MUNI | RECETOX

Bi2003 Ecotoxicology

Ecotoxicological bioassays

Jakub Hofman

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- **Types of bioassays**
- Ecotoxicological bioassays' design and results
- Aquatic bioassays - examples
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- Use of bioassays in praxis

Introduction – what, why, how, concept

Protection of environment / nature

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

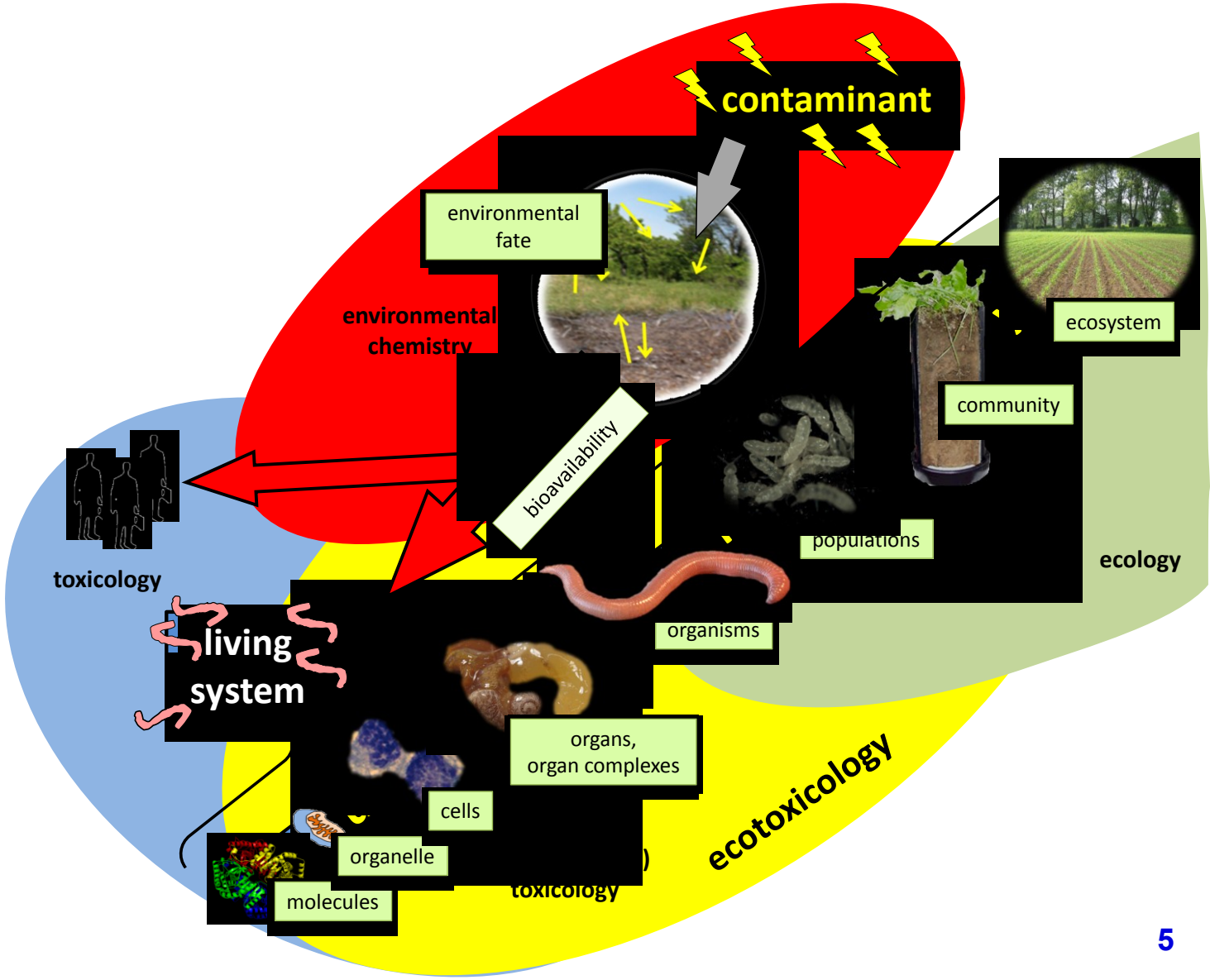
How to protect ?

