartz sand; after evaporation of solvent, this is added to soil and mixed
  - directly into soil (dry or wet) followed by evaporation and mixing
- in all carrier cases, it is necessary to include a **control for the carrier/solvent**

insoluble in water or solvent

- mixed directly with quartz sand or whole soil

Factors / conditions of the assay

- CRUCIAL !!! they affect both the organisms and the tested chemical, they significantly affect the exposure and the final results
- **must be standardized !!!**
  - temperature
  - light / photoperiod
  - oxygen (aquatic consumers)
  - pH
  - water hardness
  - clay and organic matter content in soil
  - food added/non-added
  - etc.

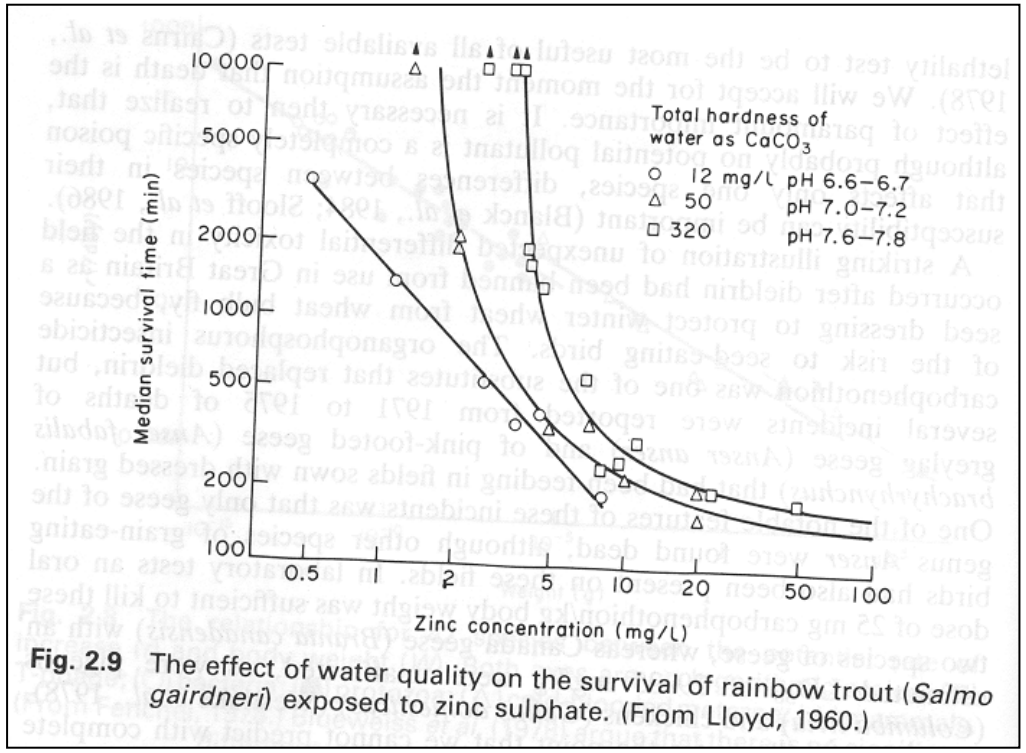


Fig. 2.9 The effect of water quality on the survival of rainbow trout (*Salmo gairdneri*) exposed to zinc sulphate. (From Lloyd, 1960.)

Results of the bioassays

Parameters of evaluation - endpoints

- **effects ~ response** = result of the exposure to toxic chemical (stressor)
  - higher/lower with increasing stressor intenzity (except hormesis)
- **endpoint** = measured (measurable) response / effect
  - original units (numbers, weight, enzyme activity etc.) or relative (% of control)

- acute effects
  - animals – letality/mortality, imobilization in case of Daphnia
  - plants – algae: growth, chlorofyl (fluorescnence); vacular: emergence, growth
  - destruents – bacteria: growth, activity ...
- chronic/sublethal effects
  - animals –growth, malformations, reproduction, behavior
  - plants – growth, reproduction...

Measures of exposure

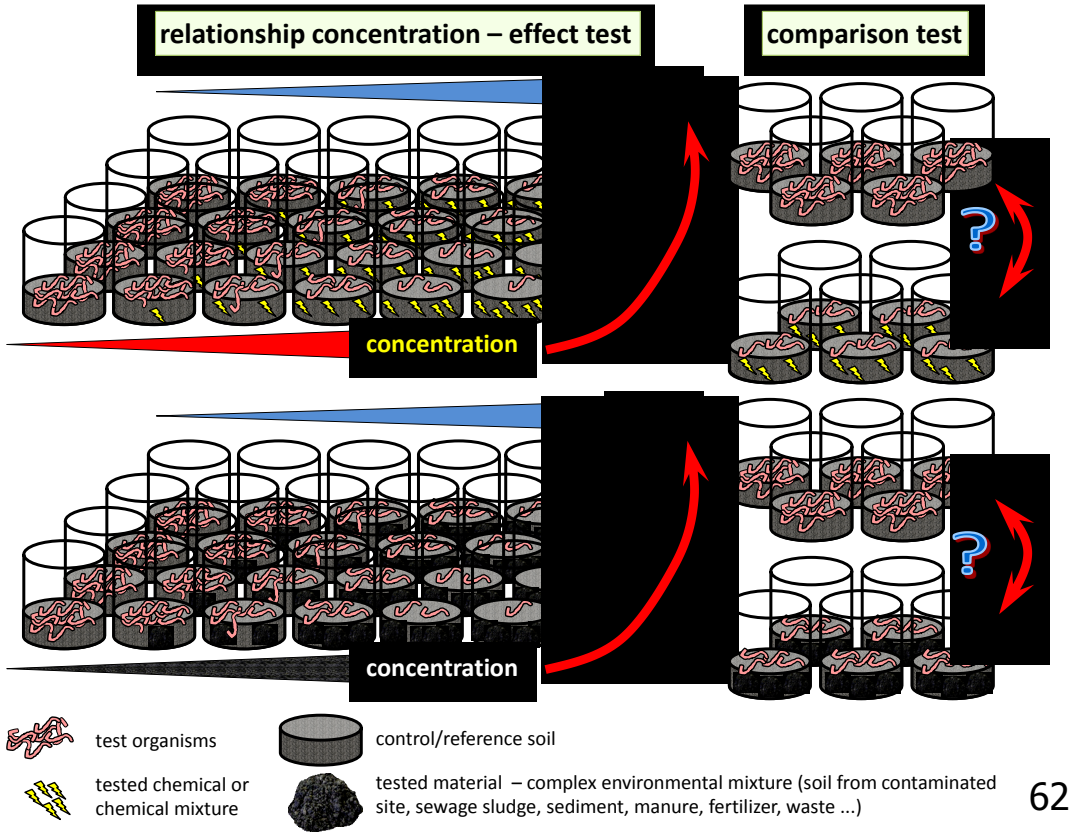
- DOSE versus CONCENTRATION
  - toxicology – dose - mg/kg b.w. - body weight, mg/kg b.w./day
  - ecotoxicology – usually the concentration in the medium - mg/L, mg/kg$_{soil}$ etc

Concentration and Dose

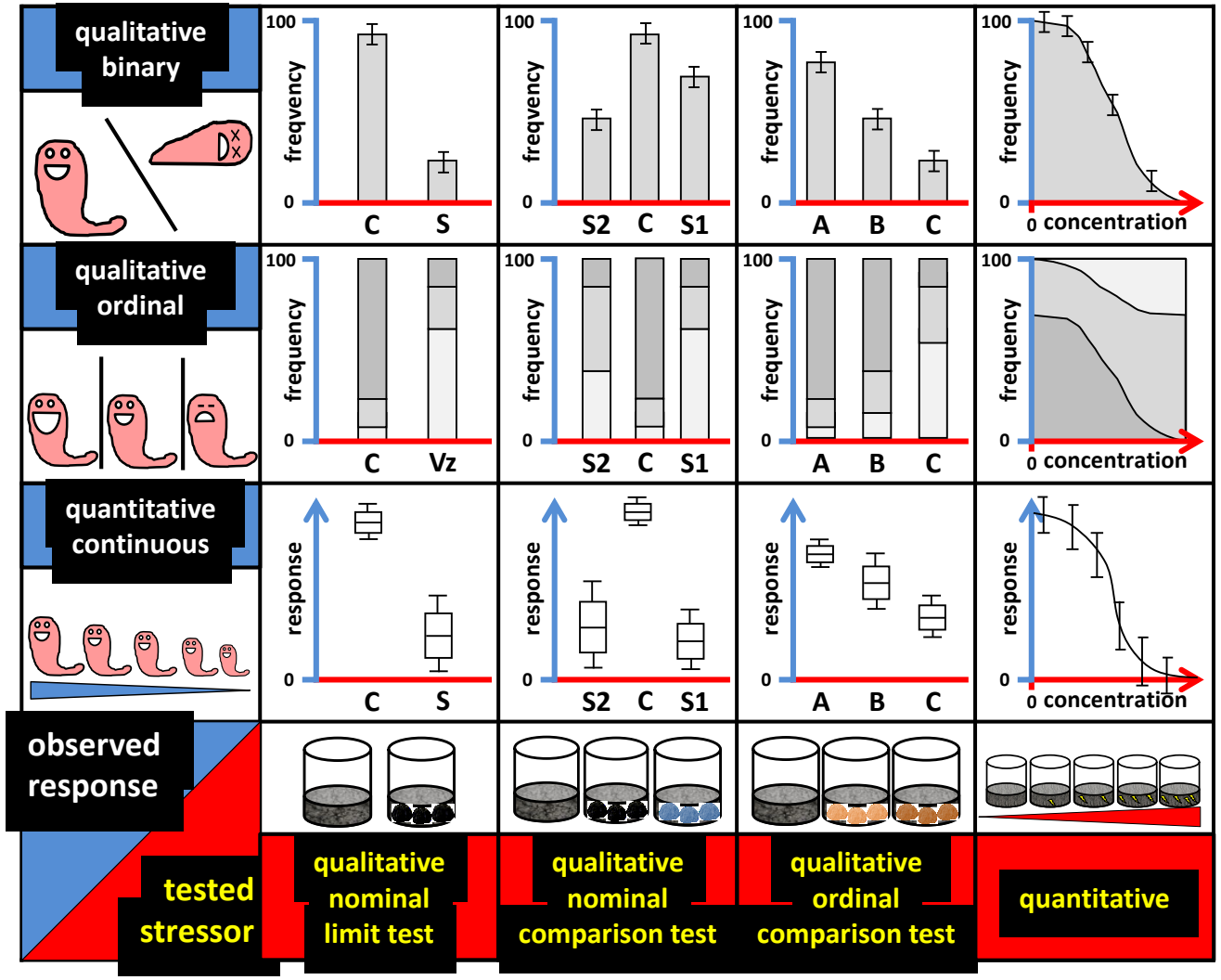
Concentration and dose both refer to the amount of test material to which the test organism is subjected. Concentrations are used to describe the amount of test material in the testing environment (e.g., mg/L in water, mg/kg in soil or mg/kg in food). Doses are used to describe the amount of test material 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.

- pure chemicals and defined mixtures
  - conc. in media - mg/L, mmol/L (= mM), mg/kg etc.

- environmental samples
  - extracts of the samples and their % dilutions
  - % of the sample in the reference material



Data from ecotoxicity bioassays



Error bars in the graphs indicate that regardless of the type of response, it is measured in several replicates and the resulting data have some variability

Data from ecotoxicity bioassays

Tested factor - qualitative, nominal
Contamination (or other stressor tested) in variants (samples) is not quantified, is not quantifiable, or is quantified, but the aim is not to study the influence of its intensity on the effect. Variants (samples) cannot be (or it is not the goal) arranged in any way. It is a comparative test of several variants (samples) each other and / or against the control (e.g. soils from monitoring from different localities, samples of different soil materials - sludge, sediments, waste). The extreme is „the limit test“ - one tested variant (sample) is compared with the control.
Tested factor - kvalitative, ordinal
Contamination (or other stressor tested) in variants (samples) is not fully quantified, but variants (samples) can be ranked based on some criteria. However, it is not possible to determine how many times the variant is larger or smaller than the previous or next one - the intensity of contamination (stress) cannot be plotted on the axis and no relationship between it and the effect can be modeled. It is a comparative test of several variants (samples) each other and / or against the control (e.g. soils little, medium and very far from the source; soils from little, medium and very damaged ecosystem, etc.).
Tested factor – quantitative
Contamination (stressor) is quantified to the extent that it is possible to say how many times or by how much its intensity is greater or less than in the previous or next variant. The contaminant concentration (stressor intensity) can be plotted on an axis and the experiment arranged and evaluated as a test of the relationship between concentration and effect, this relationship can then be graphically expressed, modeled and ecotoxicity parameters calculated. Whether the tested factor is quantified by discrete (integer) or continuous (even decimal) data is not very relevant.

Data from ecotoxicity bioassays

Observed response – qualitative, binary
The answer is not quantifiable, it is only possible to determine whether it has occurred or not - a typical example is mortality / survival of organisms, occurrence of some signs (lesions, swelling, mutations,), immobilization, escape reaction, etc. Finding on a number of organisms (biological systems), the binary result can be converted to the frequency or affected fraction and expressed as a percentage of the effect (e.g. mortality, lethality, survival, leakage) or as an affected fraction with values from 0 to 1. In this form, this type of data can be evaluated similarly to quantitative continuous data (test, model, regressions, etc.), but statistical methods designed for binomial data should be used correctly, including, for example, another formula for calculating variance.
Observed response – qualitative, ordinal
The response is quantified to the extent that the results can be ranked, eg small, medium and large damage. However, it is not possible to determine between the samples (variants) how many times or by how much the result is larger or smaller, and therefore it is not possible to model the relationship between the effect and the concentration. As with binary data, these results can be converted to fraction or frequency and expressed as a percentage in each category.
Observed response – quantitative, continuous
The response is quantified to the extent that it is possible to say how many times or how much is greater or less in one variant (concentration) than in another (eg weight, size, enzyme activity, production, number of juveniles, biomarker concentration…). The results can be plotted and the relationship between concentration and effect evaluated.

Data from ecotoxicity bioassays

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

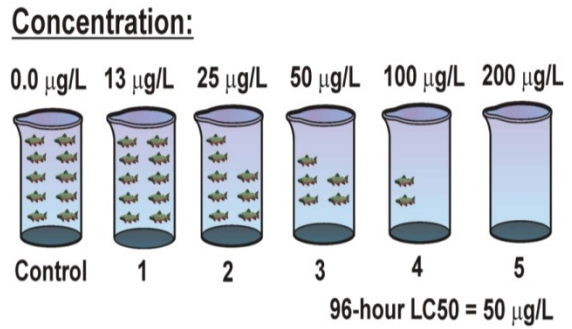
Data are continuous when they can (theoretically) take any value in an open interval, for instance any positive number. Examples include measurements of length, body weight, etc. Due to practical reasons the measured resolution depends on the quality of the measurement device. For example, if test units 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

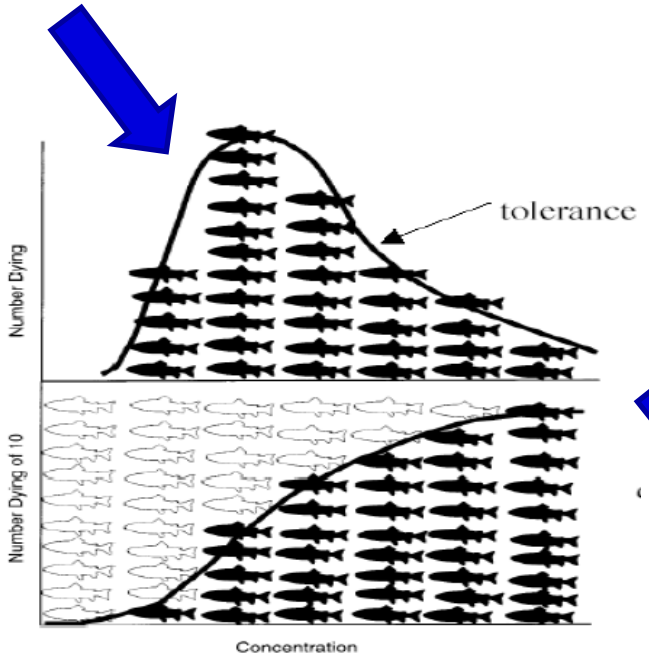
Discrete data are data that have a finite or countable number of values. There are three classes of discrete data: nominal, ordinal and interval. *Nominal data* express qualitative attributes that do not form a natural order (e.g. colours). *Ordinal data* reflect the relative magnitude from low to high (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.

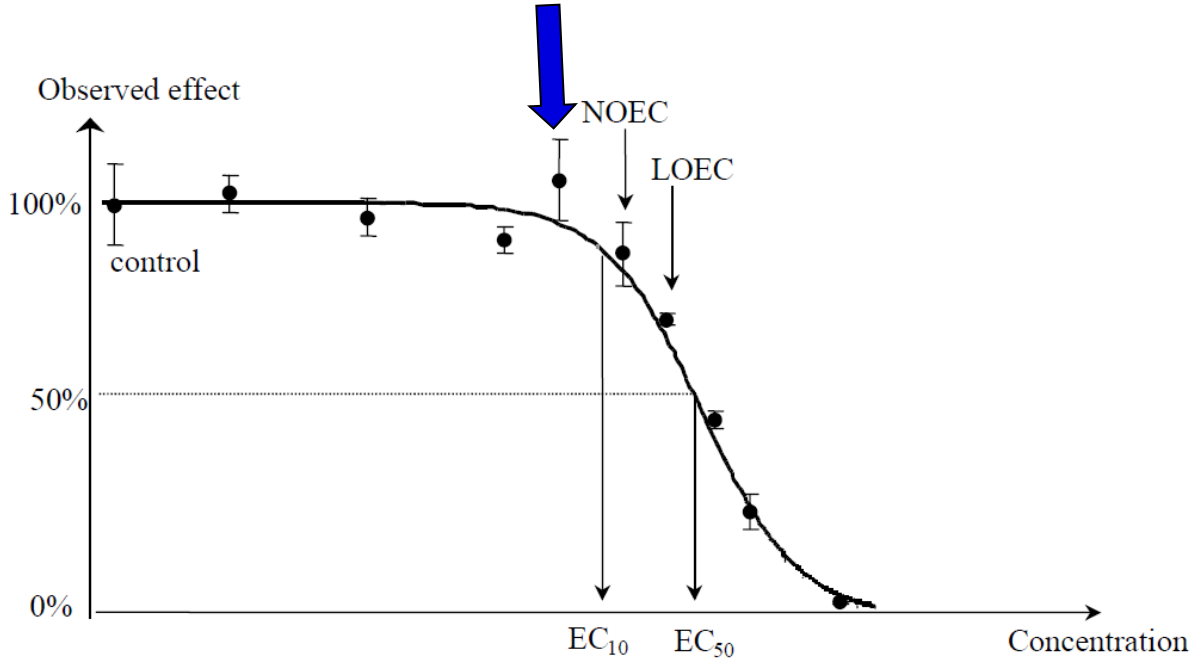
Draft Guidance Document for on the Statistical Analysis of Ecotoxicity Data. OECD Environmental Health and Safety Publications, Series on Testing and Assessment, Environment Directorate, OECD, Paris 2003.

Dose(concentration) - response relationship



each conc./dose is tested in several replicates !!!

