MUNI | RECETOX

Bi2003 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**
- Use of bioassays in praxis

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

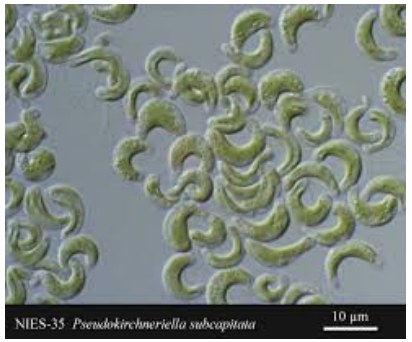
- **vascular plants – aquatic plants**
- size, length – growth, mortality

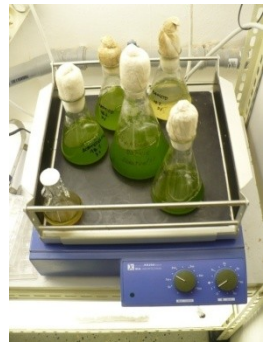
- other endpoints: concentration of pigments (eg chlorophyll a), physiological activity (nutrient intake) and metabolic activity (photosynthetic activities, enzymatic activities)

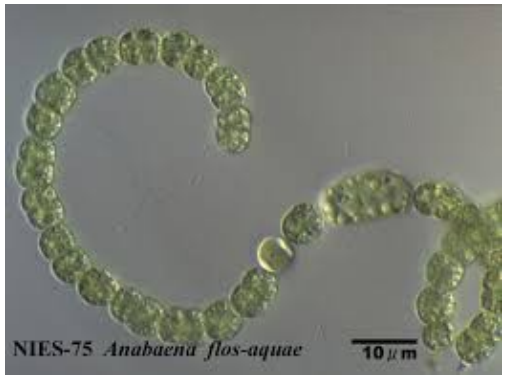
Aquatic bioassays – producers - algae

- **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





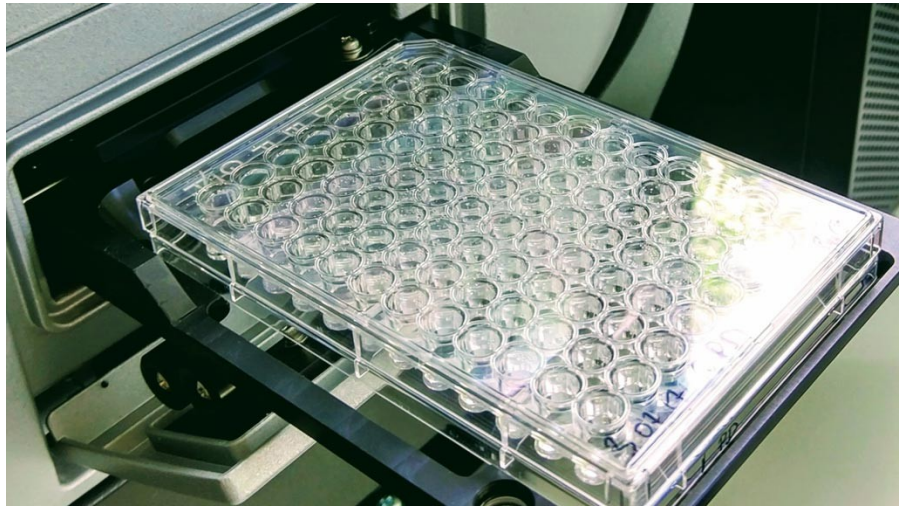


Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test 2011

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

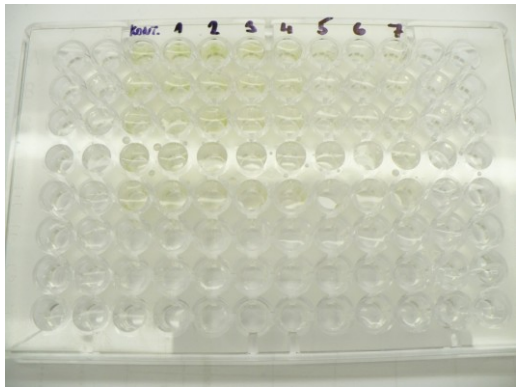
- controls:
  - exponential growth by factor of > 16 over 72 h
  - coefficient of variation (relative standard deviation, i.e. standard deviation divided by mean) < 35% (each day evaluation) and for average specific growth rate < 7%
- reference substance:
  - e.g. 3,5-dichlorophenol or potassium dichromate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

Aquatic bioassays – producers - algae

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

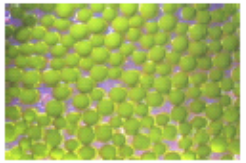
miniaturization



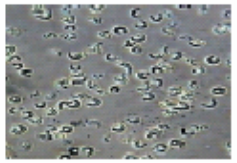
ALGALTOXKIT F™

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:

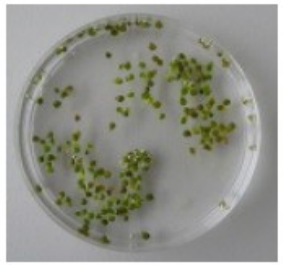
- control: doubling time < 2.5 days
- reference compound: 3,5-dichlorophenol or $K_2Cr_2O_7$ (EC50: 10-60 mg/L)