- Policy
- Legislation
- Research
- Education

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

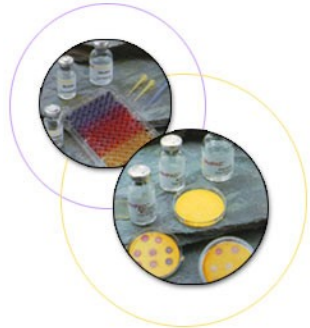
Ecotoxicology

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

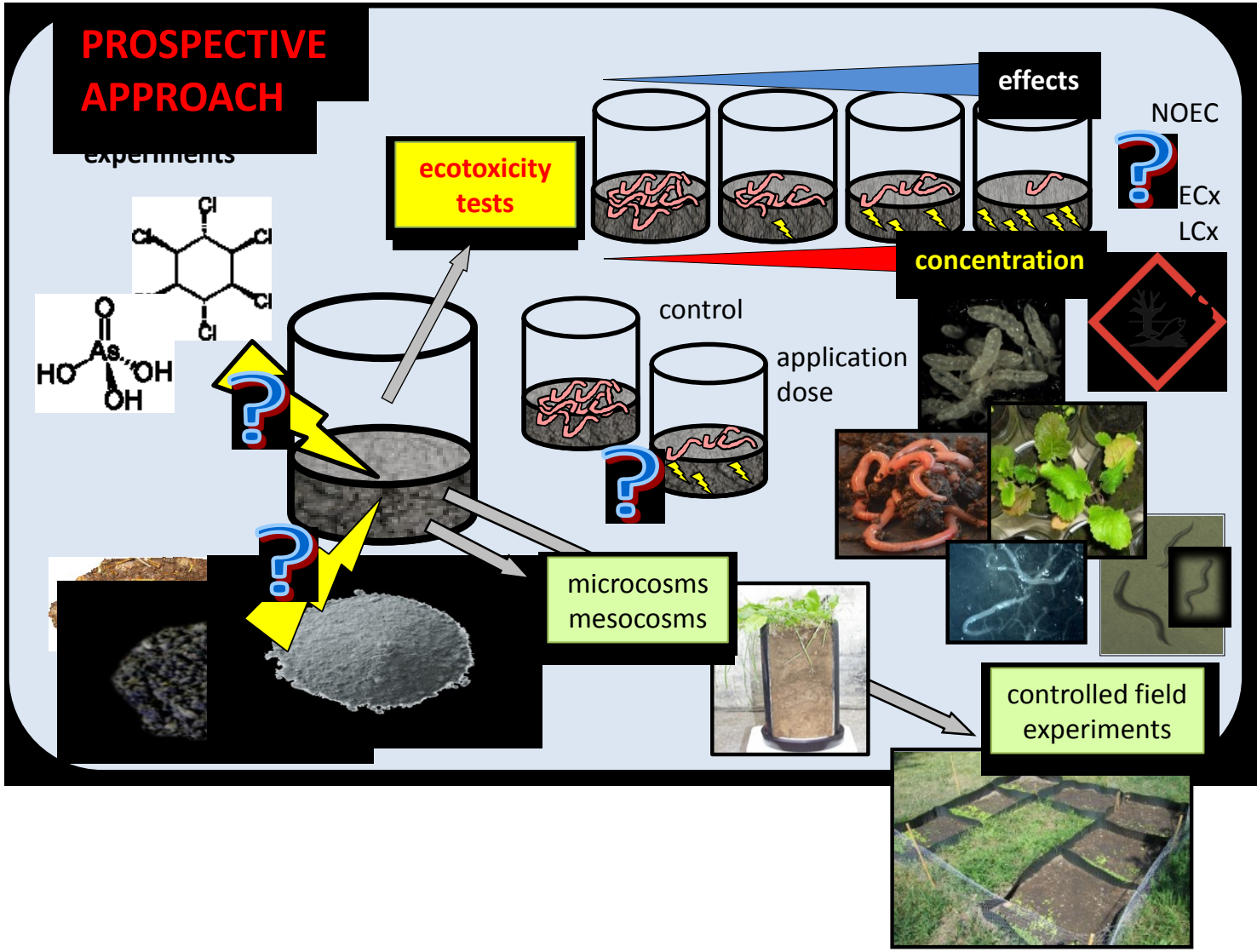
(3) negative effects of **matrix** itself, regardless of toxicant content, on organisms or interaction of matrix with effects of toxicants