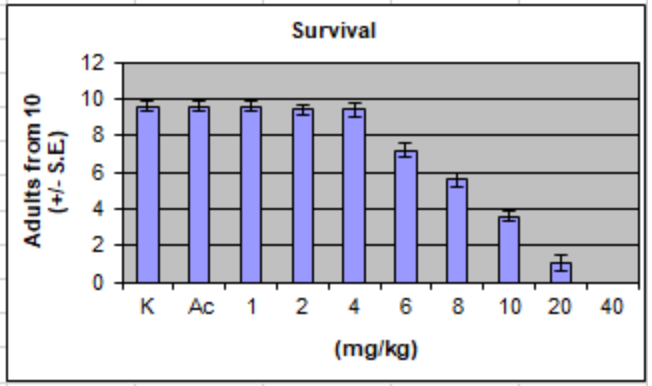




No Observed Effect Concentration (NOEC)
Lowest Observed Effect Concentration (LOEC)
ECx (x % effects concentration)
LCx (x % lethal concentration)

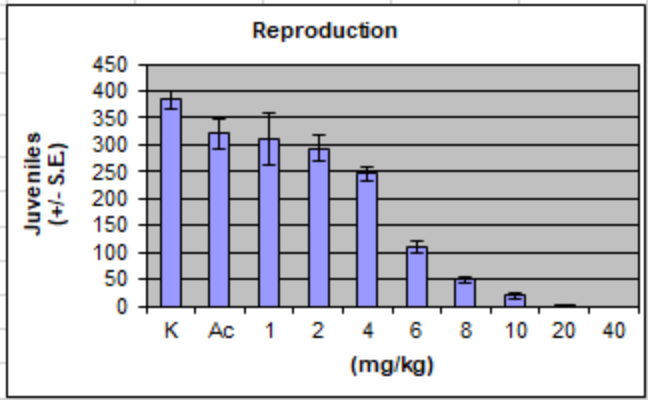
Adults

	K	Ac	1	2	4	6	8	10	20	40
1	10	10	9	10	8	6	7	4	1	
2	9	10	10	9	10	8	5	3	2	0
3	10	10	9	10	9	7	6	4	0	0
4	10	9	10	9	10	7	5	4	2	0
5	9	9	10	9	10	8	5	3	0	0
Mean	9.60	9.60	9.60	9.40	9.40	7.20	5.60	3.60	1.00	0.00
S.D.	0.55	0.55	0.55	0.55	0.89	0.84	0.89	0.55	1.00	0.00
S.E.	0.24	0.24	0.24	0.24	0.40	0.37	0.40	0.24	0.45	0.00
Adjusted to control		100.00	100.00	97.92	97.92	75.00	58.33	37.50	10.42	0.00
c.v.	5.71	5.71	5.71	5.83	9.52	11.62	15.97	15.21	100.00	########
S.D.(x) (binomial)	0.620	0.620	0.620	0.751	0.751	1.420	1.570	1.518	0.949	0.000
p	0.960	0.960	0.960	0.940	0.940	0.720	0.560	0.360	0.100	0.000
S.D.(p)	0.196	0.196	0.196	0.237	0.237	0.449	0.496	0.480	0.300	0.000
		96	96	94	94	72	56	36	10	0

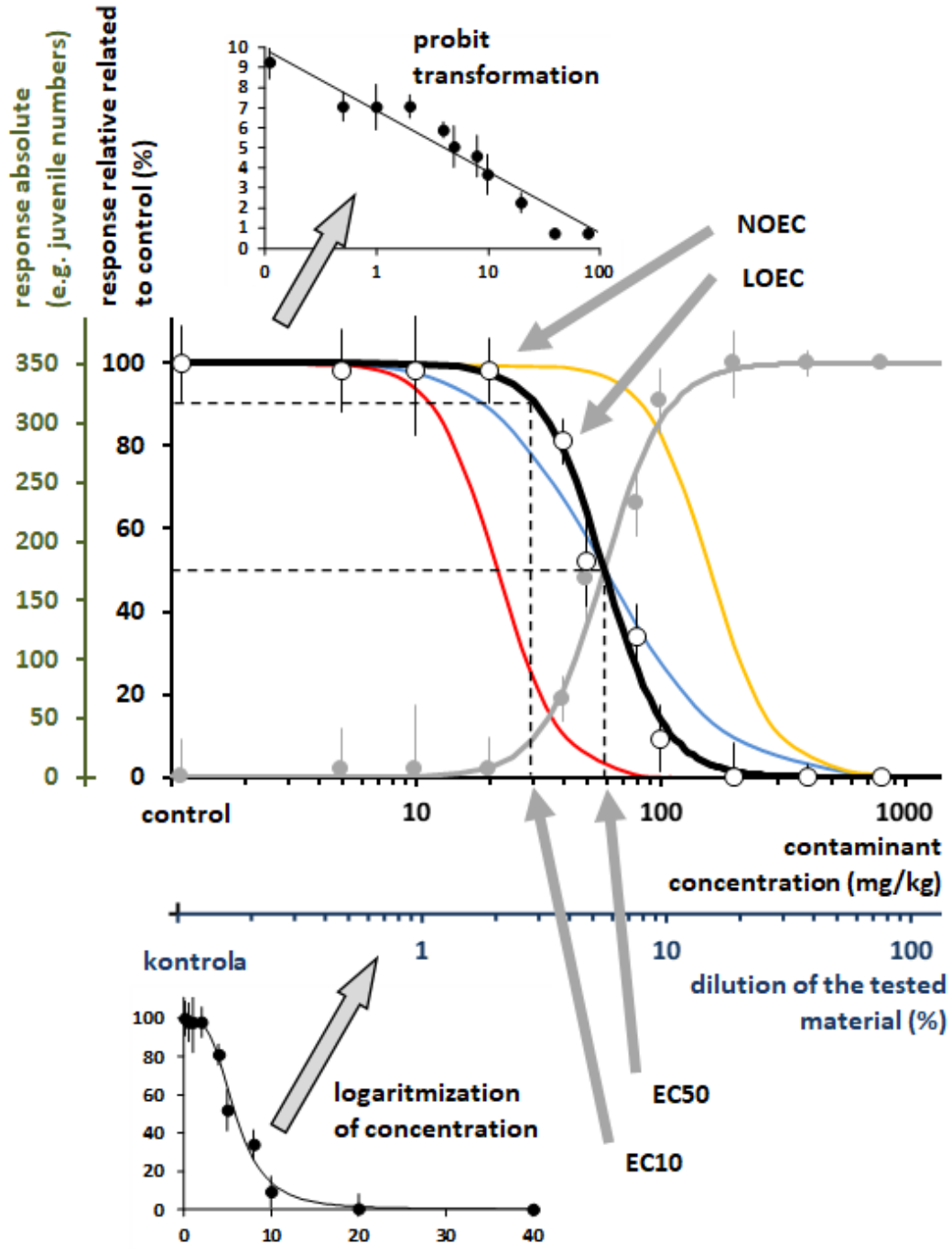


Juveniles

	K	Ac	1	2	4	6	8	10	20	40
1	372	310	227	368	201	87	57	22	5	0
2	395	402	417	319	277	79	35	38	0	0
3	368	295	314	305	247	143	45	12	4	0
4	345	233	174	220	236	123	52	25	0	0
5	442	362	420	256	271	115	53	0	0	0
Mean	384.40	320.40	310.40	293.60	246.40	109.40	48.40	19.40	1.80	0.00
S.D.	36.76	64.77	110.62	57.32	30.46	26.32	8.65	14.28	2.49	0.00
S.E.	16.44	28.97	49.47	25.63	13.62	11.77	3.87	6.38	1.11	0.00
Adjusted to control		100.00	96.88	91.64	76.90	34.14	15.11	6.05	0.56	0.00
c.v.	9.56	20.22	35.64	19.52	12.36	24.06	17.87	73.59	138.33	########



Dose(concentration) - response relationship



Aquatic bioassays - examples

Aquatic bioassays

- huge number of the tests (aquatic ecotoxicology had been for a long time the only one ecotoxicology)
- today, standardized bioassays cover the whole range of levels:
  - **suborganismal level**
    - laboratory experiments: study of toxicity mechanisms, in vitro biomarkers, specific types of toxicity (dioxin like toxicity, xenoestrogenicity ...)
  - **individual species, individuals**
    - laboratory experiments: traditional ecotoxicological bioassays with individual species, comparison of susceptibility of different species ...
  - **population effects**
    - laboratory tests - longer-term experiments - lifelong toxicity tests, tests with early developmental stages, plants - reproduction, germination ..., invertebrates - vertebrates - reproductive toxicity tests

Aquatic bioassays

- today, standardized bioassays cover the whole range of levels (cont):
  - **effects in communities**
    - laboratory microcosms - artificially established communities of organisms of various species under defined conditions (producers - consumers - destruents)
    - field multispecies manipulated in situ studies - field studies, aquatic mesocosms (flowing, standing, littoral – coastal
  - **field manipulated studies (in situ)**
    - studies with individual species - growing plants on contaminated and control areas, cage experiments in aquatic environments (molluscs, fish)
  - **ecosystem effects (these are, in fact, not „bioassays“ but „bioindication“)**
    - field observation - evaluation of effects in populations and communities in a real natural situation

Aquatic bioassays - producers

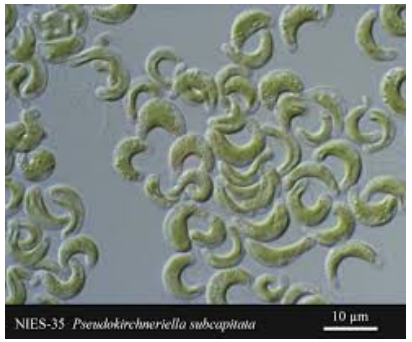
- **cyanobacteria** – photosynthetic, nitrogen-fixing eubacteria; colonies, filaments, single cells
- **unicellular algae** – eucaryotic; cells, filamentous, colonies; freshwater or marine
- both usually evaluated by the change in the number of cells (growth) – measured often as green color

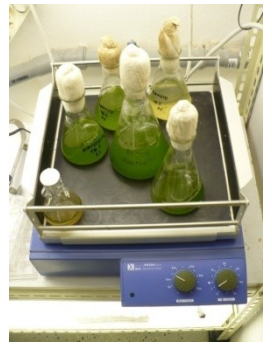
- **vascular plants – aquatic plants**
- size, length – growth, mortality

- other endpoints: concentration of pigments (eg chlorophyll a), physiological activity (nutrient intake) and metabolic activity (photosynthetic activities, enzymatic activities)

Aquatic bioassays – producers - algae

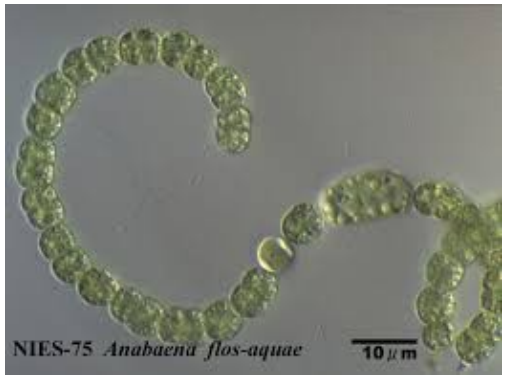
- **unicellular algae** Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum), Desmodesmus subspicatus (formerly Scenedesmus subspicatus), diatoms (Navicula pelliculosa) or cyanobacteria Anabaena flos-aquae, Synechococcus leopoliensis
- cultures or lyophylised stock may be used
- equipment needed with **high light intensity** about 60-120 µE m^{-2} s^{-1} (~4500-9000 lux) at 400-700 nm





- inicial density of the culture:

Pseudokirchneriella subcapitata:	5 x 10^3 - 10^4	cells/mL
Desmodesmus subspicatus	2-5 x10^3	cells/mL
Navicula pelliculosa	10^4	cells/mL
Anabaena flos-aquae	10^4	cells/mL
Synechococcus leopoliensis	5 x 10^4 - 10^5	cells/mL

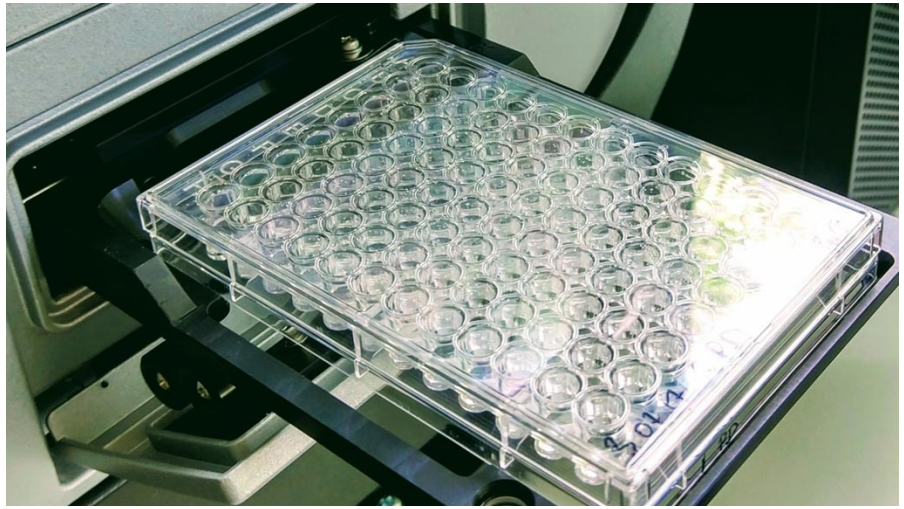


Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test 2011

ISO 8692:2012 Water quality — Fresh water algal growth inhibition test with unicellular green algae

Aquatic bioassays – producers - algae

- **defined medium →**
- **72 h exposure** at light and 20-24°C
- algal biomass determination: **cell counts** (electronic or microscope or flow cytometer) or **fluorescence or color** (fluorimeter, spectrophotometer)



Component	AAP		OECD	
	mg/L	mM	mg/L	mM
$NaHCO_3$	15.0	0.179	50.0	0.595
$NaNO_3$	25.5	0.300		
NH_4Cl			15.0	0.280
$MgCl_2 \cdot 6(H_2O)$	12.16	0.0598	12.0	0.0590
$CaCl_2 \cdot 2(H_2O)$	4.41	0.0300	18.0	0.122
$MgSO_4 \cdot 7(H_2O)$	14.6	0.0592	15.0	0.0609
K_2HPO_4	1.044	0.00599		
KH_2PO_4			1.60	0.00919
$FeCl_3 \cdot 6(H_2O)$	0.160	0.000591	0.0640	0.000237
$Na_2EDTA \cdot 2(H_2O)$	0.300	0.000806	0.100	0.000269*
H_3BO_3	0.186	0.00300	0.185	0.00299
$MnCl_2 \cdot 4(H_2O)$	0.415	0.00201	0.415	0.00210
$ZnCl_2$	0.00327	0.000024	0.00300	0.0000220
$CoCl_2 \cdot 6(H_2O)$	0.00143	0.000006	0.00150	0.00000630
$Na_2MoO_4 \cdot 2(H_2O)$	0.00726	0.000030	0.00700	0.0000289
$CuCl_2.2(H_2O)$	0.000012	0.00000007	0.00001	0.00000006
pH	7.5		8.1	

Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test 2011

ISO 8692:2012 Water quality — Fresh water algal growth inhibition test with unicellular green algae

Aquatic bioassays – producers - algae

validity

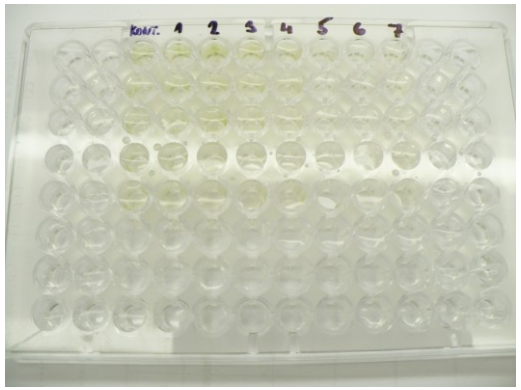
- controls:
  - exponential growth by factor of > 16 over 72 h
  - coefficient of variation (relative standard deviation, i.e. standard deviation divided by mean) < 35% (each day evaluation) and for average specific growth rate < 7%
- reference substance:
  - e.g. 3,5-dichlorophenol or potassium dichromate should be tested at least twice a year
  - effect (EC50) must be within the prescribed range

Aquatic bioassays – producers - algae

OECD	Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test	2011
ISO	ISO 8692:2012 Water quality — Fresh water algal growth inhibition test with unicellular green algae	

Aquatic bioassays – producers - algae

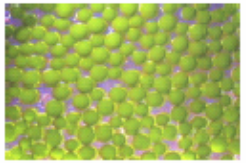
miniaturization



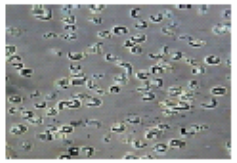
ALGALTOXKIT F™

MICROBIOTESTS

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

http://ebpi.ca/_slideshows_Algaltoxkit%20F%20slide%20show.pdf

OECD Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test 2011

ISO ISO 8692:2012 Water quality — Fresh water algal growth inhibition test with unicellular green algae

Aquatic bioassays – producers - plants

- „duckweed“, Lemna minor or gibba
- start with 10 leaves (fronds) per 1 beaker
- several recommended special media
- 96 h; 6500 - 10000 lux; pH 6.5; 24°C
- growth: biomass – weight and no. of fronds (image analysis possible)

validity:

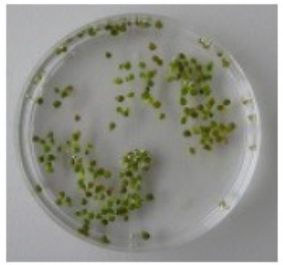
- control: doubling time < 2.5 days
- reference compound: 3,5-dichlorophenol or $K_2Cr_2O_7$ (EC50: 10-60 mg/L)

Test No. 221: Lemna sp. Growth Inhibition Test		2006
ISO 20079:2005	Water quality — Determination of the toxic effect of water constituents and waste water on duckweed (Lemna minor) — Duckweed growth inhibition test	

Aquatic bioassays – producers - plants

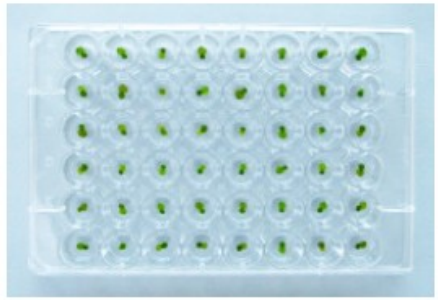
- miniaturization with Spirodela

TEST PROCEDURE OF THE DUCKWEED *SPIRODELA* MICROBIOTEST



3 days germination of the turions at 25°C and with 6000 lux illumination

Transfer of 1 germinated turion into each cup of a 6 x 8 multiwell, containing the toxicant concentrations



Taking of a photo of the multiwell at the start of the test (t0h)

Incubation for 3 days at 25°C with 6000 lux illumination

Taking of a photo of the multiwell at the end of the test (t72h)

Measurement of the area of the first fronds in each cup at t0h and at t72h with an Image Analysis Programme

Calculation of "the growth" of the first fronds in the controls and in the 5 test concentrations, and calculation of the percentage growth inhibition + the 72h EC50

https://www.microbiotests.com/wp-content/uploads/2019/07/duckweed-toxicity-test_duckweed-toxkit-f_standard-operating-procedure.pdf

ISO 20227:2017	Water quality — Determination of the growth inhibition effects of waste waters, natural waters and chemicals on the duckweed Spirodela polyrhiza — Method using a stock culture independent microbiotest

Aquatic bioassays – producers - plants

Test No. 238: Sediment-Free Myriophyllum Spicatum Toxicity Test		2014
Test No. 239: Water-Sediment Myriophyllum Spicatum Toxicity Test		2014
ISO 16191:2013	Water quality — Determination of the toxic effect of sediment on the growth behaviour of Myriophyllum aquaticum	
ISO 10710:2010	Water quality — Growth inhibition test with the marine and brackish water macroalga Ceramium tenuicorne	

Aquatic bioassays – consumers - invertebrates

- are very very common - sometimes ecotoxicology is confused with "Daphnia bioassays„
- standard layouts:
  - beakers/vessels, acute tests 1-few days, extended tests 21 d
  - evaluation of lethality, growth ... short-term - usually static
  - evaluation of reproduction etc. ... longer exposures, need for food, well controlled **supply of oxygen** ...
- aquatic planktonic crustaceans - the most common
  - Daphnia magna, Ceriodaphnia dubia, Artemia salina (marine)
- other invertebrates
  - benthic - Gammarus, Hyallela azteca
  - oligochaetic worms - Tubifex, Lumbriculus
  - snails – sand snail
  - insects - midges (Chironomus), mayflies ...

Aquatic bioassays – consumers - invertebrates

- also many of them developed to **microbiotests**

DAPHTOXKIT F

THAMNOTOXKIT F

CERIODAPHTOXKIT F

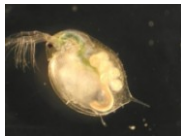
ARTOXKIT M

http://ebpi.ca/_slideshows_Daphtoxkit%20F%20magna%20slide%20show.pdf

https://www.microbiotests.com

OSTRACODTOXKIT F

Aquatic bioassays – consumers - invertebrates



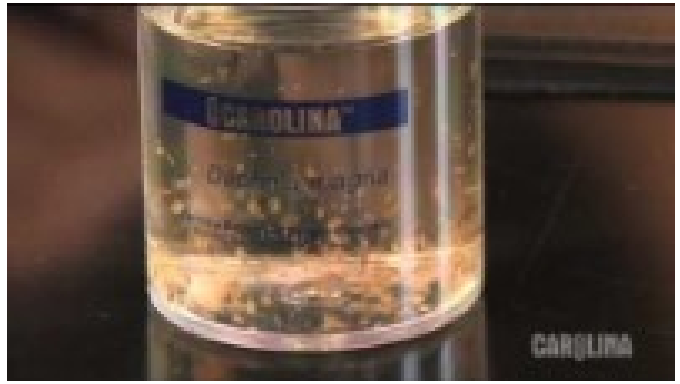
Daphnia magna acute test

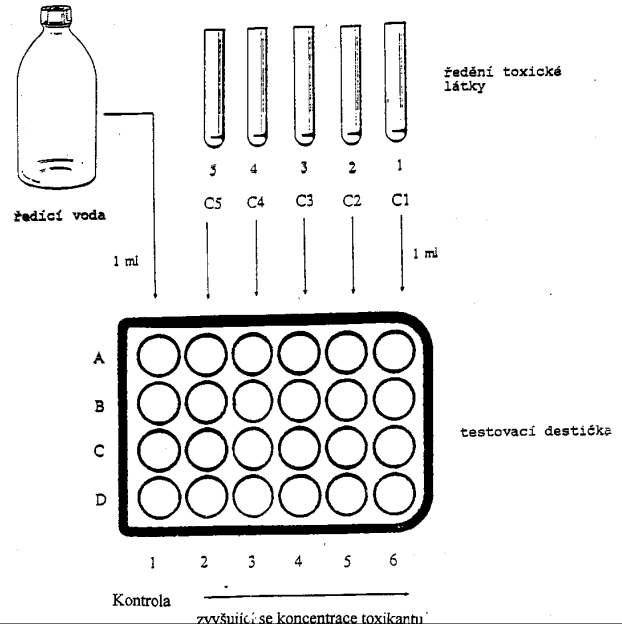
- 5 juvenile daphnids per replicate (min 2 ml)
- **medium** = so called reconstituted water
  1. $CaCl_2 \cdot 2H_2O$ 11,76 g/l
  2. $MgSO_4 \cdot 7H_2O$ 4,93 g/l
  3. $NaHCO_3$ 2,59 g/l
  4. KCl 0,23 g/l

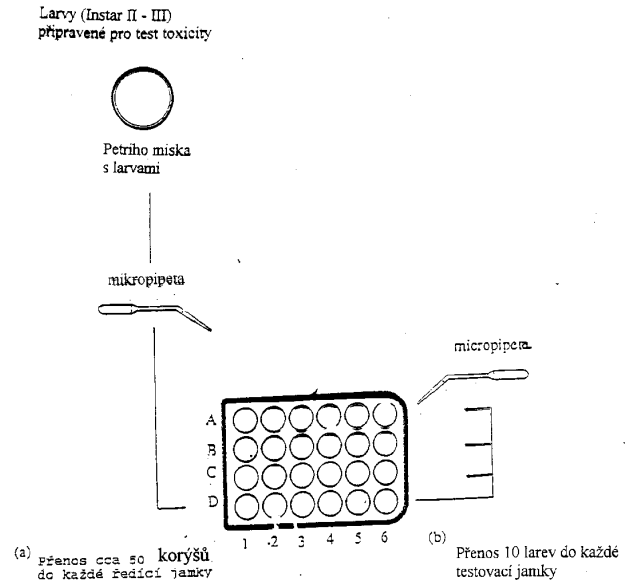
     mix 25 ml of each to 1 L, pH 7.8, aeration
- 24h, 48h; dark or 16h light / 8h dark;
  O2 > 80 % (2 mg/l); 20°C; no food
- mortality = immobilization

validity:

- control: mortality< 10 %;
- reference compound: $K_2Cr_2O_7$ (LC50: mg/L)

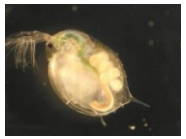






Aquatic bioassays – consumers - invertebrates

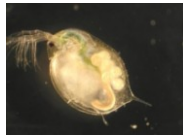
Daphnia magna acute test



other videos:
https://www.youtube.com/watch?v=ElENqCeGNSA
https://www.youtube.com/watch?v=3AxO36DLjsU

Test No. 202: Daphnia sp. Acute Immobilisation Test		2004
ISO 6341:2012	Water quality — Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) — Acute toxicity test	

Aquatic bioassays – consumers - invertebrates

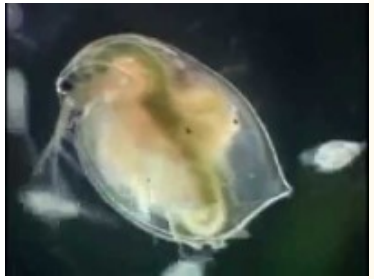


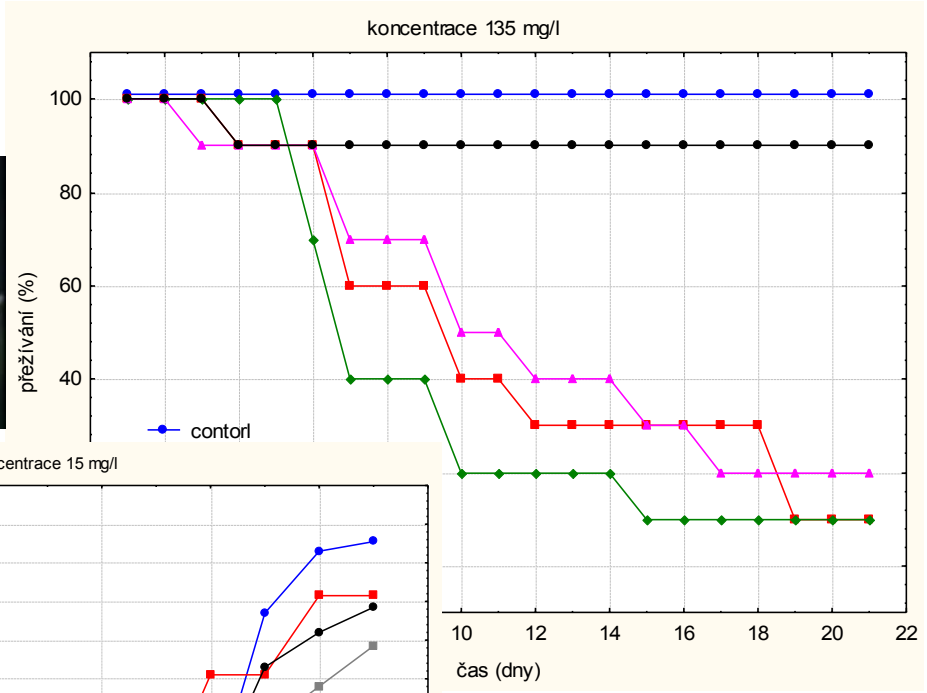
Daphnia magna reproduction test

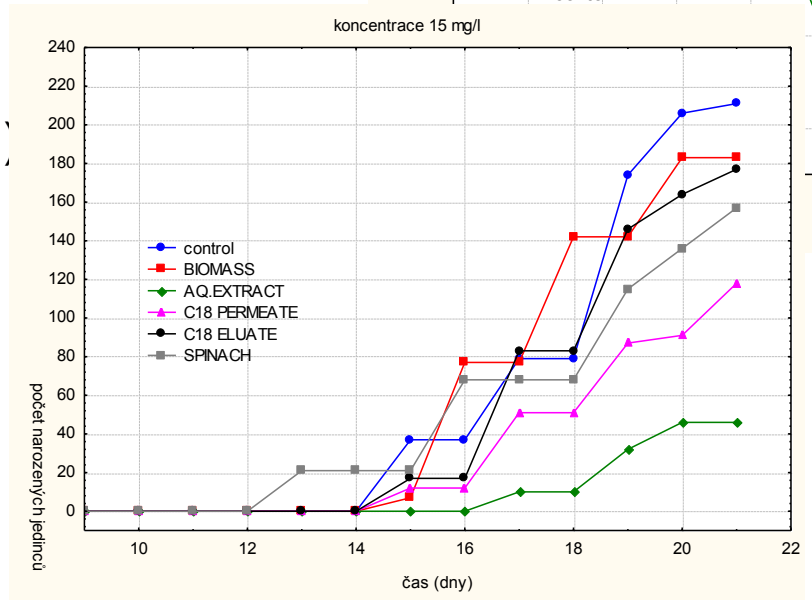
- 24 h juveniles, 10 per replicate, 50 ml or more
- **medium** reconstituted water or M4/M7
- 21 days; 16/8 light/dark; O2 > 3 mg/l); 20°C; **food (algae)**
- week check, aeration or medium change
- mortality + number of juveniles + other parameters (behavior, malformations ...)

validity:

- control: mortality< 10 %;
- reference compound: $K_2Cr_2O_7$ (LC50: mg/L)

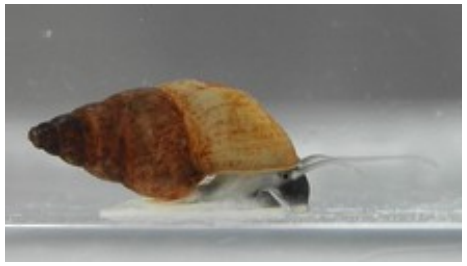


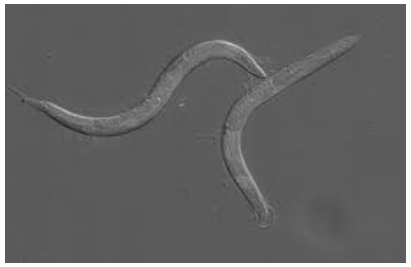




Aquatic bioassays – consumers - invertebrates

other invertebrates used in the bioassays








OECD	Test No. 242: Potamopyrgus antipodarum Reproduction Test	2016
	Test No. 243: Lymnaea stagnalis Reproduction Test	2016
ISO	ISO 10872:2020	Water and soil quality — Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of Caenorhabditis elegans (Nematoda)
	ISO 14380:2011	Water quality — Determination of the acute toxicity to Thamnocephalus platyurus (Crustacea, Anostraca)
	ISO 14669:1999	Water quality — Determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea)
	ISO 20665:2008	Water quality — Determination of chronic toxicity to Ceriodaphnia dubia
	ISO/TS 18220:2016	Water quality — Larval development test with the harpacticoid copepod Nitocra spinipes
	ISO 16778:2015	Water quality — Calanoid copepod early-life stage test with Acartia tonsa
	ISO 17244:2015	Water quality — Determination of the toxicity of water samples on the embryo-larval development of Japanese oyster (Crassostrea gigas) and mussel (Mytilus edulis or Mytilus galloprovincialis)

Aquatic bioassays – consumers - fish

Species[6]	Temperature[7] (°C)	Salinity[8] (‰)	pH	Hardness (mg/L $CaCO_3$)	Photoperiod (hours light)	Recommended length range[9] (cm)
Danio rerio *Zebrafish*	21-25	<0.2	6.0-8.5	40- 250, preferably <180	12-16	1-2
Pimephales promelas Fathead minnow	21-25	<0.2	6.0-8.5	40-250, preferably <180	12-16	1-3
Cyprinus carpio Carp	20-24	<0.2	6.0-8.5	40-250, preferably <180	12-16	2-4
Oryzias latipes Japanese Medaka	23-27	<0.2	6.0-8.5	40-250, preferably <180	12-16	1-2
Poecilia reticulata Guppy	21-25	<0.2	6.0-8.5	40-250, preferably <180	12-16	1-2
Lepomis macrochirus Bluegill	21-25	<0.2.	6.0-8.5	40-250, preferably <180	12-16	1-3
Oncorhynchus mykiss Rainbow trout	10-14[10]	<0.2	6.0-8.5	40-250, preferably <180	12-16	3-6
Gasterosteus aculeatus Three-spined stickleback	13-19	0-35	6.0-8.5	40-7500	12-16	1-2
Cyprinodon variegatus Sheepshead minnow	23-27	15-35	6.0-8.5	3000-7500	12-16	1-2
Dicentrarchus labrax European sea bass	18-22	15-35	6.0-8.5	3000-7500	12-16	4-8
Pagrus major Red sea bream	18-22	30-35	6.0-8.5	5000-7500	12-16	2-4

...

Aquatic bioassays – consumers - fish

- **fish cultures** require specific equipment – culture labs with very controlled conditions
- **need for special approval to work experiments with vertebrates !!!**

TANGUAY LAB
THINKING OUTSIDE THE TANK

Aquatic bioassays – consumers - fish

- **fish cultures** require specific equipment – culture labs with very controlled conditions

Aquatic bioassays – consumers - fish

- **standard layout:**
  - aquaria smaller or larger, **aeration needed**
  - various arrangements (static, flow-through ...)
  - acute tests - 96 h, prolonged tests - days to months
  - lethality, growth, reproduction
  - lot of sublethal endpoints possible: behavior, spasms, food intake, breathing, health, histology, bioaccumulation, teratogenicity, carcinogenicity, xenoestrogenicity ➔ ➔ ➔

Clinical sign	Definition	Synonyms
LOSS OF EQUILIBRIUM (sub-categories below)		
Abnormal horizontal orientation	Loss of balance displaying as abnormal horizontal orientation/posture in water column	Keeling, lost righting reflex
Abnormal vertical orientation	Head-up or head-down posture	
Loss of buoyancy control	Floating at surface or sinking to the bottom	
ABNORMAL SWIMMING BEHAVIOUR (sub-categories below)		
Hypoactivity	Decrease in spontaneous activity	Torpid, apathy, lethargy, weak, immobility, inactivity, ceased swimming, quiescent
Hyperactivity	Increase in spontaneous activity	Erratic swimming, skittering
Corkscrew swimming	Rotation around long axis; erratic movements, often in bursts	Rolling, spiralling, spiral swimming, tumbling, circling movements
Convulsions	Abnormal involuntary and uncontrolled contraction of muscles	Seizures, twitching, muscle spasms, shaking, shuddering, vibration
Tetany	Rigid body musculature (intermittent or permanent)	Paralysis
Irritated skin behaviours		Flashing, scraping, rubbing
Abnormal surface distribution/behaviour	Abnormal depth selection, close to water/air interface	Jumping, surfacing; on/at/near/just below surface/top
Abnormal bottom distribution/behaviour	Abnormal depth selection, close to base of tank	Diving, sounding; Lying on/ orientation to / collecting at / near / just above bottom
Over-reactive to stimulus	Flight (startle) or avoidance response to: visual (hand passing over top of tank, light beam), tactile (touch) or vibration (tank rapped lightly) stimulus	Hyperexcitability; hyperactivity after stimulus/threat
Under-reactive to stimulus		Not responsive to external stimulation; inactivity after stimulus/ threat
Loss of schooling / shoaling behaviour	Individual fish show loss of aggregating and social interactions	Isolation, social isolation
Dense schooling / shoaling behaviour	Increase in clumped association of fish	Crowding
ABNORMAL VENTILATORY (RESPIRATORY) FUNCTION (sub-categories below)		
Hyperventilation	Increased frequency of opercular ventilatory movements, with possible open mouth and extended operculae	Rapid/strong respiratory rate/ function. Heavy gill movements, strong ventilation, strongly extended gills, abnormal opercular activity, operculae spread apart, mouth open
Hypoventilation	Decreased frequency of (and possibly shallow) opercular ventilatory movements	Reduced/laboured/weak/slow respiration/respiratory action/ventilation
Irregular ventilation	Irregular opercular ventilatory movements	Sporadic / spasmodic respiration / gill movement
Coughing	Fast reflex expansion of mouth and operculae not at water surface - assumed to clear ventilatory channels	Gasping, abnormal opercular activity, yawn
Gulping	Mouth (and opercular) movements at water surface, resulting in intake of water and air	Piping
Head shaking	Rapid lateral head movements	
ABNORMAL SKIN PIGMENTATION (sub-categories below)		
Darkened		Changed / increased / dark(ened) colour / pigmentation / melanistic markings
Lightened		Pallor, pale/changed/weak pigmentation
Mottled		Discoloured patches
OTHER VISIBLE (APPEARANCE & BEHAVIOUR) ABNORMALITIES (sub-categories below)		
Exophthalmia	Swelling within orbital socket(s) resulting in bulging of one or both eyes	Exophthalmos, exophthalmus, popeye, protruding eyeball
Oedema	Abdominal swelling due to accumulation of fluid. May cause protruding scales and/or fissure in abdominal wall	Distended/swollen/bloated abdomen/gut area; dropsy
Haemorrhage	Petechias (pinhead sized spots) and/or haematoma (area of blood) due to intradermal or sub-mucus bleeding	
Mucus secretion	Excess mucus production	Mucus build-up (pay close attention to eyes); increased secretion (mucus on skin or in water); mucus loss
Faecal (anal) casts	String of faeces hanging from anus or on tank floor	
Aggression and/or cannibalism		Aggression, direct attack, domination of choice tank locations, pick at or eat bodies of dead fish

Aquatic bioassays – consumers - fish

Acute toxicity test

- juvenile fish, acclimatized
- medium: reconstituted water, groundwater or clean water
- 24-96 h
- conditions depend on species, e.g. pH, temp, photoperiod
- disolved O_2 >60% saturation
- no feeding
- mortality, size, weight

validity

- controls: mortality < 10%, O2 etc.
- dissolved O_2 ≥ 60% of air saturation
- analytical measurement of test concentrations is compulsory

Parameter	Maximum concentration
Particulate matter	5 mg/L
Total organic carbon (TOC)[11]	2 mg/L
Un-ionised ammonia (NH_3)	1 µg/L
Nitrate (NO_3)	<9 mg/L[12]
Residual chlorine	10 µg/L
Total organophosphorus pesticides	50 ng/L
Total organochlorine pesticides plus polychlorinated biphenyls	50 ng/L
Total organic chlorine	25 ng/L
Aluminium (Al)	1 µg/L
Arsenic (As)	1 µg/L
Chromium (Cr)	1 µg/L
Cobalt (Co)	1 µg/L
Copper (Cu)[13]	1 µg/L
Iron (Fe)	1 µg/L
Lead (Pb)	1 µg/L
Nickel (Ni)	1 µg/L
Zinc (Zn)	1 µg/L
Cadmium (Cd)	100 ng/L
Mercury (Hg)	100 ng/L
Silver (Ag)	100 ng/L
Chemical oxygen demand (COD)[14]	5 mg/L

Test No. 203: Fish, Acute Toxicity Test		2019
ISO 7346-1:1996	Water quality — Determination of the acute lethal toxicity of substances to a freshwater fish (Brachydanio rerio ...) - Part 1: Static method	
ISO 7346-2:1996	Part 2: Semi-static method	
ISO 7346-3:1996	Part 3: Flow-through method	

Aquatic bioassays – consumers - fish

Prolonged, chronic toxicity tests

- **prolonged** = longer exposure with mortality endpoint, sublethal endpoints also recorded
- from 14 to 28 d
- **chronic** = also other endpoints like reproduction
- 7-200 d

OECD	Test No. 210: Fish, Early-life Stage Toxicity Test	2013
	Test No. 215: Fish, Juvenile Growth Test	2000
	Test No. 229: Fish Short Term Reproduction Assay	2012
	Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study	1984
	Test No. 230: 21-day Fish Assay	2009
	Test No. 240: Medaka Extended One Generation Reproduction Test (MEOGRT)	2015
ISO	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))