(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



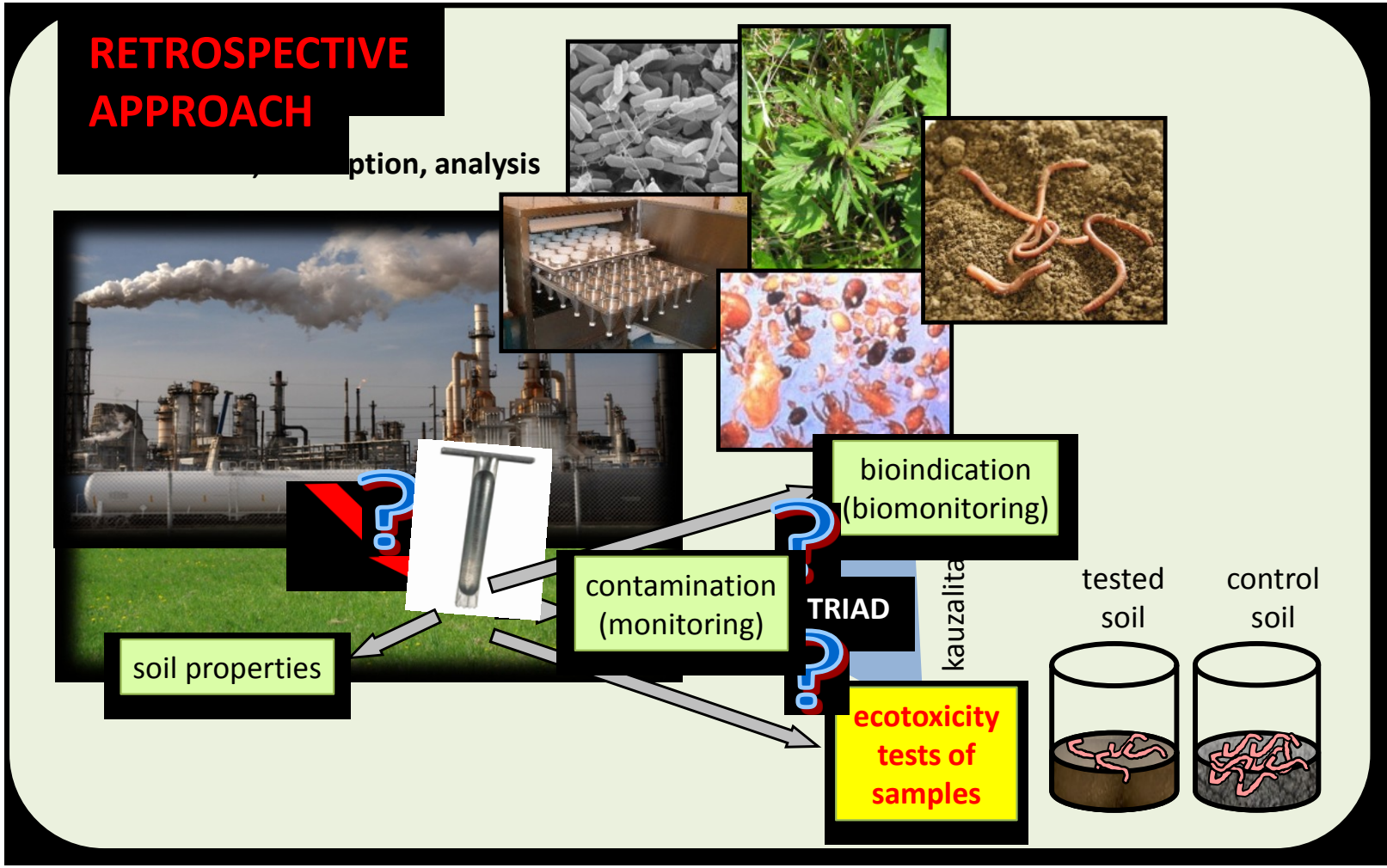
Bioassays useful for:

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



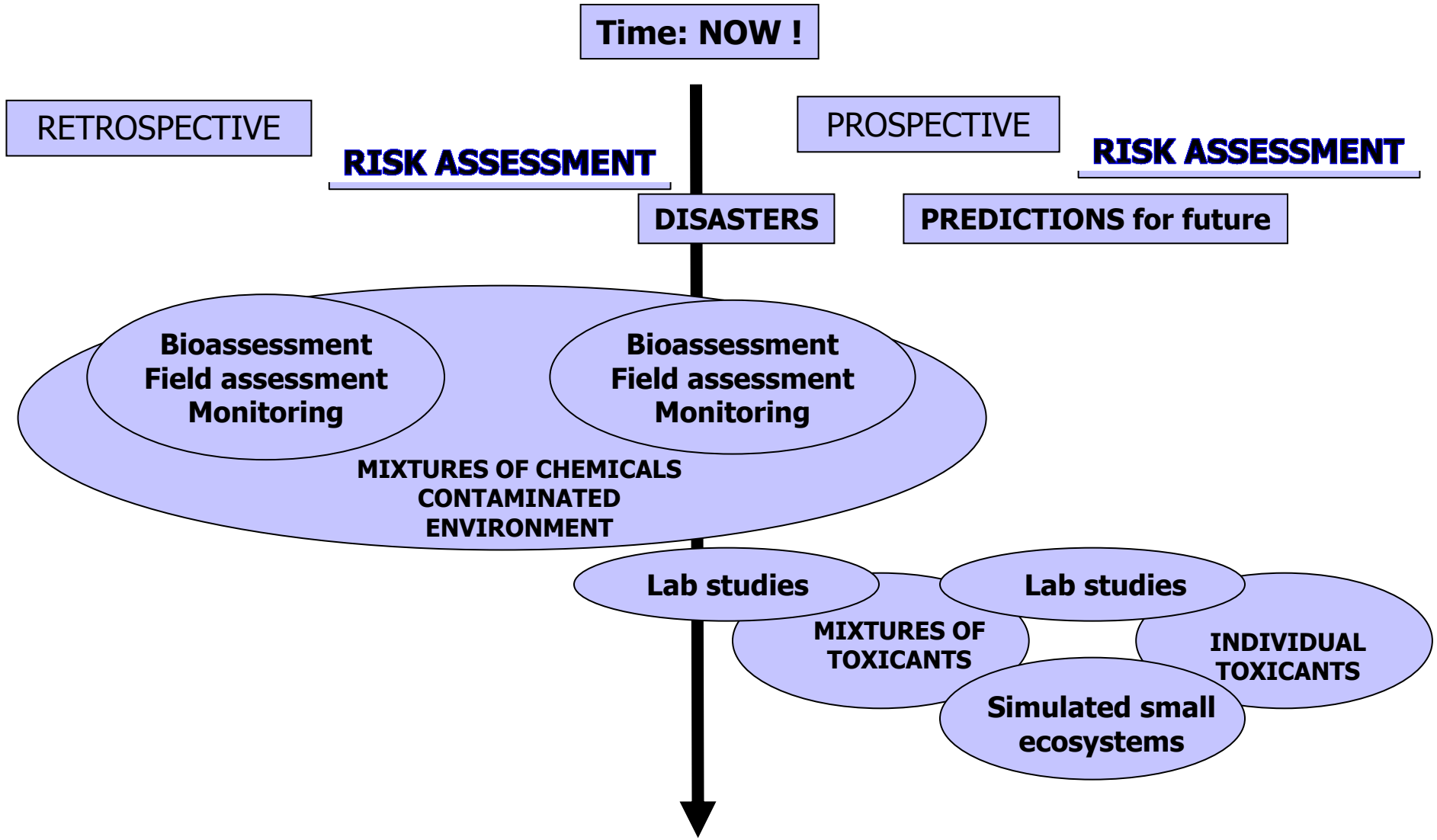
ISO 19204:2017 Soil quality — Procedure for site-specific ecological risk assessment of soil contamination (soil quality TRIAD approach)

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

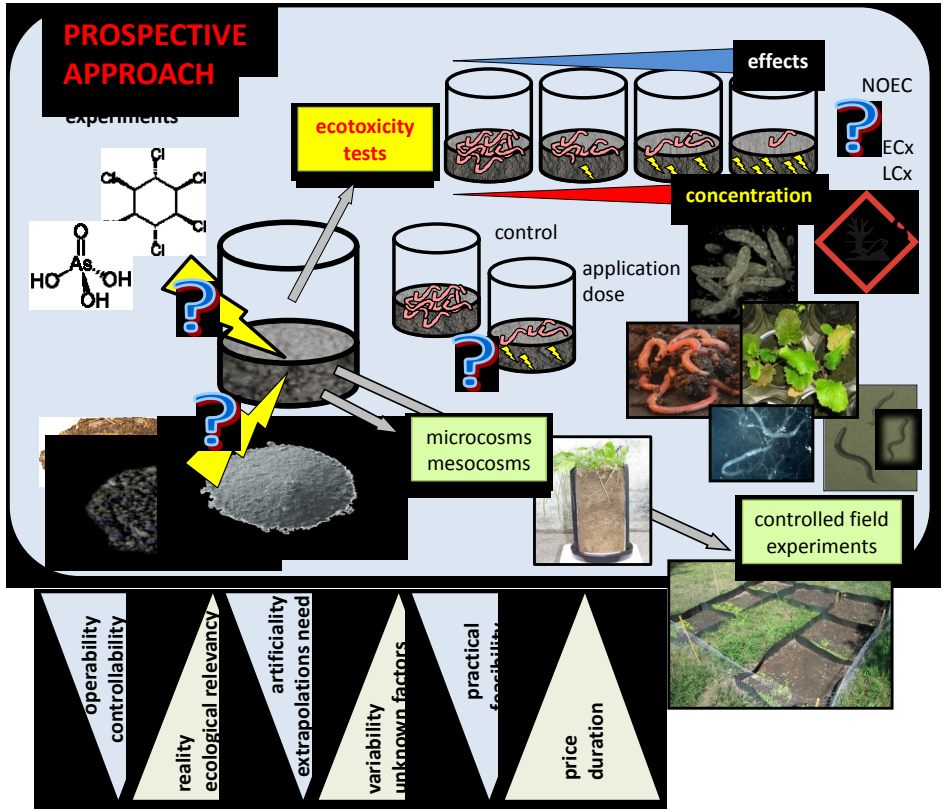
Bioassays useful for:

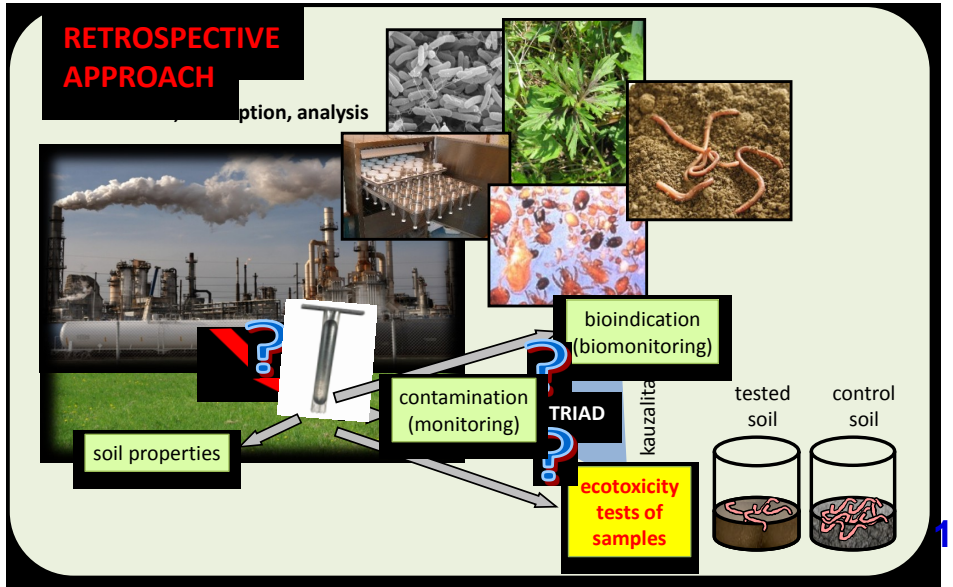


Bioassays useful for:

- 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

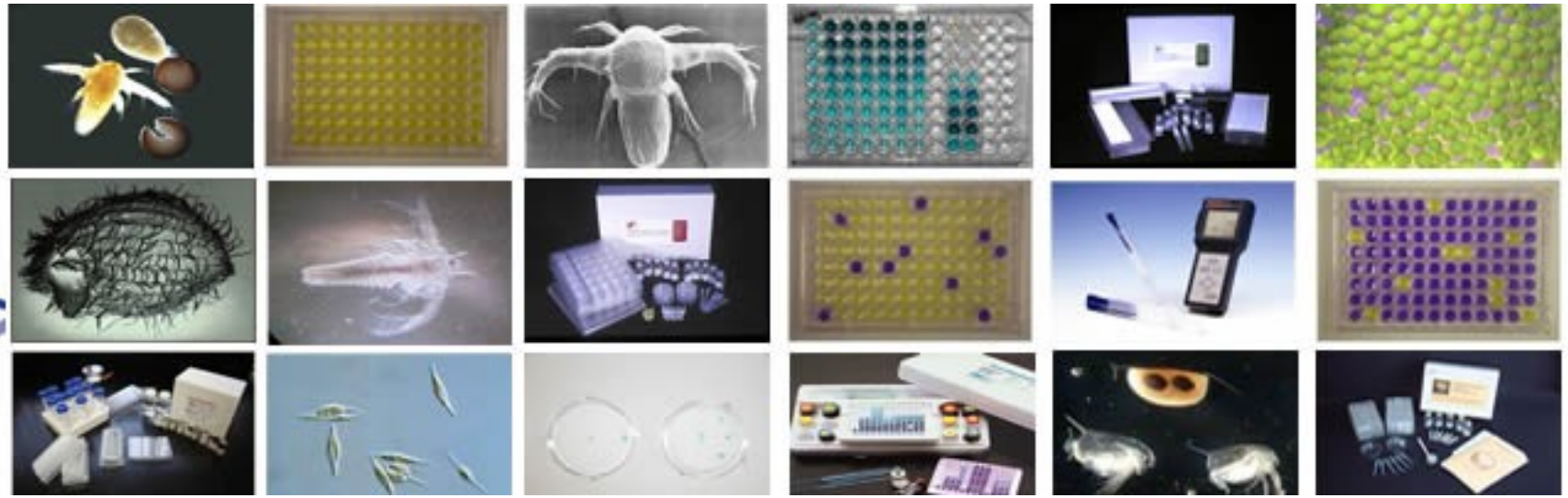
Bioassay development

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

Microbiotests

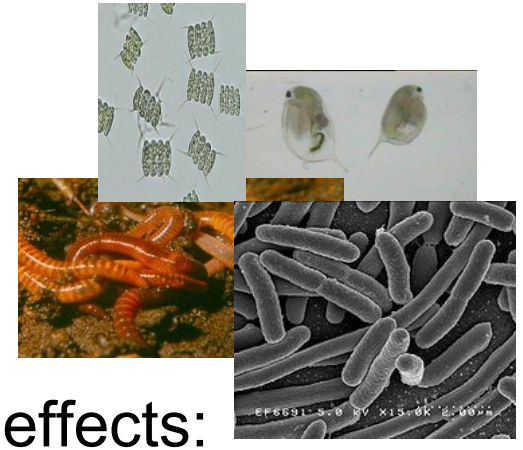
- they use some dormant stages of testing organisms
- practical = everything in one box