Aquatic bioassays – consumers - fish

Embryonal, embryolarval tests

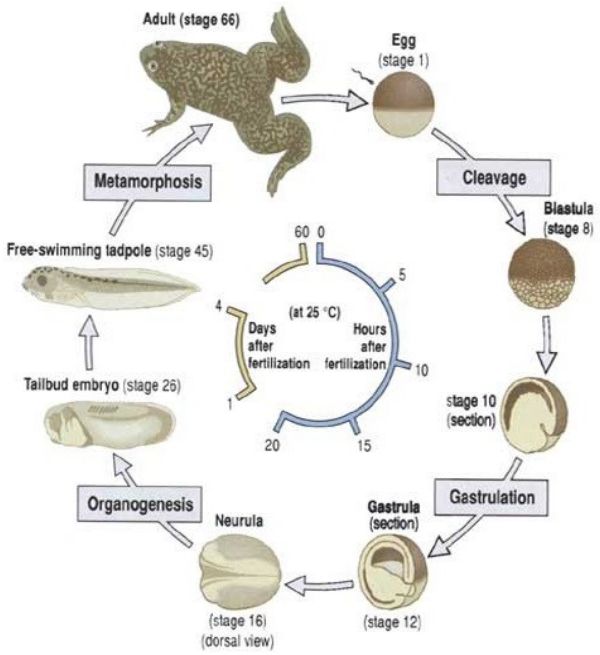
- fertilized eggs are exposed to chemicals for several days
- effects on development = **teratogenicity**
- prolonged to hatching and larvae development
- endpoints: hatching, survival, development, behavior, size

Test No. 210: Fish, Early-life Stage Toxicity Test		2013
Test No. 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages		1998
Test No. 236: Fish Embryo Acute Toxicity (FET) Test		2013
ISO 15088:2007	Water quality — Determination of the acute toxicity of waste water to zebrafish eggs (Danio rerio)	

Aquatic bioassays – consumers - frog

FETAX – Frog Embryo Teratogenicity Assay Xenopus

- fertilized eggs are exposed to chemicals for several days
- effects on development = **teratogenicity**



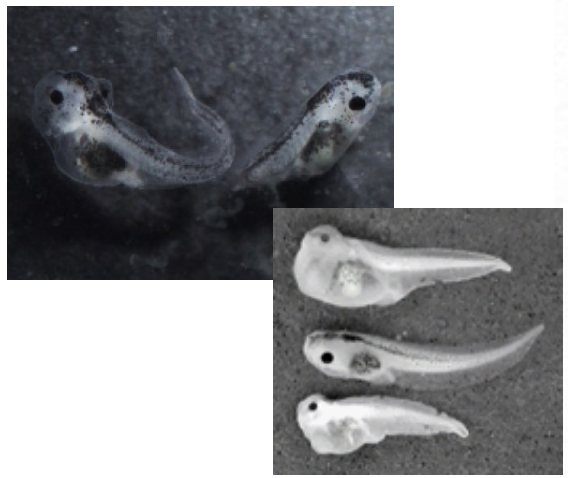
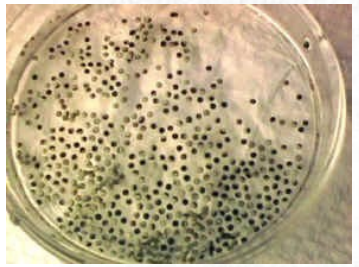


Table 4.11 The Frog Embryo Teratogenesis Assay: Xenopus (FETAX)

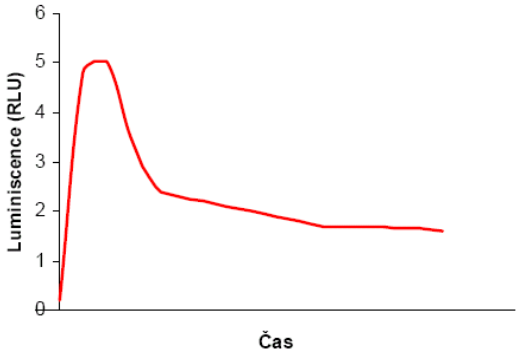
Test type	96 h static renewal
Organism	*Xenopus laevis*
Age of parent organism	Adult male: at least 2 years of age Adult female: at least 3 years of age
Size of parent organism	Adult male: 7.5–10 cm in crown-rump length Adult female: 10–12.5 cm in length
Feeding	Adult: three feedings per week of ground 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)



Aquatic bioassays – destruents - bacteria

Vibrio fisheri luminescece test

- marine bacteria - bad for samples with high minerals or organic matter - stimulation
- very quick - 5-30 min
- luminiscence inhibition
- problem with particles and colour ➔ flash test

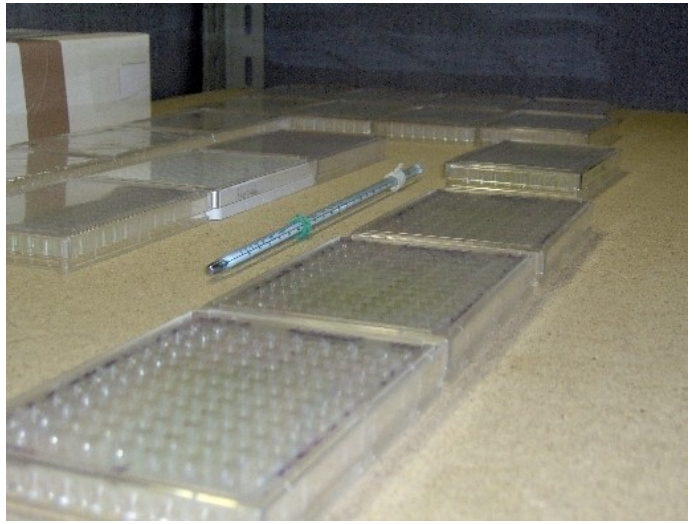


ISO 11348-1:2007/Amd 1:2018	Water quality — Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) — Part 1: Method using freshly prepared bacteria — Amendment 1
ISO 11348-2:2007/Amd 1:2018	Part 2: Method using liquid-dried bacteria — Amendment 1
ISO 11348-3:2007/Amd 1:2018	Part 3: Method using freeze-dried bacteria — Amendment 1

Aquatic bioassays – destruents - bacteria

Pseudomonas putida growth inhibition test

- ...



Aquatic bioassays – destruents - bacteria

Mutation and genotoxicity tests

- with bacteria, but used also for general testing of mutagenicity and genotoxicity
- with liver fraction S9 simulate activation of xenobiotics within de-toxification in vertebrates

Salmonella sp. – Ames test

- mutants cannot live in medium without histidine
- in case of mutagenic chemical the reverse mutation is induces – they grow

Umu-C

- transgenic bacteria with luciferase gene introduced to operon for DNA reparation
- genotoxicity causes activation of reparation and thus luciferase and light

ISO 11350:2012	Water quality — Determination of the genotoxicity of water and waste water — Salmonella/microsome fluctuation test (Ames fluctuation test)
ISO 16240:2005	Water quality — Determination of the genotoxicity of water and waste water — Salmonella/microsome test (Ames test)
ISO 13829:2000	Water quality — Determination of the genotoxicity of water and waste water using the umu-test

Aquatic bioassays – destruents - bacteria

Testing the effects on activated sludge

- important because of biotechnologies – water treatment plants
- **complex microbial community** and its activities: **respiration, nitrification**
- in erlenmeyer flasks etc.
- measurements of biological oxygen demand (BOD) or respirometry

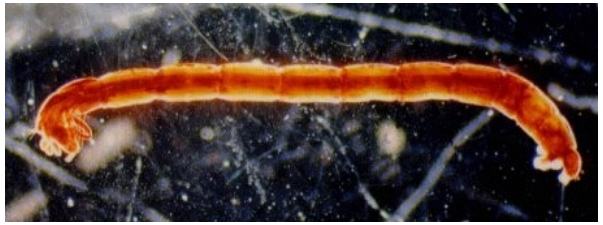
Test No. 224: Determination of the Inhibition of the Activity of Anaerobic Bacteria		2007
Test No. 209: Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation)		2010
Test No. 244: Protozoan Activated Sludge Inhibition Test		2017
ISO 15522:1999	Water quality — Determination of the inhibitory effect of water constituents on the growth of activated sludge microorganisms	
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	

Aquatic bioassays – sediments

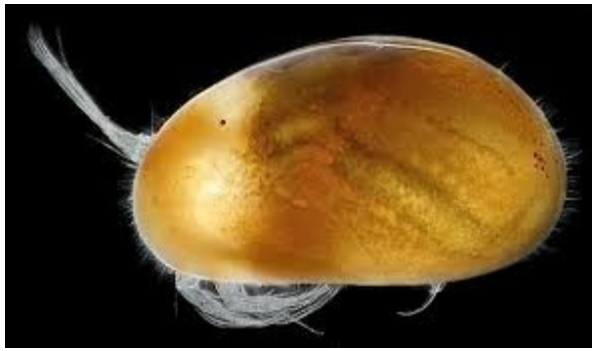
- sediment = aquatic equivalent of the soil ➔ very **heterogenic matrix**, contains **solid phase** (mineral particles and organic matter) and **pore water**
- sediment is often potential long-term reservoir / source of contaminants
- **distribution of contaminatns between water and sediment (sorption)** – sediment organisms are exposed to contaminants in solid and/or liquid components of sediment
- benthic organisms

Aquatic bioassays – sediments

Chironomus riparius



Heterocypris incongruens



Chironomus tentans

Lumbriculus variegatus

Tubifex tubifex

Hyalela aztecaincongruens

Aquatic bioassays – consumers - invertebrates

Tests with chironomids

OECD 235

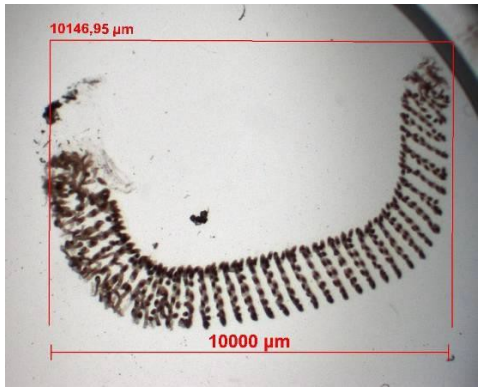
- same as Daphnia acute test, just with Chironomus larvae

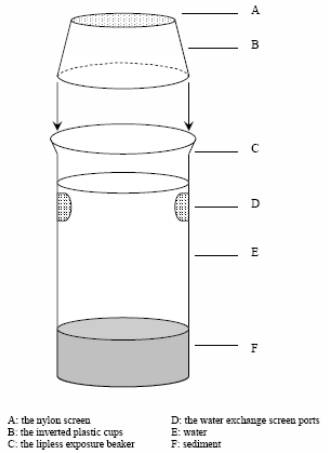
OECD 218+219

- 10 larvae (cca 10d old) per beaker
- OECD artificial sediment
- 100 ml sediment / 175 ml water
- 21-28 d; 16/8 h light/dark; controled pH, O_2
- food
- survival and growth

OECD 233

- reproduction = development to midges





Test No. 235: Chironomus sp., Acute Immobilisation Test	2011
Test No. 218: Sediment-Water Chironomid Toxicity Using Spiked Sediment	2004
Test No. 219: Sediment-Water Chironomid Toxicity Using Spiked Water	2004
Test No. 233: Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment	2010

Soil bioassays - examples

Soil bioassays – producents - plants

- large number of different tests

various endpoints

- seed germination and root elongation
- emergence
- seedling growth
- biomass production
- life cycle (changes in weight, size, number of flowers, seeds ..)
- physiological tests (photosynthesis, respiration)
- enzymatic tests
- symbiosis – N fixation, mycorhiza

Soil bioassays – producents - plants

species

- usually needed at least **monocolyledenous + dicolyledenous**
- Sinapis alba, Lactuca sativa, Lepidium sativum, Hordeum vulgare, Zea mays
-

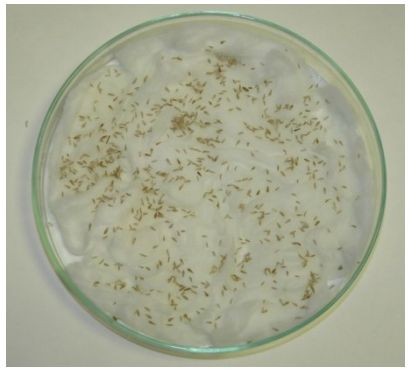


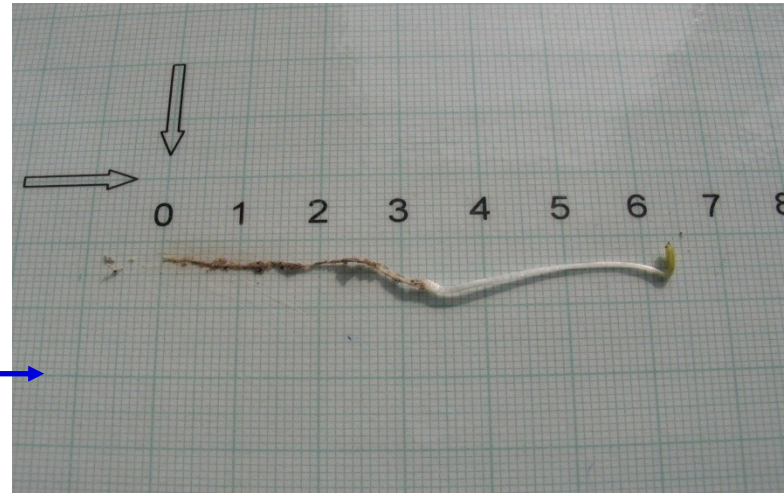


Soil bioassays – producents - plants

Root elongation inhibition

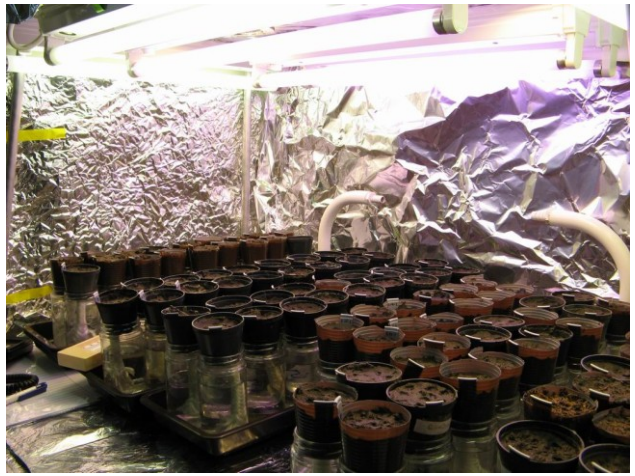
- preparation of seeds to 2 mm
- 15 seeds to 200-300 g soil
- 3-5 d; soil pH; dark; 24°C
- root length

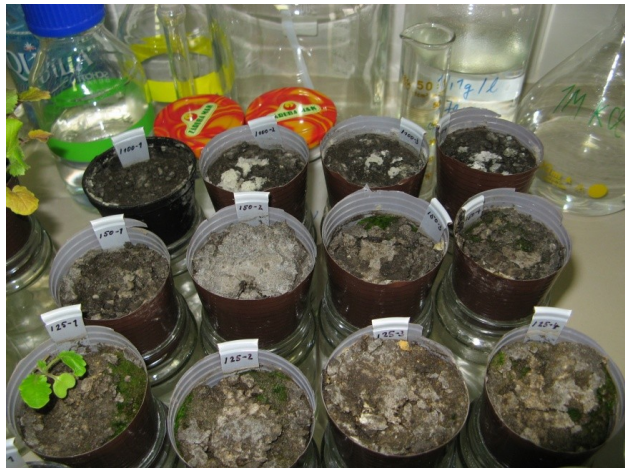




Soil bioassays – producents - plants

Emergence, early growth, growth, chronic





Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test	2006
Test No. 227: Terrestrial Plant Test: Vegetative Vigour Test	2006

ISO 11269-2:2012	Soil quality — Determination of the effects of pollutants on soil flora — Part 2: Effects of contaminated soil on the emergence and early 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 18763:2016	Soil quality — Determination of the toxic effects of pollutants on germination and early growth of higher plants
ISO 22030:2005	Soil quality — Biological methods — Chronic toxicity in higher plants

Soil bioassays – producents - plants

https://www.microbiotests.com

http://ebpi.ca/_slideshows_Phytotoxkit%20slide%20show.pdf

PHYTOTOXKIT
SOLID
SAMPLES

Soil bioassays – consumers - invertebrates

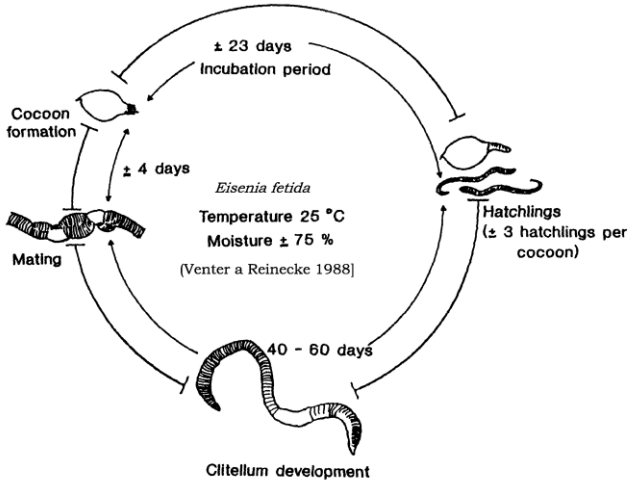
Earthworms

- earthworms are the most and longest time used representative of soil organisms in ecotoxicology – the oldest test OECD from 1984
- Benefits and reasons:
  - the whole development cycle takes place in the soil - **a typical geobiont**
  - consume large amounts of soil (**high food exposure and accumulation of contaminants**)
  - have very **close physical contact with soil** (skin exposure + oral)
  - they have significant bioaccumulation and bioconcentration character – **macroconcentrators**
  - **very significant role in soil formation, decomposition processes, soil fertility**
  - key role in the transfer of pollutants in **food chains**
  - occurrence in almost all soils in high numbers and weights
  - well introduced in laboratory tests (easy breeding)
  - easily identified in real samples (thanks to size)...

Soil bioassays – consumers - invertebrates

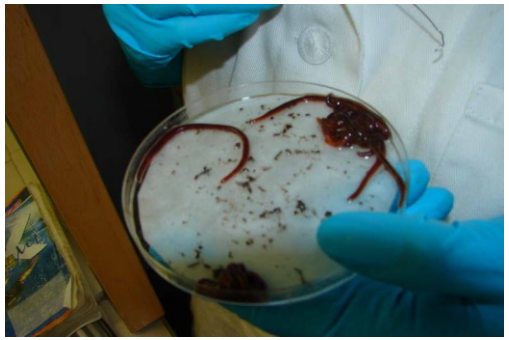
Earthworms - acute

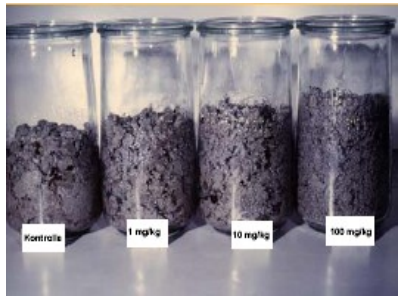
- Eisenia fetida / Eisenia andrei
- culcure in mixture manure/soil/garden-substrate/peat
- 7,14 days
- 500 g soil; 50-60 % water holding capacity
- artificial soil or LUFA 2.2 or other



Soil bioassays – consumers - invertebrates

Earthworms - acute

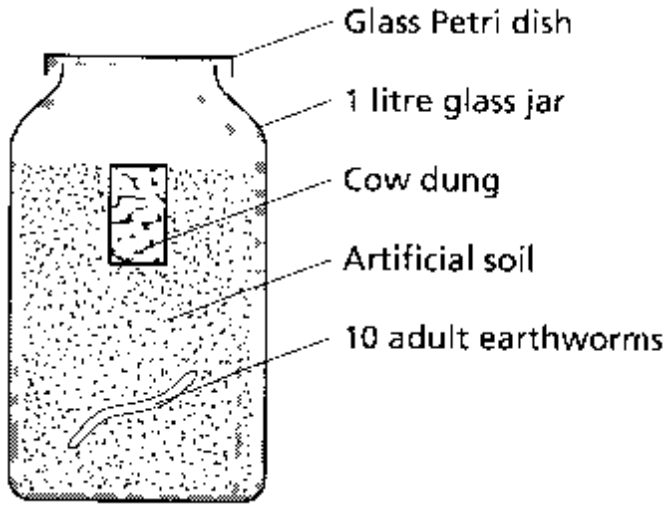




Soil bioassays – consumers - invertebrates

Earthworms - reproduction

- 2 months: after 1 month adults removed, after 2nd month juveniles counted
- soil and conditions same as for acute test but **food added (dung)**
- various techniques how to extract juveniles from soil (usually heat)



Soil bioassays – consumers - invertebrates

Earthworms - reproduction

Soil preparation

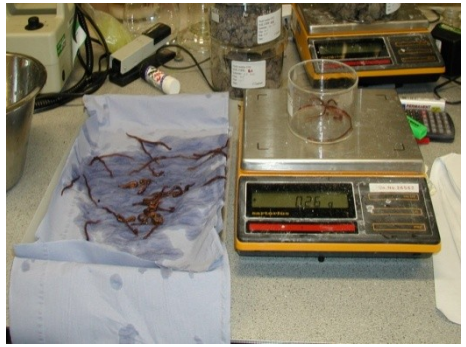
WHC measurement

Water added

Soil weighted to jars

10 adults from culture

Washed



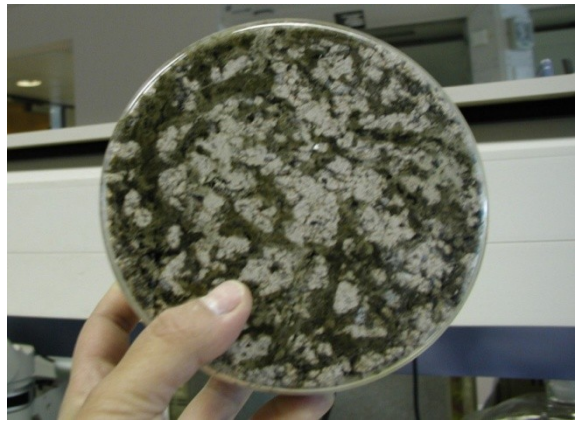
Weighting worms

10 adults to 1 jar

Soil bioassays – consumers - invertebrates

Earthworms - reproduction

Temperated room



Control of the jars, activity markers

Mortality assessment

Weighting the worms

Soil bioassays – consumers - invertebrates

Earthworms - reproduction

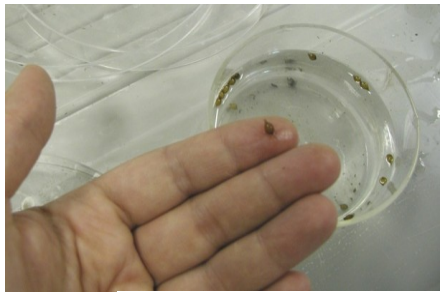
After 20 min juveniles appear

Collecting and counting juveniles

Water bath, increasing temperature 40ºC - 60ºC

Sieving the soil

Hand sorting of cocoons

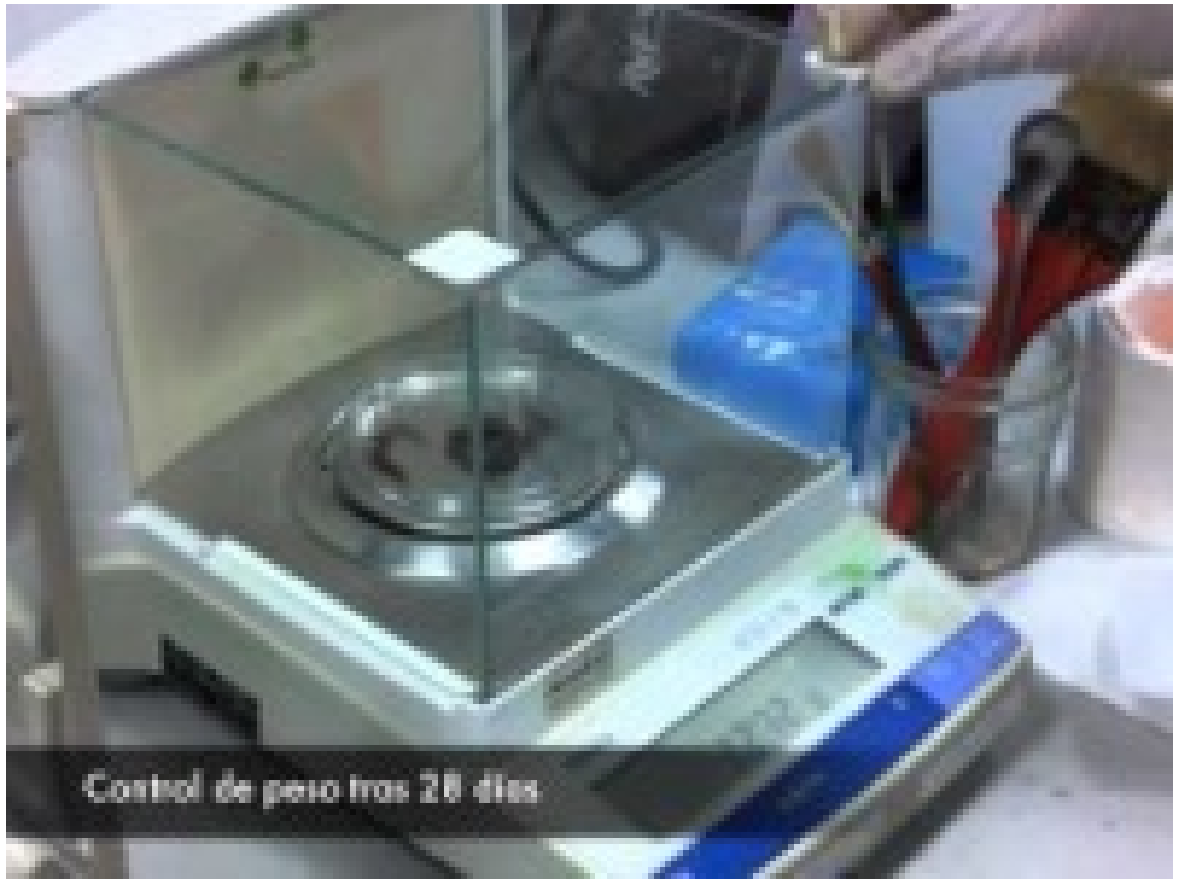


Counting

Soil bioassays – consumers - invertebrates

Earthworms - reproduction

- …

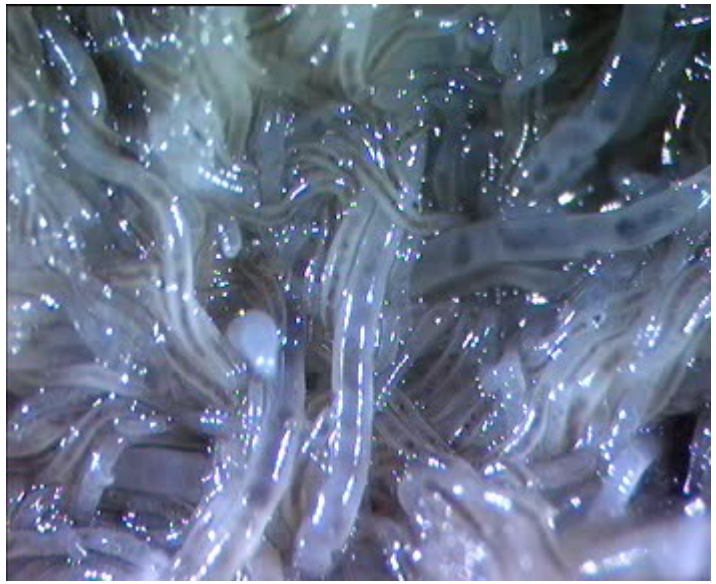


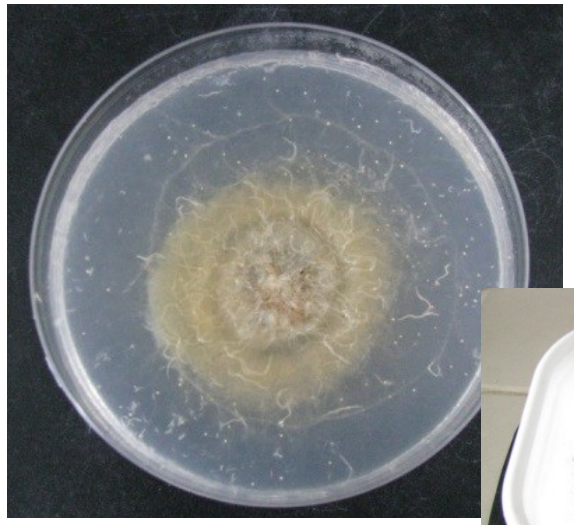
Test No. 222: Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)		2016
ISO 11268-2:2012	Soil quality — Effects of pollutants on earthworms — Part 2: Determination of effects on reproduction of Eisenia fetida/Eisenia andrei	

Soil bioassays – consumers - invertebrates

Enchytraeids

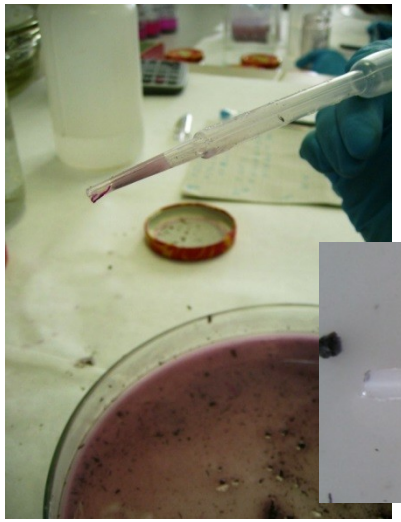
- Enchytraeus albidus or Enchytraeus crypticus
- 28 d; 20-30 g soil; 50-60% water holding capacity
- artificial soil or LUFA 2.2 or other

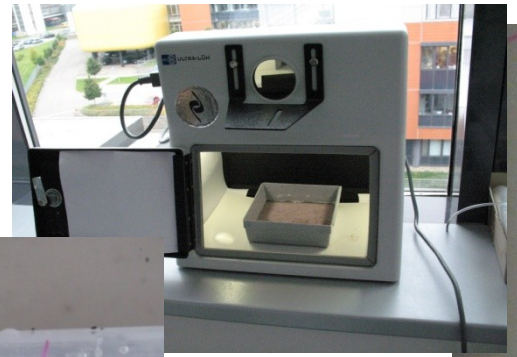


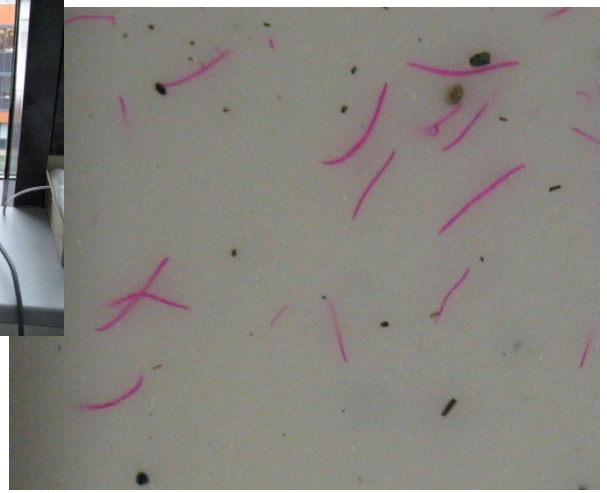


Soil bioassays – consumers - invertebrates

Enchytraeids

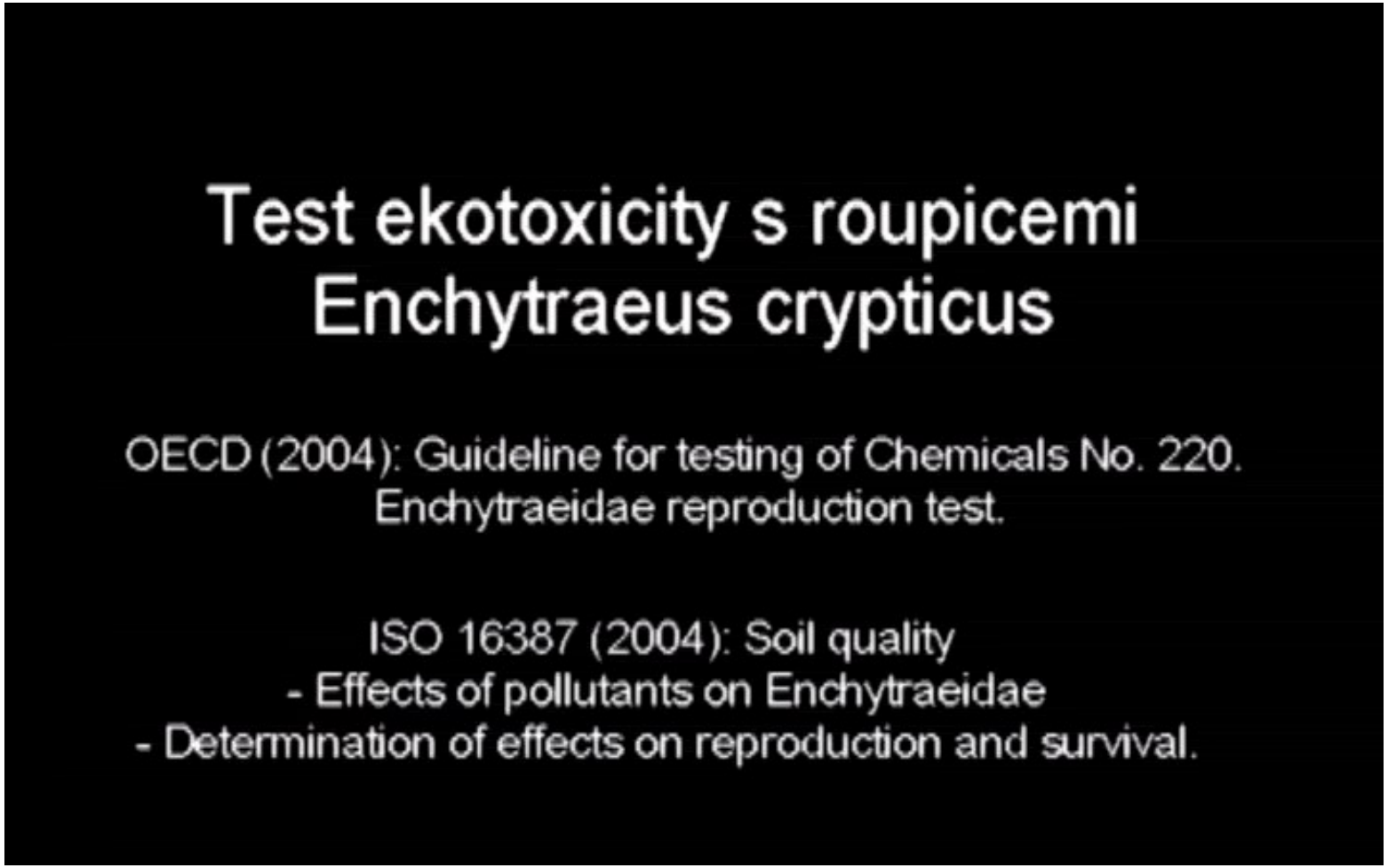






Soil bioassays – consumers - invertebrates

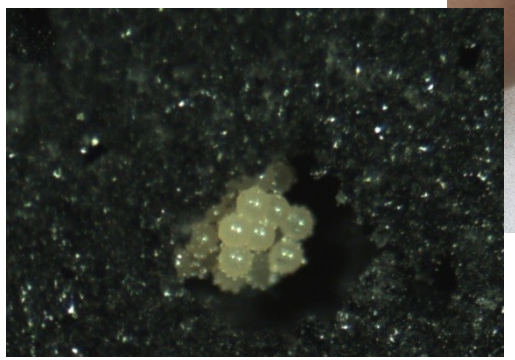
Enchytraeids



Soil bioassays – consumers - invertebrates

Springtails

- Folsomia candida partenogenetic collembola or Folsomia fimetaria
- 28 d; 20-30 g soil; 50-60% water holding capacity
- artificial soil or LUFA 2.2 or other



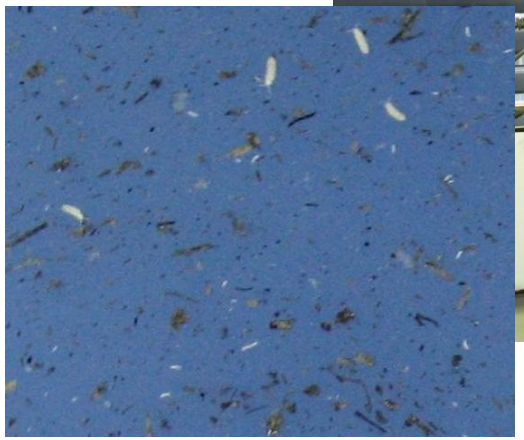
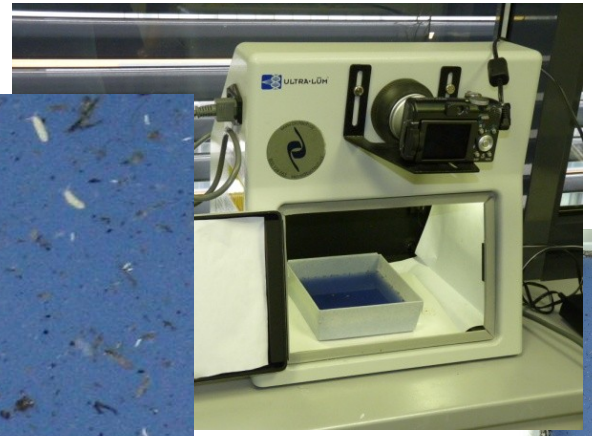
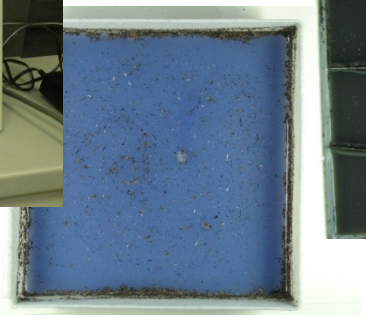
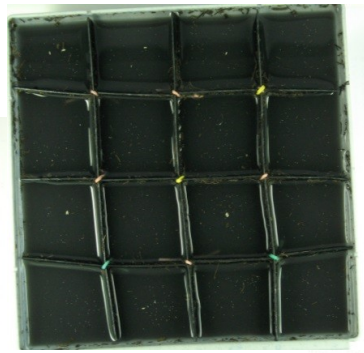
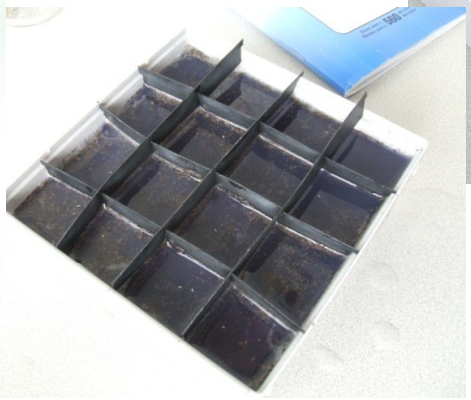
Soil bioassays – consumers - invertebrates