- cheap and easy, however, often not very relevant for real situations
- http://ebpi.ca/index.php?option=com_content&view=article&id=20&Itemid=50

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

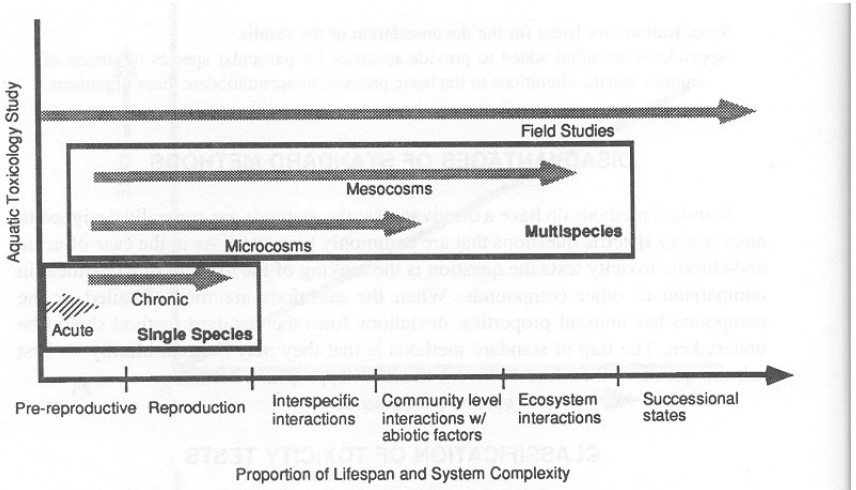
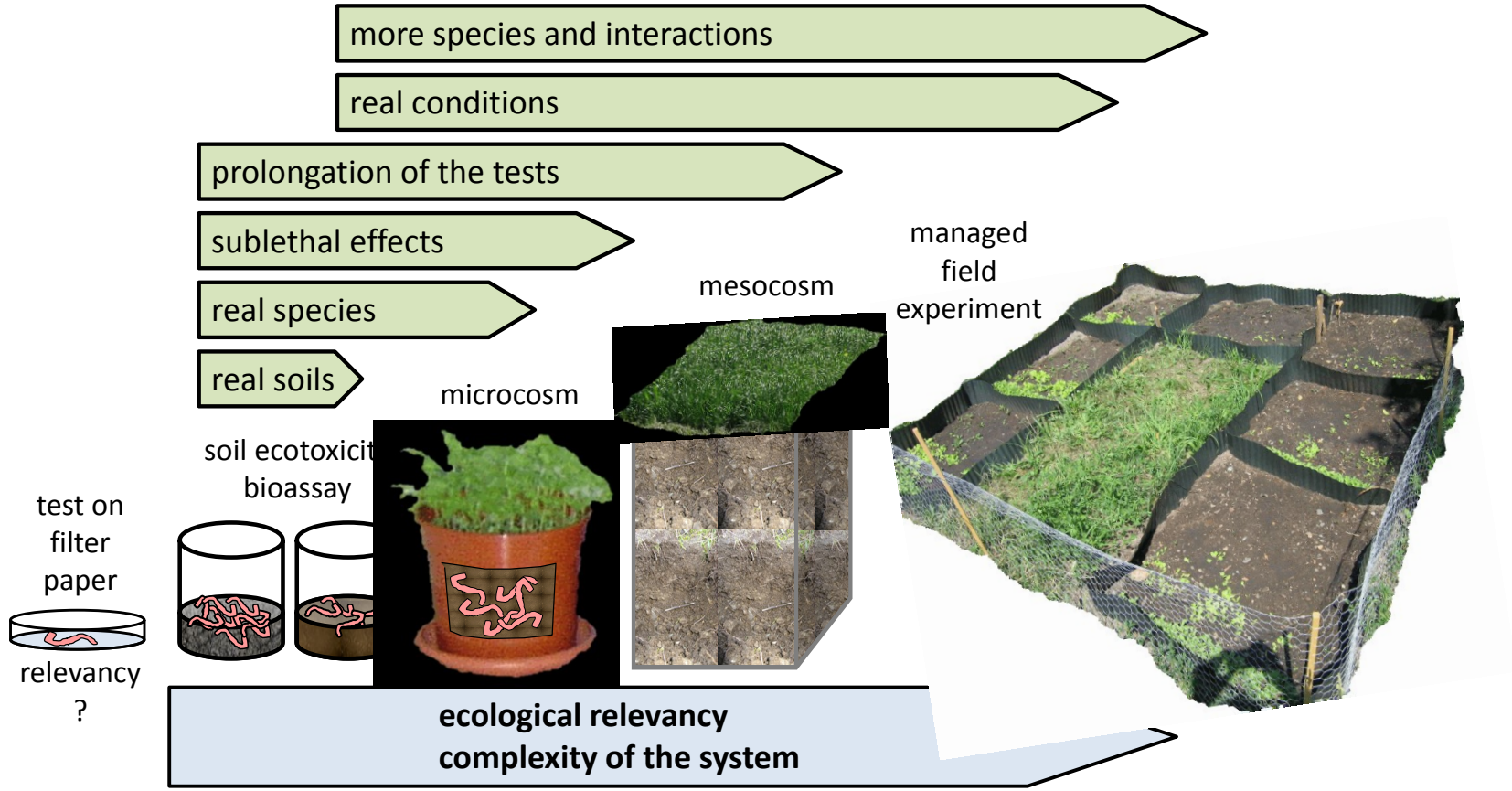