Springtails



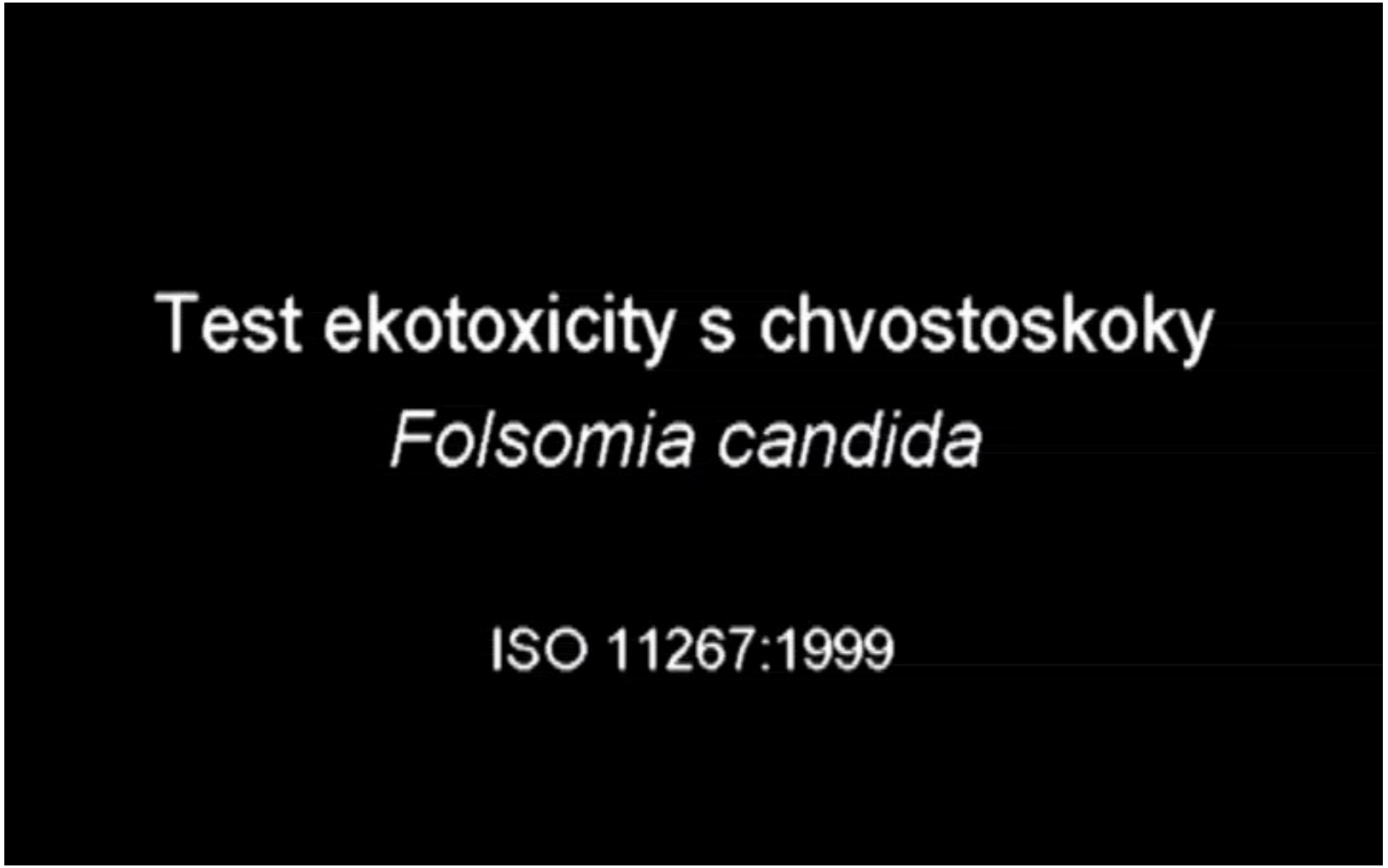




Soil bioassays – consumers - invertebrates

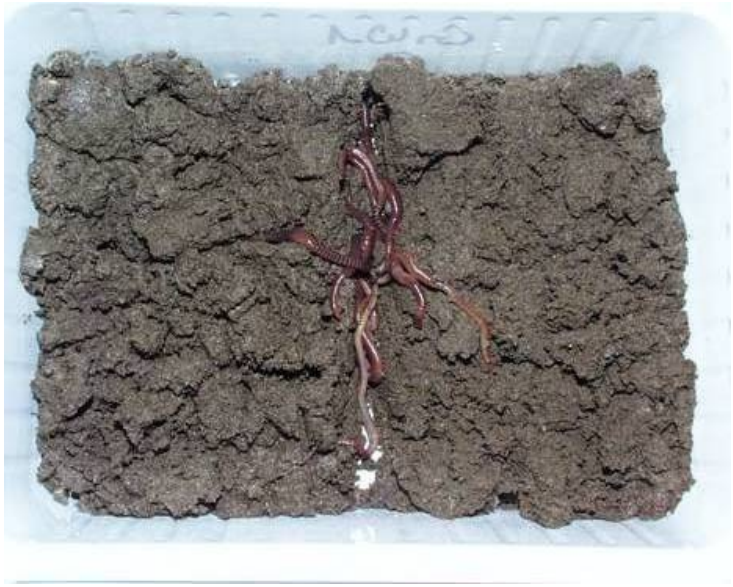
Springtails

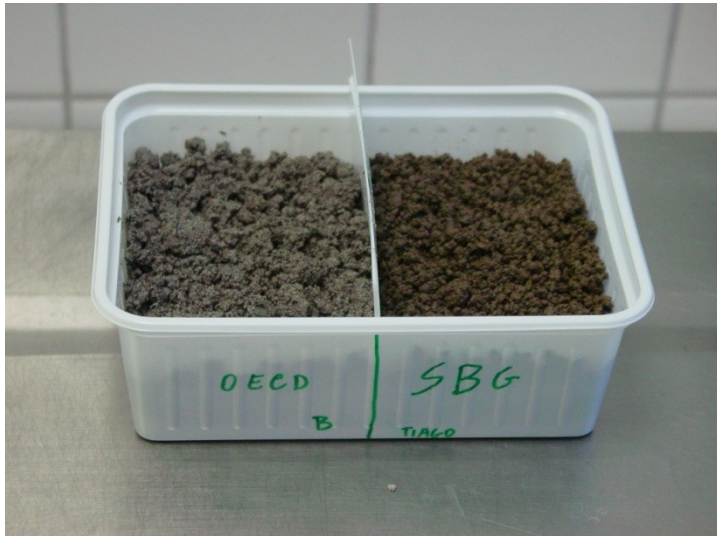


Soil bioassays – consumers - invertebrates

Avoidance tests

- with earthworms or springtails
- very quick (1-2 days) - screening





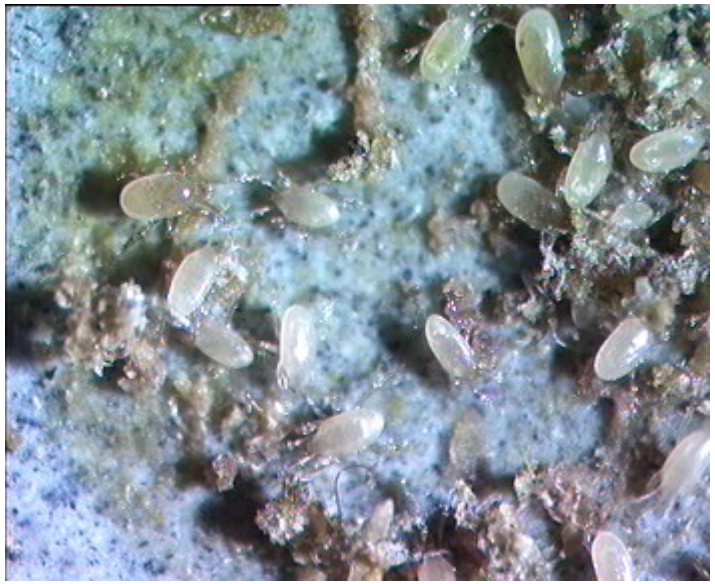
Soil bioassays – consumers - invertebrates

Mites

- ...

Hypoaspis aculeifer

predator



cont. soil

prey

Test No. 226: Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil		2016
ISO 21285:2019	Soil quality — Inhibition of reproduction of the soil mite (Hypoaspis aculeifer) by soil contaminants	
ISO 23266:2020	Soil quality — Test for measuring the inhibition of reproduction in oribatid mites (Oppia nitens) exposed to contaminants in soil	

Soil bioassays – consumers - invertebrates

Snails

- ...

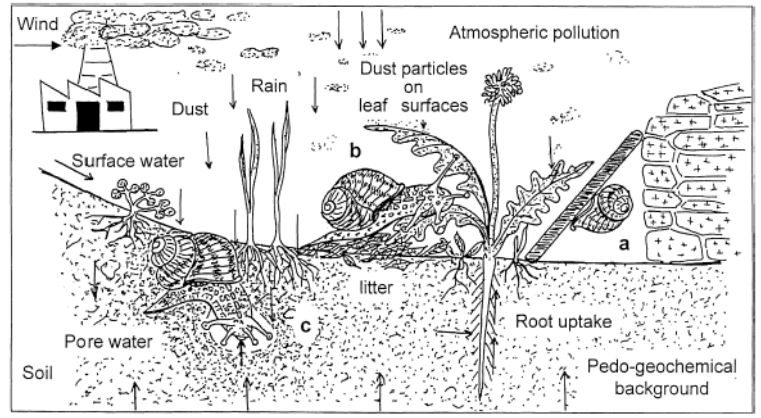
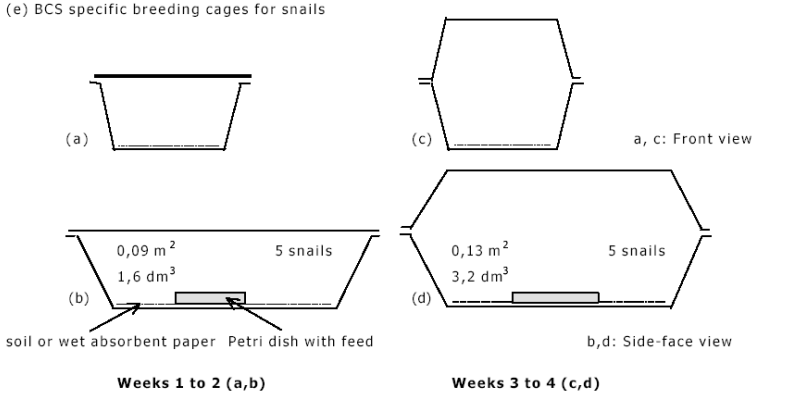


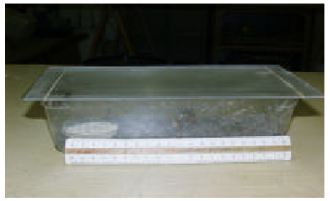
Fig. 6. Possible routes of direct and indirect exposure of land snails to contaminants: (a) contamination by breathing air; (b) contamination at soil surface by eating plants, soil contact, breathing air, rain and surface water contact; (c) contamination in the soil by swallowing soil particles, soil contact and interstitial air and pore water.

(a, b) A transparent plexiglass cover held in place by two rubber bands (weeks 1 and 2 of the test): volume 1.6 dm3.

(c, d) The flat cover replaced by another box up-side down (weeks 3 and 4 of the test): volume 3.2 dm3.

(e) BCS specific breeding cages for snails



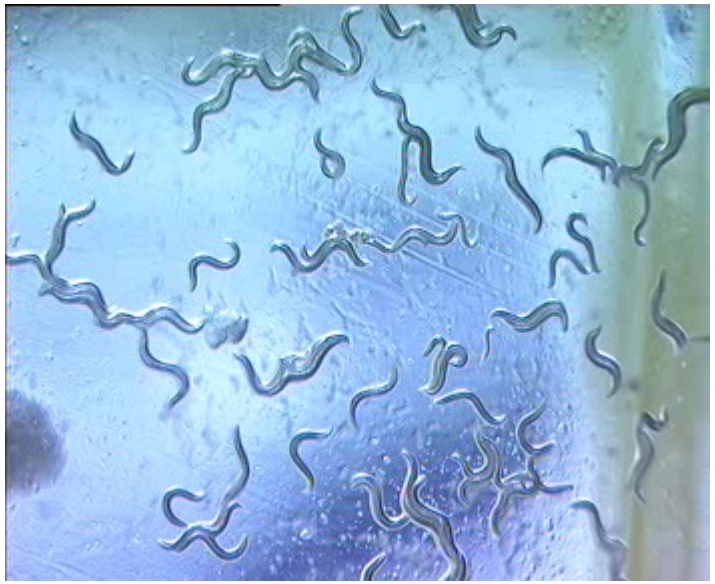


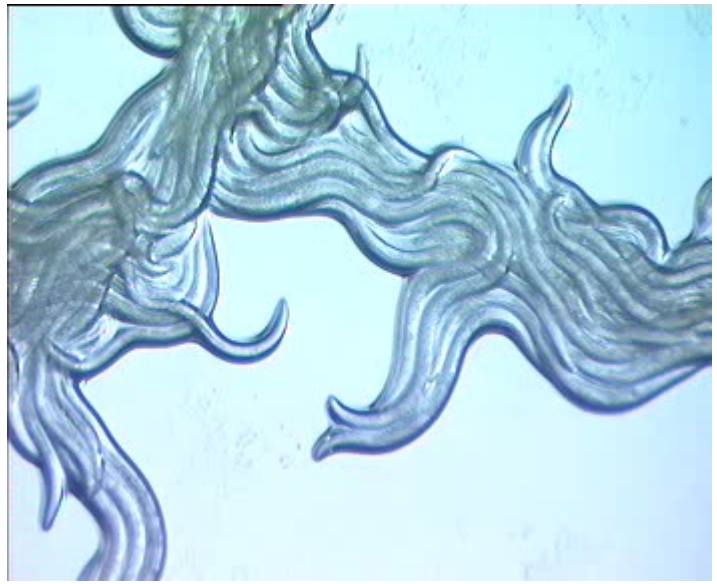
(e)

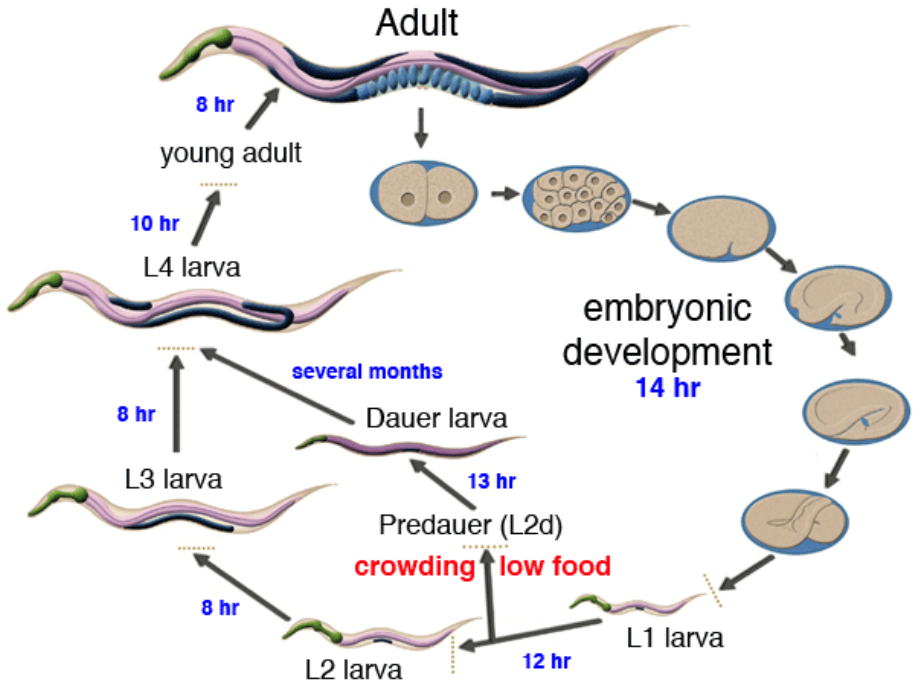
Soil bioassays – consumers - invertebrates

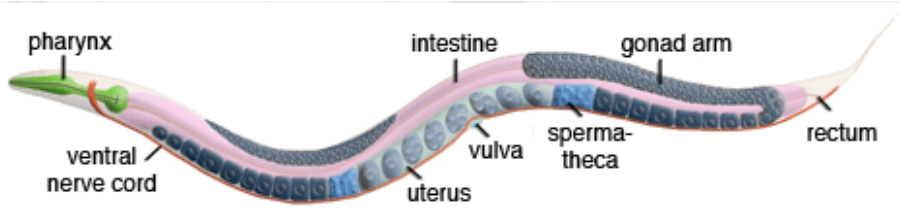
Nematodes

- the most abundant soil invertebrates
- in fact aquatic organisms – pore water
- very fast tests - short life cycle - screening





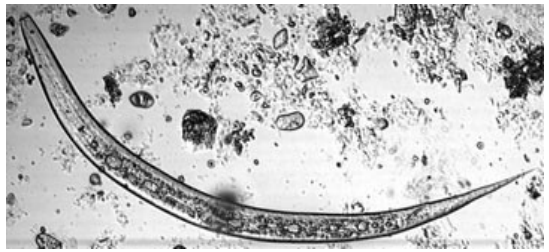


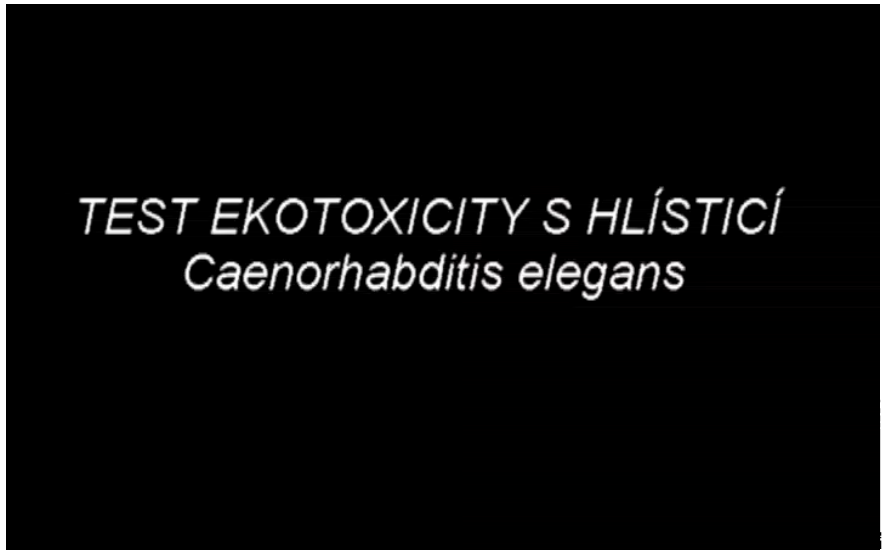


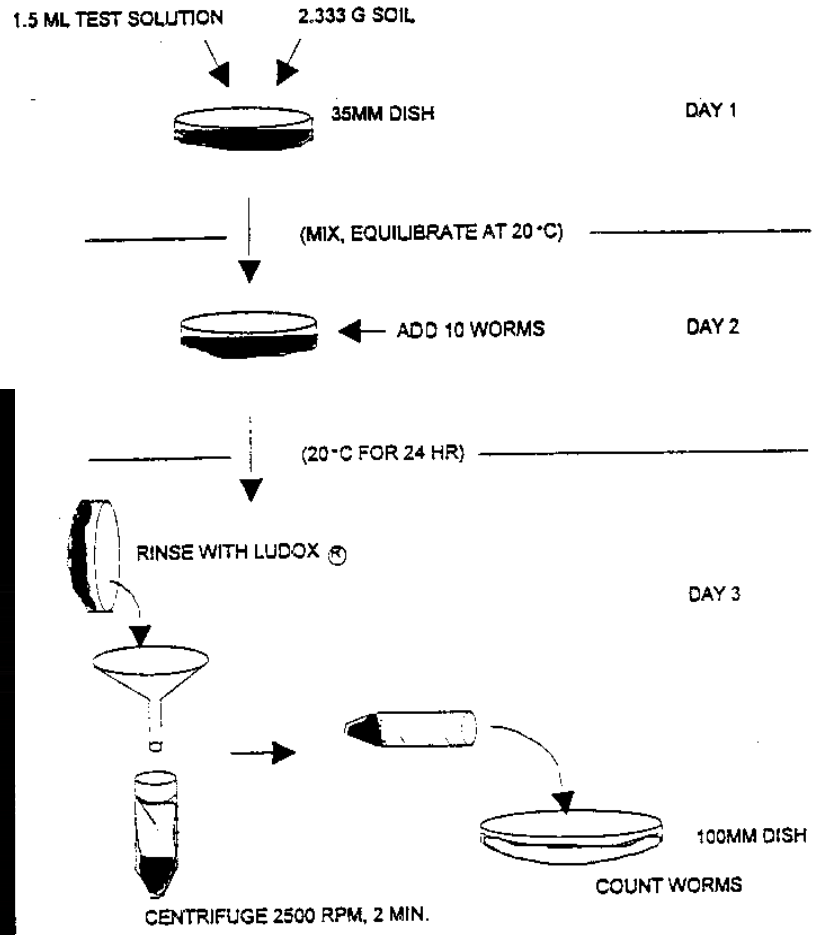
Soil bioassays – consumers - invertebrates

Nematodes

- Caenorhabditis elegans
- grown on agar plates with E. coli
- aseptic techniques and careful handling needed !
- 24-48 h exposure







ASTM E 2172 – 01 Standard Guide for Conducting Laboratory Soil Toxicity Tests with the Nematode Caenorhabditis elegans

ISO 10872:2020	Water and soil quality — Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of Caenorhabditis elegans (Nematoda)

Soil bioassays – consumers - invertebrates

Beneficial arthropods

- testing of pesticides
- natural enemies of pests

Lithobius mutabilis

Philonthus cognatus

Poecilus cupreus

Linyphiidae

Soil bioassays – destruents – microorganisms

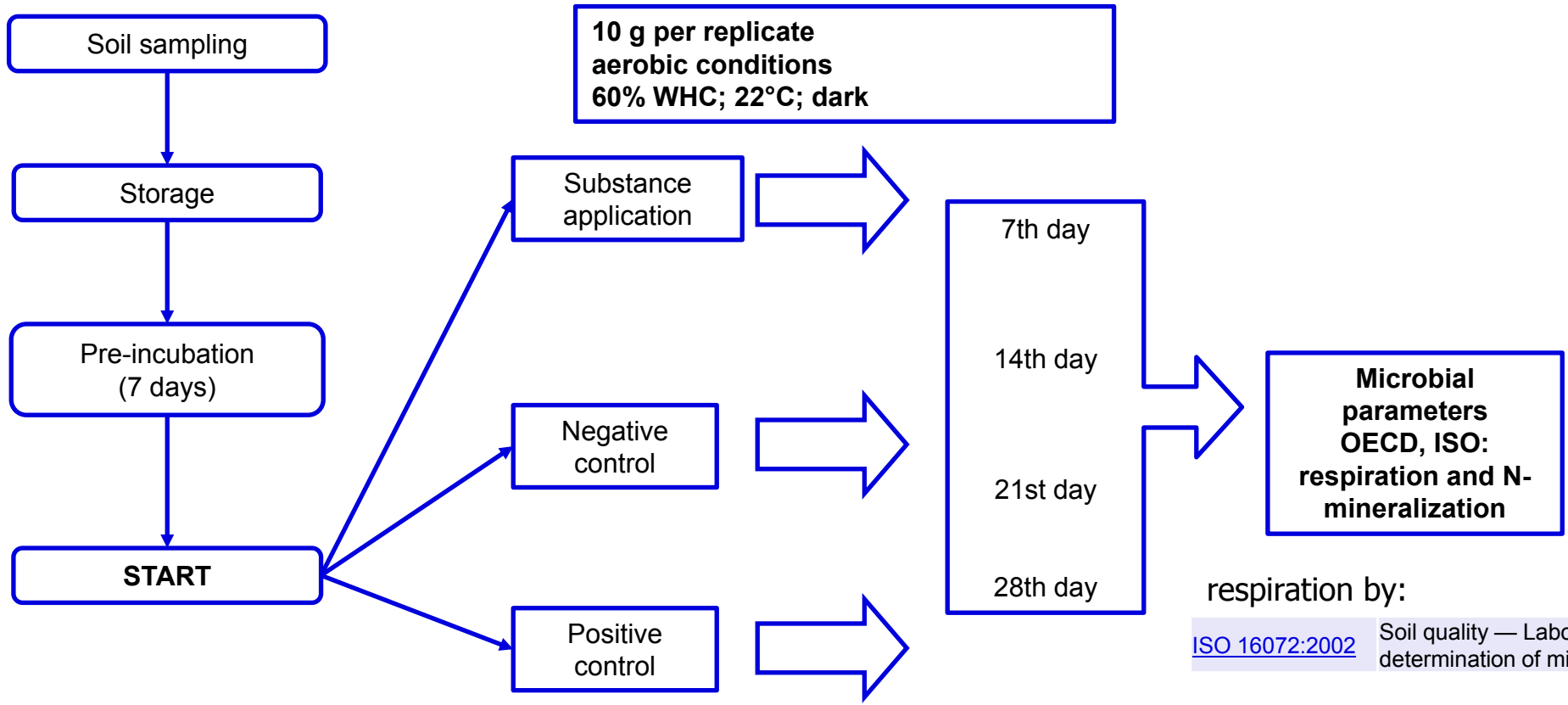
Whole community testing for microbial activities

- real uncontaminated agricultural soil with indigenous microflora of desired properties:
  - sand > 70%; TOC = 1.5%; pH(KCl) = 7–7.5
  - microbial biomass 400-700 µg C . g_{dw}^{-1}; basal respiration 0.5-0.7 µg CO_2-C . h^{-1} . g_{dw}^{-1}

Test No. 216: Soil Microorganisms: Nitrogen Transformation Test		2000
Test No. 217: Soil Microorganisms: Carbon Transformation Test		2000
ISO 14238:2012	Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes	

Soil bioassays – destruents – microorganisms

Whole community testing for microbial activities

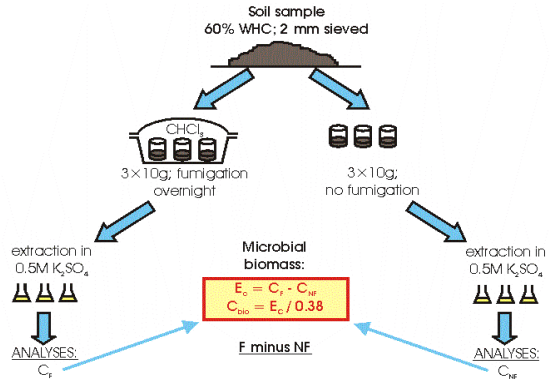


Test No. 216: Soil Microorganisms: Nitrogen Transformation Test		2000
Test No. 217: Soil Microorganisms: Carbon Transformation Test		2000
ISO 14238:2012	Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes	

Soil bioassays – destruents – microorganisms

Whole microbial community testing

- same design of exposure as in OECD 216, 217
- also other parameters can be measured !!!



biomass	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
enzyme activity	ISO 23753-1:2019	Soil quality — Determination of dehydrogenases activity in soils — Part 1: Method using triphenyltetrazolium chloride (TTC)
	ISO 23753-2:2019	Soil quality — Determination of dehydrogenases activity in soils — Part 2: Method using iodotetrazolium chloride (INT)
diversity • structural • genetic • functional	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/TS 29843-2:2011	Soil quality — Determination of soil microbial diversity — Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method
	ISO 11063:2020	Soil quality — Direct extraction of soil DNA
	ISO 17601:2016	Soil quality — Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil
	ISO 20130:2018	Soil quality — Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates
denitrification	ISO/TS 20131-1:2018	Soil quality — Easy laboratory assessments of soil denitrification, a process source of N2O emissions — Part 1: Soil denitrifying enzymes activities
	ISO/TS 20131-2:2018	Soil quality — Easy laboratory assessments of soil denitrification, a process source of N2O emissions — Part 2: Assessment of the capacity of soils to reduce N2O

Soil bioassays – destruents – microorganisms

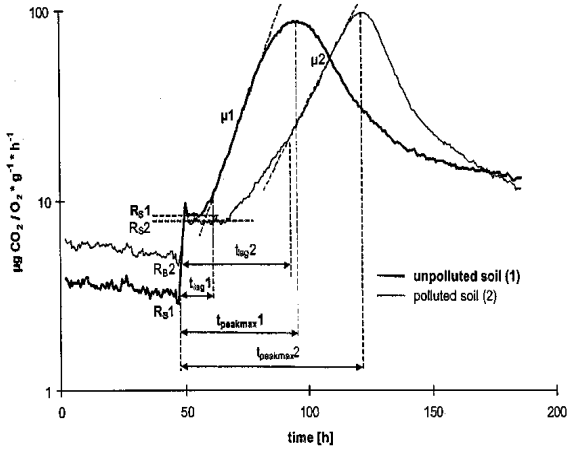
Whole community testing for microbial activities – quick tests

Potential ammonium oxidation

- = ammonification, first step of nitrification
- 3 h oxidation of ammonium sulfate
- sodium chlorate added – stops nitrite oxidation to nitrate
- nitrite measured by colorimetric test

Respiration curves

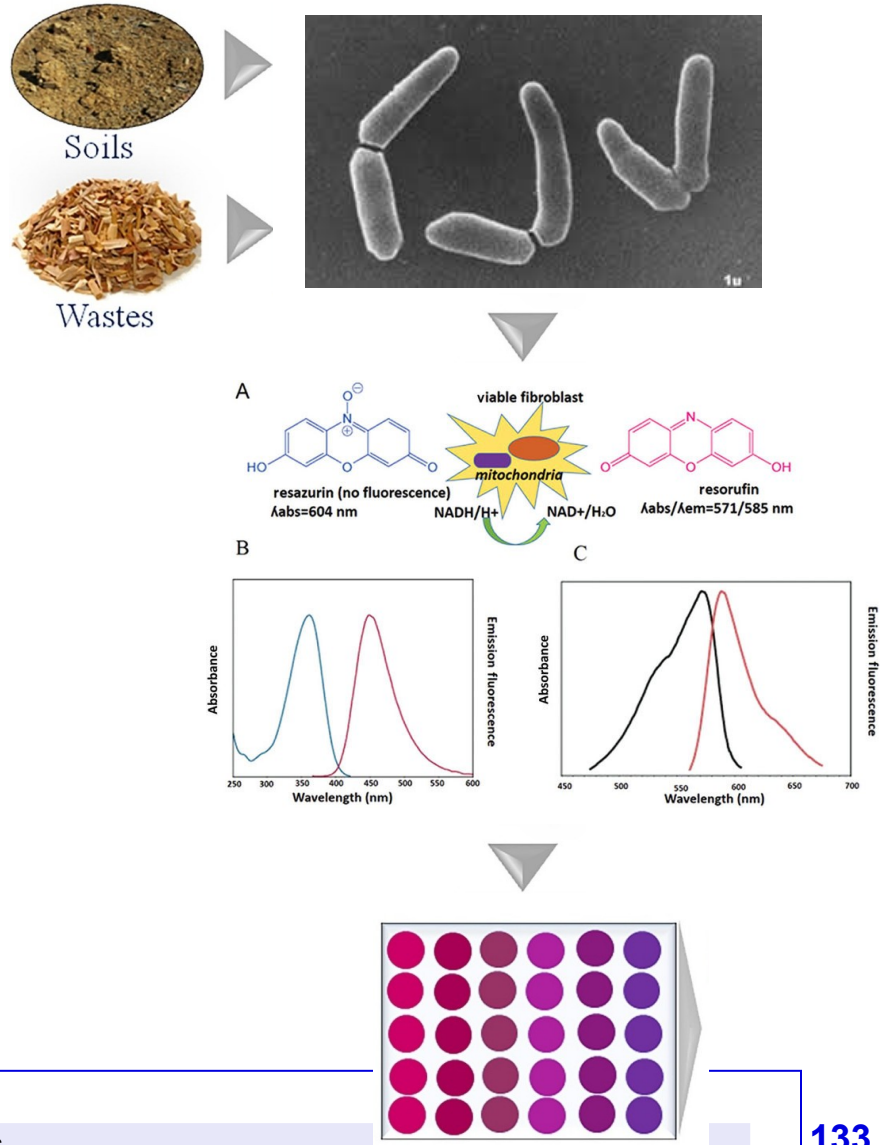
- they indicate the growth of microbial community
- respirometry – increase of CO_2 or decrease of O_2



Soil bioassays – destruents – microorganisms

Arthrobacter globiformis test

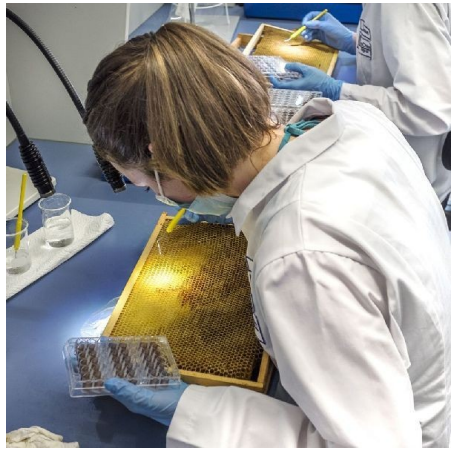
- introduced bacteria species NOT whole community
- contaminated soil or other solid materials (waste) or soil spiked with tested chemical(s)
- A. globiformis added and dehydrogenase activity measured



Terrestrial species bioassays - examples

Terrestrial species bioassays - polinators

- **bees or bumblebees**
- brood development of cells (eggs or larvae)
- acute oral or contact tests, prolonged-chronic tests – mortality, behavior
- semi-field tests [tunnel tests (~100 m^2)] and field tests - mortality, flight density, development of bee colonies (brood and feed), behavior of bee colonies
- residue analysis studies in relevant feed matrices



Terrestrial species bioassays - polinators

Acute oral test

- adult worker honey bees
- doses of test substance in sugar solution (100-200 µl, 50% sugar solution)
- after consuming food (3-4 hours), the feeding device is removed
- mortality is recorded daily for at least 48 h

Acute contact test

- test substance dissolved in a suitable vehicle
- applied directly to anesthetized bees – 1 µl droplets to back area of each bee chest
- control for carrier necessary

Test No. 213: Honeybees, Acute Oral Toxicity Test	1998
Test No. 214: Honeybees, Acute Contact Toxicity Test	1998
Test No. 245: Honey Bee (Apis Mellifera L.), Chronic Oral Toxicity Test (10-Day Feeding)	2017
Test No. 246: Bumblebee, Acute Contact Toxicity Test	2017
Test No. 247: Bumblebee, Acute Oral Toxicity Test	2017

Terrestrial species bioassays

Birds

- dietary toxicity tests
- dietary dosage + 14 days of observation
- reproduction tests - long-term

Japanese quail

Bobwhite quail

Feral pigeon

Malard duck

Test No. 223: Avian Acute Oral Toxicity Test	2016
Test No. 205: Avian Dietary Toxicity Test	1984

Terrestrial species bioassays

Birds

- 20 weeks of feeding by contaminated feed
- reproduction + 14 days observation of juveniles

Table 4.9 Summary for Conducting Reproductive Studies with Avian Species

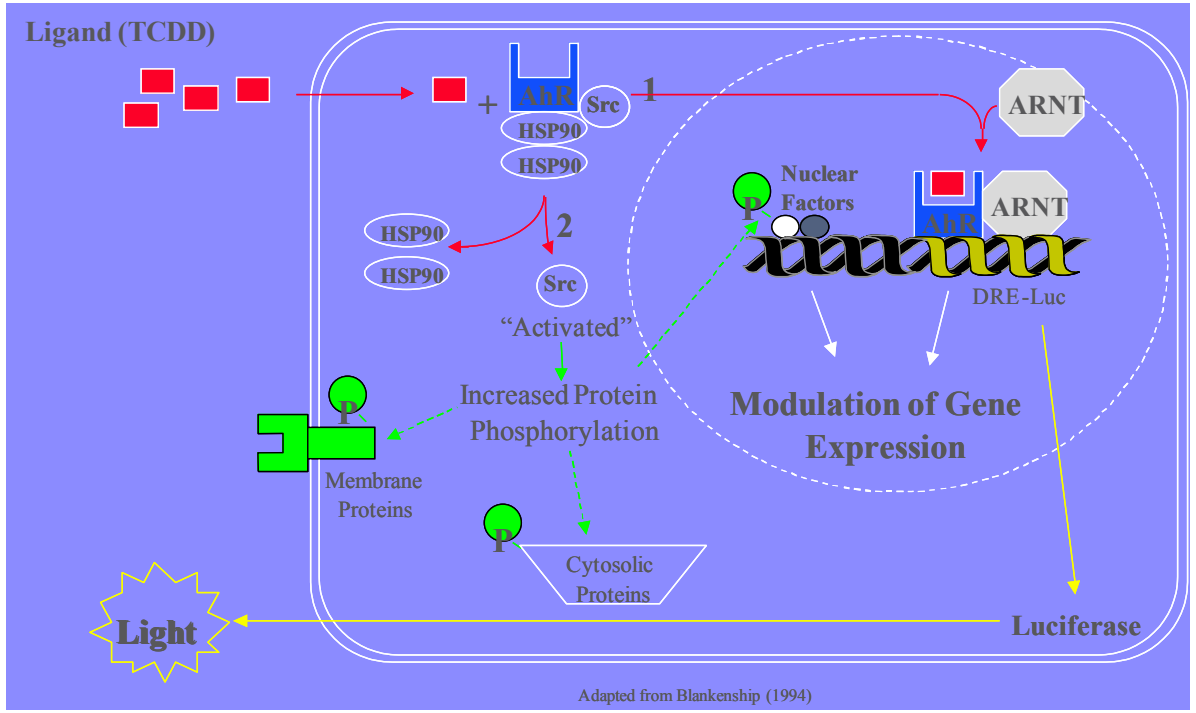
Test type	Avian reproduction
Organisms	Ring-necked pheasant (*Phasianus colchicus*), bobwhite (*Colinus virginianus*), Japanese quail (*Coturnix japonica*), chicken (*Tympanuchus cupido*), mallard (*Anas platyrhynchos*), black duck (*Anas rubripes*), screech owl (*Otus asio*), American kestrel, ring dove (*Streptopelia risoria*), gray partridge, crowned guinea-fowl
Age of organism	Should be within ±10% of the mean age of the group
Feeding	Feed and water should be available *ad libitum*. Feed consumption should be measured for 7-day periods throughout the study
Experimental design	
Test chamber type and size	Materials that can be dissolved by water or loosened by pecking should not be used; stainless steel, galvanized steel, or materials coated with perfluorocarbon plastics are acceptable; any design is acceptable such that the birds are able to move about freely and the pens kept clean
Test concentration	(1) At least one concentration must produce an effect (2) The highest test concentration must contain at least 0.1% (1000 ppm) (3) The highest test concentration must be 100 times the highest measured or expected field concentration
Number of test groups	A minimum of 16 pens per test concentration and control group should be used
Number of organisms per chamber	Pairs or groups containing no more than one male
Exposure to test substance	Mix test substance directly into feed
Clinical examinations	Eggs laid; normal eggs; fertile eggs; hatchability; normal young; survival; weight of young; eggshell thickness; residue analysis
Physical and chemical parameters	
Temperature	About 21°C for adults. For hatchlings, the amount and duration of heat is species-specified. A temperature gradient should be established from an appropriate heat source and range down to about 21°C
Humidity	45–70% (higher relative humidities may be appropriate for waterfowl)
Light quality	Should emit a spectrum simulating daylight
Light intensity	65 lux (6 fc)
Photoperiod	For adults: 8 hr light/16 h dark prior to photostimulation; 17 hr light/7 h dark from onset of photostimulation For hatchlings: at least 14 h of light for precocial species
Endpoint	Reproduction

In vitro bioassays

In vitro tests

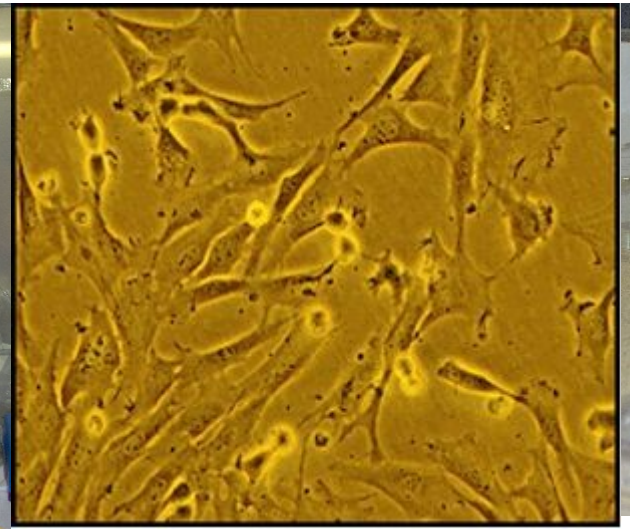
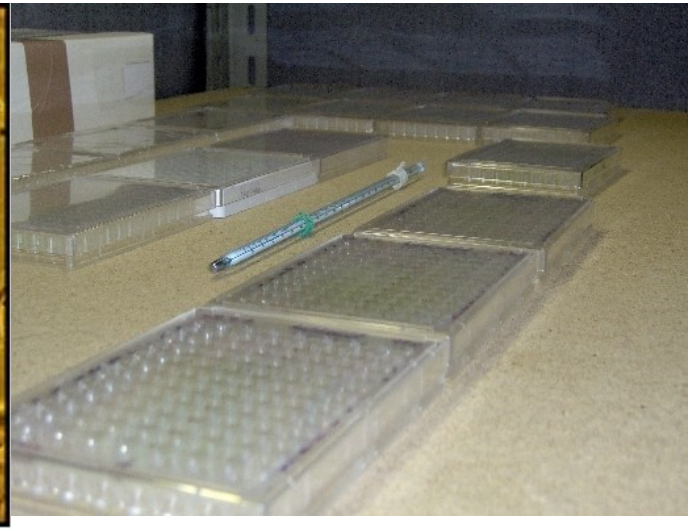
- special cell lines
- specific compound ➔ specific cellular or intracellular reporter ➔ reporter gene
- dioxin-like toxicity (AhR), estrogenicity (ER), thyroid-hormone-like toxicity ...