Figure 3.9 Classification of toxicity tests in environmental toxicology. Generally, the two parameters that are involved are the length of the test relative to the test organism and the species composition of the test system.



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

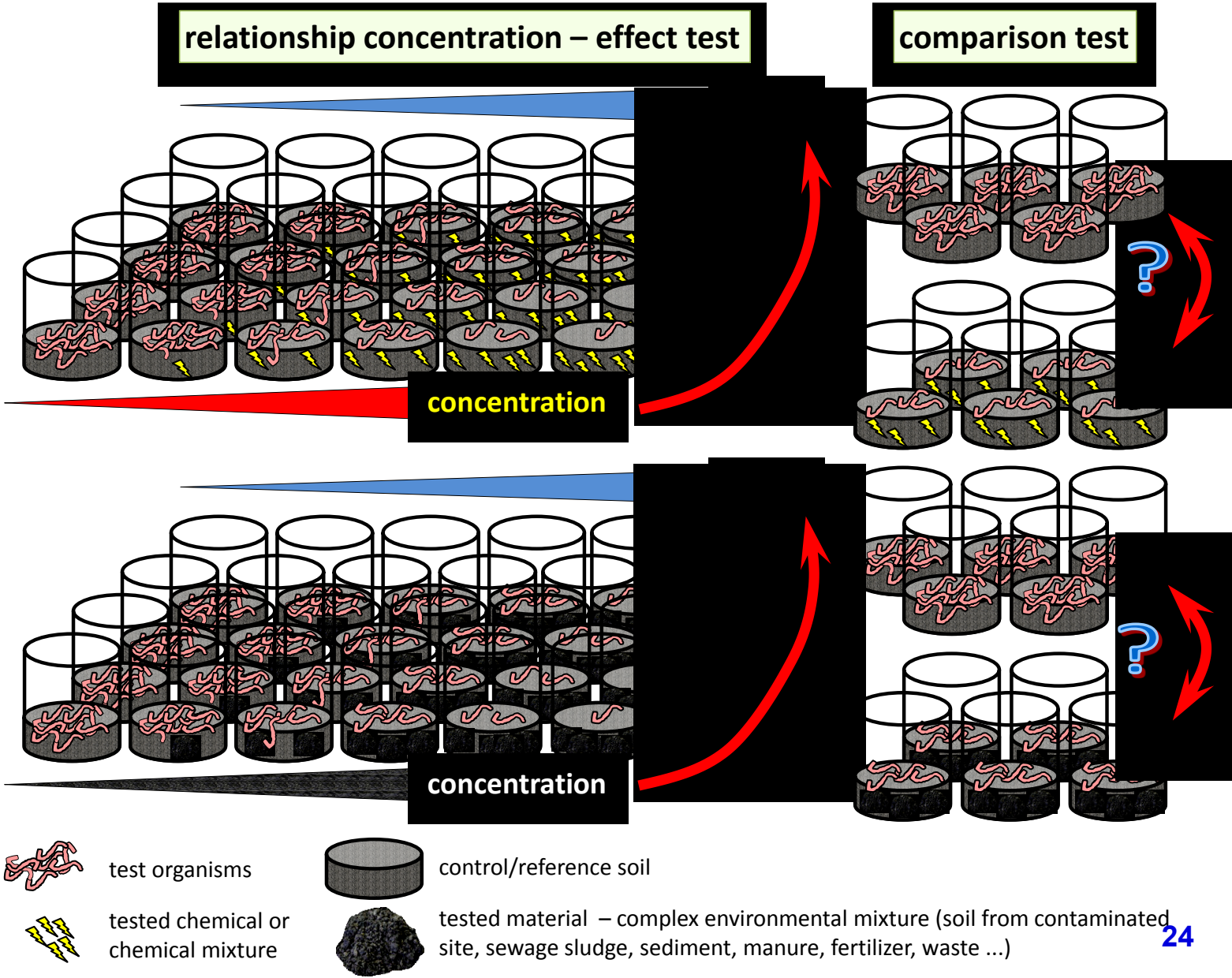
žížala dlouhá
Aporrectodea longa

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

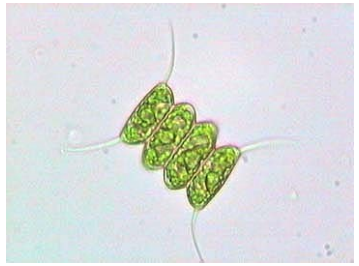
OECD guidelines - other

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

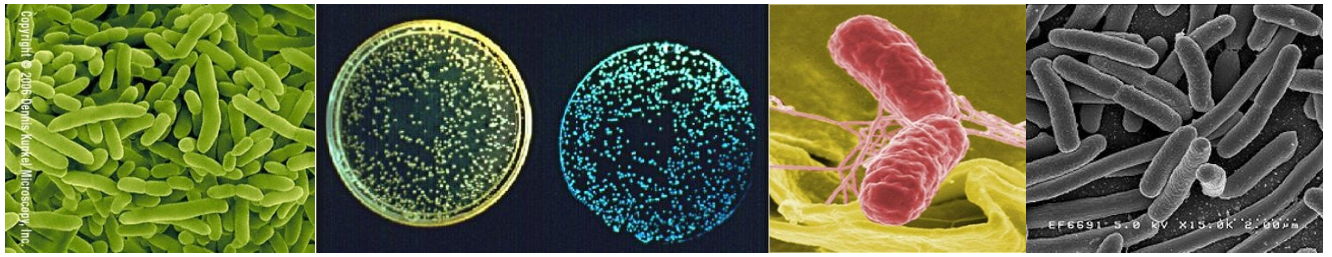
ISO 6341:2012	Water quality — Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) — Acute toxicity test
ISO 10706:2000	Water quality — Determination of long term toxicity of substances to Daphnia magna Straus (Cladocera, Crustacea)
ISO 10872:2020	Water and soil 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

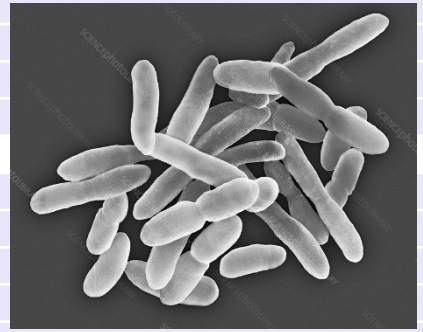
ISO 23611-1:2018	Soil quality — Sampling of soil invertebrates — Part 1: Hand-sorting and extraction of earthworms
ISO 23611-2:2006	Soil quality — Sampling of soil invertebrates — Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina)
ISO 23611-3:2019	Soil quality — Sampling of 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



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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

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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

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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)

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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