ISO 19040-1:2018	Water quality — Determination of the estrogenic potential of water and waste water — Part 1: Yeast estrogen screen (Saccharomyces cerevisiae)
ISO 19040-2:2018	Water quality — Determination of the estrogenic potential of water and waste water — Part 2: Yeast estrogen screen (A-YES, Arxula adeninivorans)
ISO 19040-3:2018	Water quality — Determination of the estrogenic potential of water and waste water — Part 3: In vitro human cell-based reporter gene assay
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/Cor 1:2009	Water quality — Evaluation of genotoxicity by measurement of the induction of micronuclei — Part 2: Mixed population method using the cell line V79 — Technical Corrigendum 1
ISO/CD 24295	Water quality — Determination of the dioxin-like potential of water and wastewater — Method using in vitro mammalian cell-based reporter gene assay



In vitro tests

- in vitro special cell lines: mammalian or fish
- specific compound ➔ specific cellular or intracellular receptor ➔ reporter gene conected with luciferase ➔ light
- dioxin-like toxicity (AhR), estrogenicity (ER), androgenicity (AR), thyroid-hormone-like toxicity (RAR) ...

Microcosms / mesocosms

Microcosms / mesocosms

- micro < 1 m^3, meso > 1 m^3
- usually **multi-species** – big benefit
- **optimum:** less uncontrolled than field but more realistic than lab tests
- considers also **indirect effects**
- **enable also environmental fate endpoints**
- many possible desings

Aquatic microcosms
- Benthic-pelagic microcosm
- Compartmentalized lake
- Mixed flask culture microcosm
- Pond microcosm
- Sediment core microcosm
- Ecocore microcosm
- Ecocore II microcosm
- Standard aquatic microcosm
- Stream microcosm
- Waste treatment microcosm

Terrestrial microcosms
- Root microcosm system
- Soil core microcosm
- Soil in a jar
- Terrestrial microcosm chamber
- Terrestrial microcosm system
- Versacore

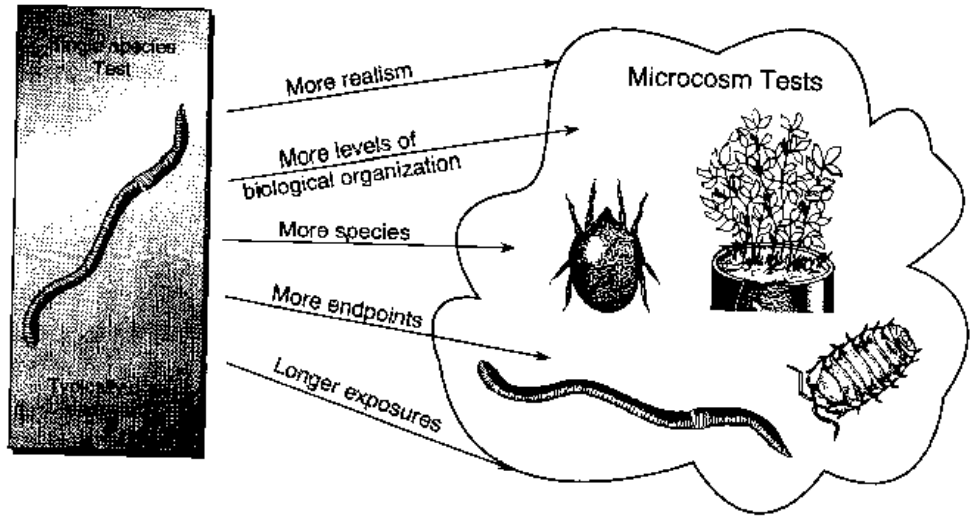


Figure 1 Microcosm tests can be defined by how they differ from single-species tests. Most importantly, there are more species. This usually also means more levels of biological organization (population and community effects) and more toxicity endpoints. They may also provide more realism and allow longer exposures.

Microcosms / mesocosms

- **Water:** large aquariums, tanks, ponds, or artificial stream ecosystems

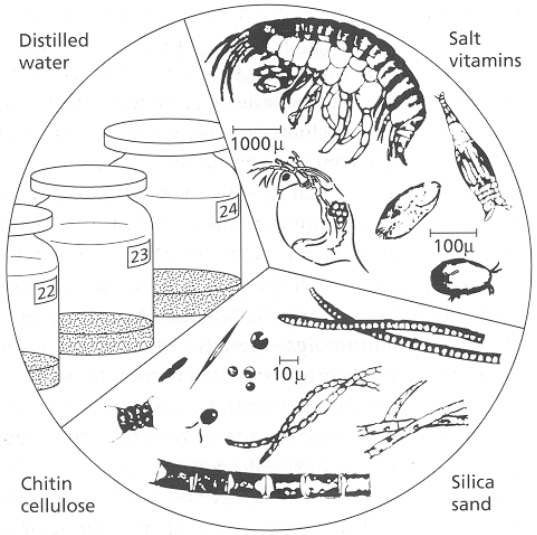


Fig. 5.2 Components of a standardized aquatic microcosm.

Test type	Multispecies
Organisms	
Type and number of test organisms per chamber	Algae (added on day 0 at initial concentration of 10^3 cells for each algae species): *Anabaena cylindrica*, *Ankistrodesmus* sp., *Chlamydomonas reinhardi* 90, *Chlorella vulgaris*, *Lyngbya* sp., *Nitzschia kutzigiana* (Diatom 216), *Scenedesmus obliquus*, *Selenastrum capricornutum*, *Stigeoclonium* sp., and *Ulothrix* sp. Animals (added on day 4 at the initial numbers indicated in parentheses): *Daphnia magna* (16/microcosm), *Hyalella azteca* (12/microcosm), *Cypridopsis* sp. or *Cyprinotus* sp. (ostracod) (6/microcosm), Hypotrichs [protozoa] (0.1/ml) (optional), and *Philodina* sp. (rotifer) (0.03/ml)
Experimental design	
Test vessel type and size	1-gal (3.8-l) glass jars are recommended; soft glass is satisfactory if new containers are used; measurements should be 16.0 cm wide at the shoulder, 25 cm tall with 10.6-cm openings
Medium volume	500 ml added to each container
Number of replicates	6
Number of concentrations	4
Reinoculation	Once per week add one drop (ca 0.05 ml) to each microcosm from a mix of the ten species; 5×10^2 cells of each alga added per microcosm
Addition of test materials	Add material on day 7; test material may be added biweekly or weekly after sampling
Sampling frequency	2 times each week until end of test
Test duration	63 days
Physical and chemical parameters	
Temperature	Incubator or temperature controlled room is required providing an environment 20 to 25°C with minimal dimensions of 2.6 × 0.85 × 0.8 m high.
Work surface	Table at least 2.6 × 0.85 m and having a white or light colored top or covering
Light quality	Warm white light
Light intensity	80 µE m^{-2} photosynthetically active radiation s^{-1} (850–1000 fc)
Photoperiod	12 h light/12 h dark
Microcosm medium	Medium T82MV
Sediment	Composed of silica sand (200 g), ground, crude chitin (0.5g), and cellulose powder (0.5 g) added to each container.
pH level	Adjust to pH 7
Endpoint	Population dynamics, chemistry, etc.

Microcosms / mesocosms

- **Water:** large aquariums, tanks, ponds, or artificial stream ecosystems

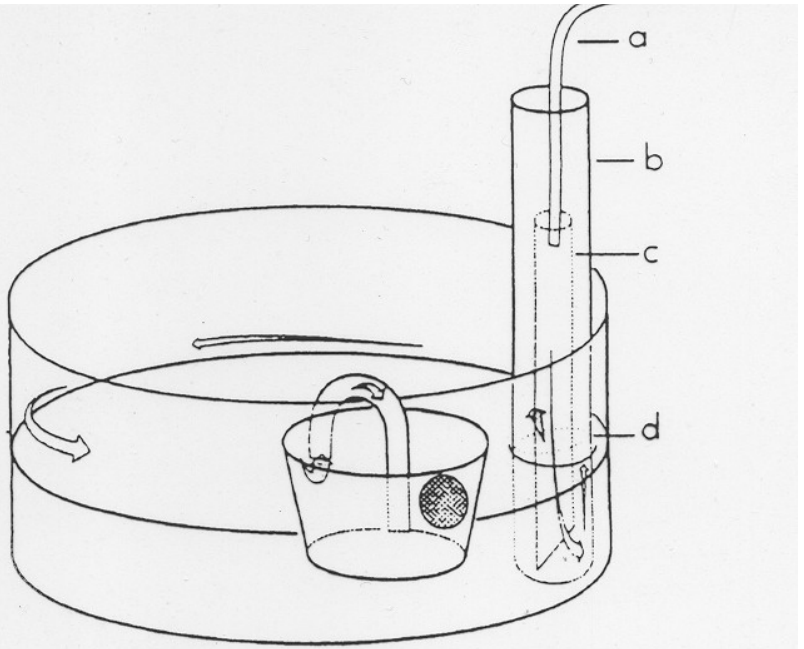


Figure 7. Flow-through exposure chamber for flow-through tests with polychaetes. The exposure chamber is a glass crystallizing dish with an inflow of water over the sediment surface. Arrows show flow of water into the test tube (b) through silicone tubing (a), which has a piece of glass tubing (c) attached at the bottom, then through an elliptical opening (d) cut in the side of the test tube and into the dish just above the sediment surface. Water circulates around the dish and leaves through a siphon and catch cup. (Reprinted with permission from Pesch, C. E., Munns, W. R. Jr., Gutjahr-Gobell, R.: Effects of a contaminated sediment on life history traits and population growth rate of *Neanthes arenaceodentata* (Polychaeta: Nereidae) in the laboratory. Environmental Toxicology and Chemistry 10(6):805–815. Copyright 1991, SETAC.)

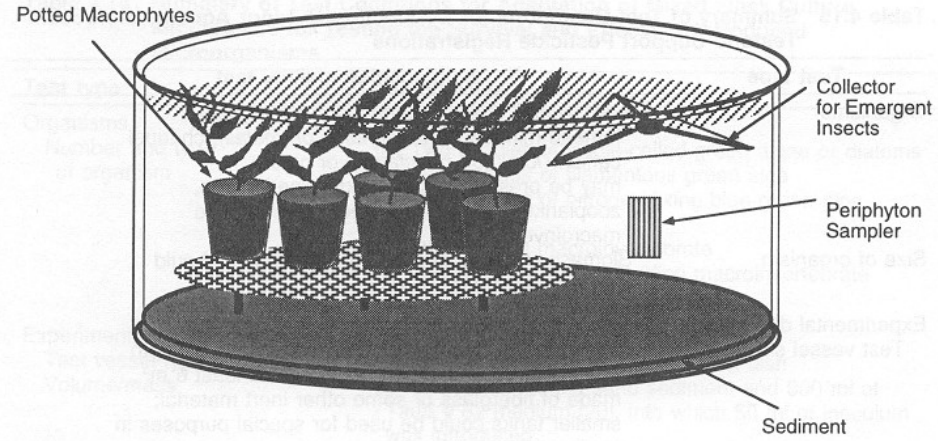


Figure 4.2 FIFRA microcosm experimental unit. An example of a microcosm experimental unit designed to test the effects of a herbicide on an aquatic environment. This particular setup does not include fish since the predatory effects would tend to hide lower trophic level effects upon the invertebrate populations. Typically, a FIFRA microcosm experiment includes fish species, particularly when acetylcholinesterase inhibitors or other toxicants particularly effective against animal species are tested.

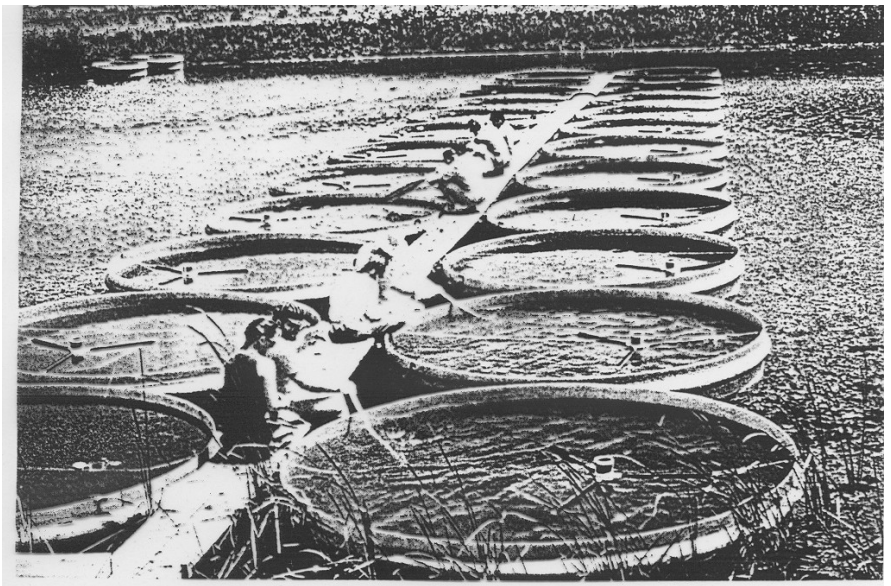


Figure 6. Photograph of outdoor microcosm test systems (10,000-L tanks) located at the University of Kansas. These fiberglass tanks have been used by Springborn Laboratories, Wareham, MA. to evaluate the impact of pesticides on aquatic communities.

Microcosms / mesocosms

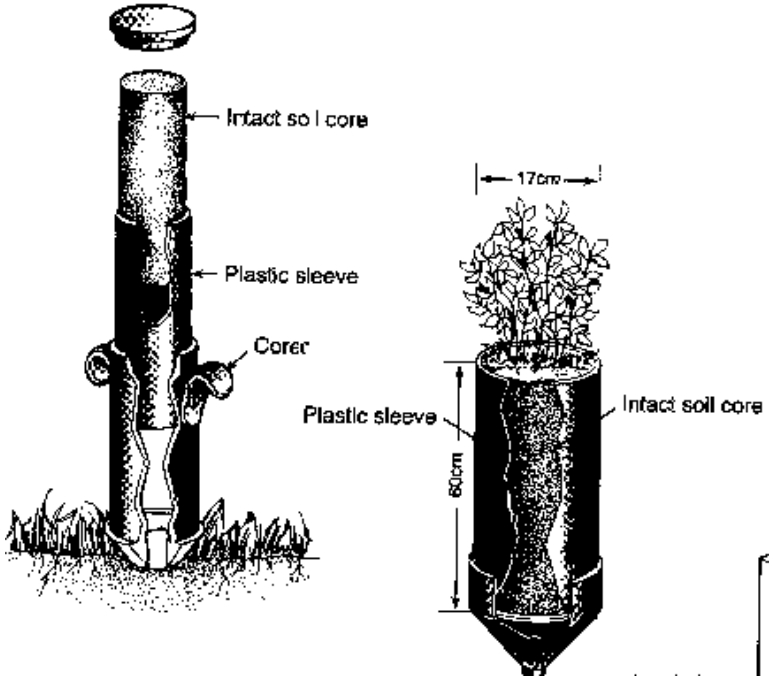
- **Soil:** usually core/column (made of plastic, wood, metal) of soil with vegetation, contains standard soil or real soil; can be outside (open system) or in the climate chamber (also gas monitoring)

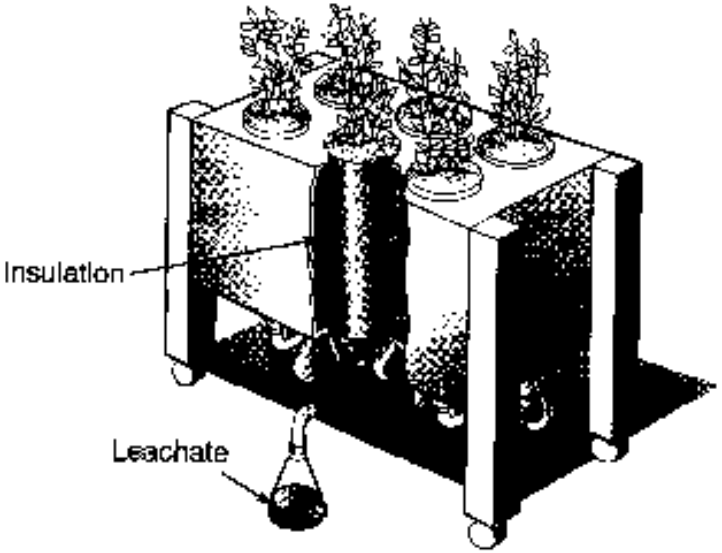
Table 9.1 Classification of Various Semi-Field Tests

1. Model ecosystem segments (= "microcosms") *Natural or artificially assembled units; a few centimeters in size — up to approx. 1 m³ (contents up to a few hundred liters); closed and open systems are both possible.*	
Specialized techniques:	e.g. the plant metabolism box of the NATEC (FIGGE, 1992) or small "artificial streams" (CLEMENTS et al., 1989).
Integrated techniques:	e.g. the Terrestrial Model Ecosystem (TME) (VAN VORIS et al., 1984; KNACKER et al., 1990, 1991) or the Standardized Aquatic Microcosm (SAM) (TAUB et al., 1986; EPA, 1987).
2. Ecosystem segments in the field (= "mesocosms") *Field segments which remain exposed to normal environmental conditions; various sizes ranging between 1 m³ and several hundred m³.*	
Specialized techniques:	Partial enclosures in lakes or rivers, e.g. plastic bags with algae coenoses (EIDE et al., 1979). Lysimeter (usually about 1 m3 in size), e.g. tests on the mobility of pesticides in natural soil cores (e.g. BBA, 1990a). Semi-field tests (usually tests with beneficial organisms), e.g. effects of pesticides on ground beetles (carabids) in cultivated soil system segments (ABEL & HEIMBACH, 1992).
Integrated techniques (very rarely in the terrestrial medium):	Artificial testing systems, e.g. "artificial streams" — reconstructions of real streams including sediment (EATON et al., 1985). Natural enclosures, e.g. "Bremerhaven-Caissons" in wadden seas (FARKE et al., 1984).

Microcosms / mesocosms

- **Soil core microcosm**





Microcosms / mesocosms

- **Soil core microcosm**

Table 4.16 Summary of Test Conditions for Conducting A Terrestrial Soil-Core Microcosm Test

Test type	Multispecies toxicity test
Organisms	Varies; dependent on site being tested
Experimental design	
Microcosm size and type	60-cm-deep by 17-cm-diameter plastic pipe made of ultra-high molecular weight, high-density, and nonplasticized polyethylene and contains an intact soil core covered by homogenized topsoil; tube sits on a Buchner funnel covered by a thin layer of glass wool
Soil volume	40 cm intact soil core; 20 cm homogenized topsoil
Number of replicates	Each cart holds 6–8 microcosms; place microcosms paired for analyses in different carts to ensure that all microcosms are housed under similar conditions.
Number of concentrations	3
Leaching	At least once before dosing and once every 2 or 3 weeks after dosing
Test duration	12 or more weeks
Physical and chemical parameters	
Temperature	Based on season of region being tested; insulated cart is used to prevent drastic temperature changes
Lighting	Based on season of region being tested
Watering	Determined on the basis of site history; use either purified laboratory water or rainwater that has been collected, filtered, and stored in a cooler at 4°C
Endpoint	Many