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

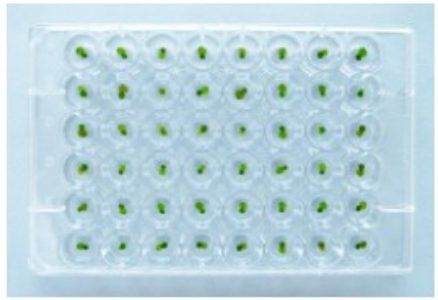
- miniaturization with Spirodela

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

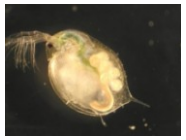
ARTOXKIT M

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

https://www.microbiotests.com

OSTRACODTOXKIT F

Aquatic bioassays – consumers - invertebrates



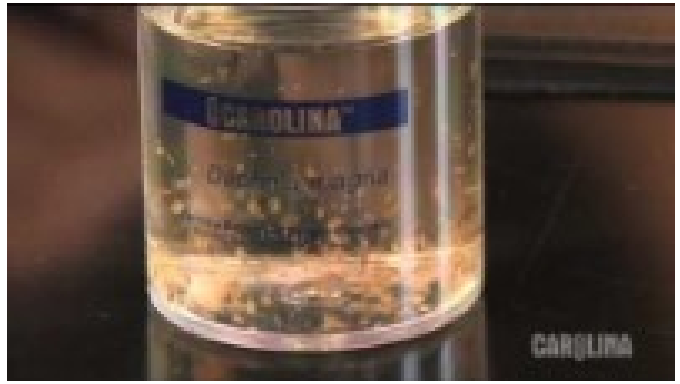
Daphnia magna acute test

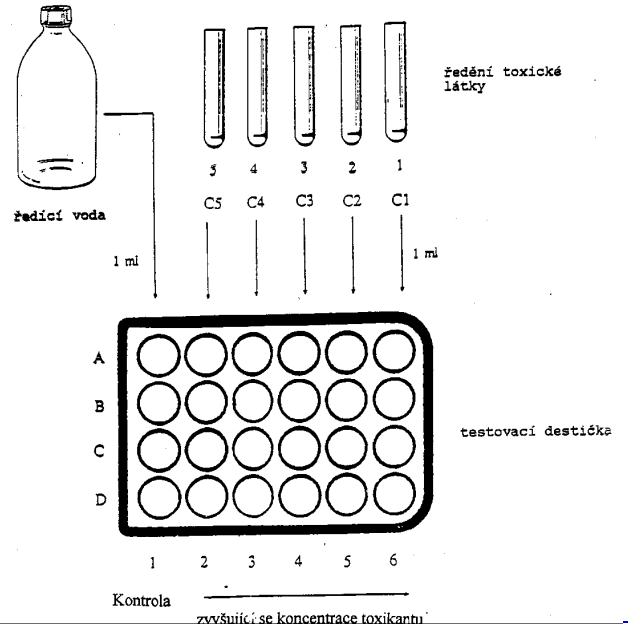
- 5 juvenile daphnids per replicate (min 2 ml)
- **medium** = so called reconstituted water
  1. $CaCl_2 \cdot 2H_2O$ 11,76 g/l
  2. $MgSO_4 \cdot 7H_2O$ 4,93 g/l
  3. $NaHCO_3$ 2,59 g/l
  4. KCl 0,23 g/l

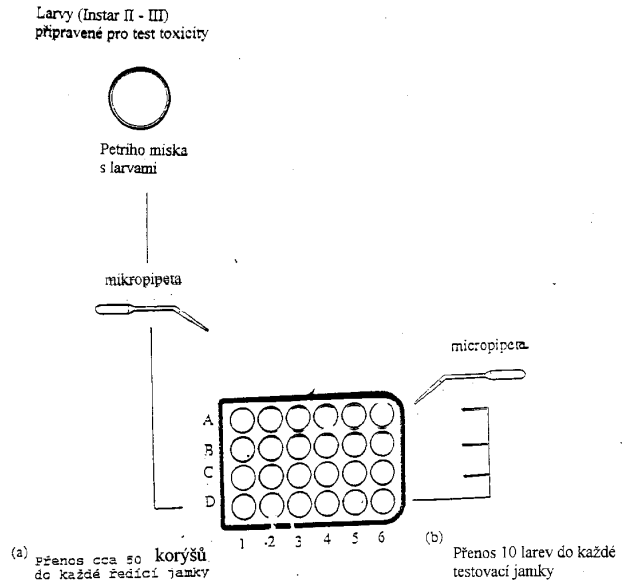
     mix 25 ml of each to 1 L, pH 7.8, aeration
- 24h, 48h; dark or 16h light / 8h dark;
  O2 > 80 % (2 mg/l); 20°C; no food
- mortality = immobilization

validity:

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

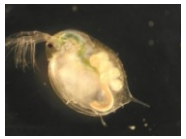






Aquatic bioassays – consumers - invertebrates

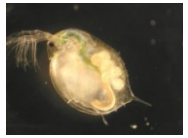
Daphnia magna acute test



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

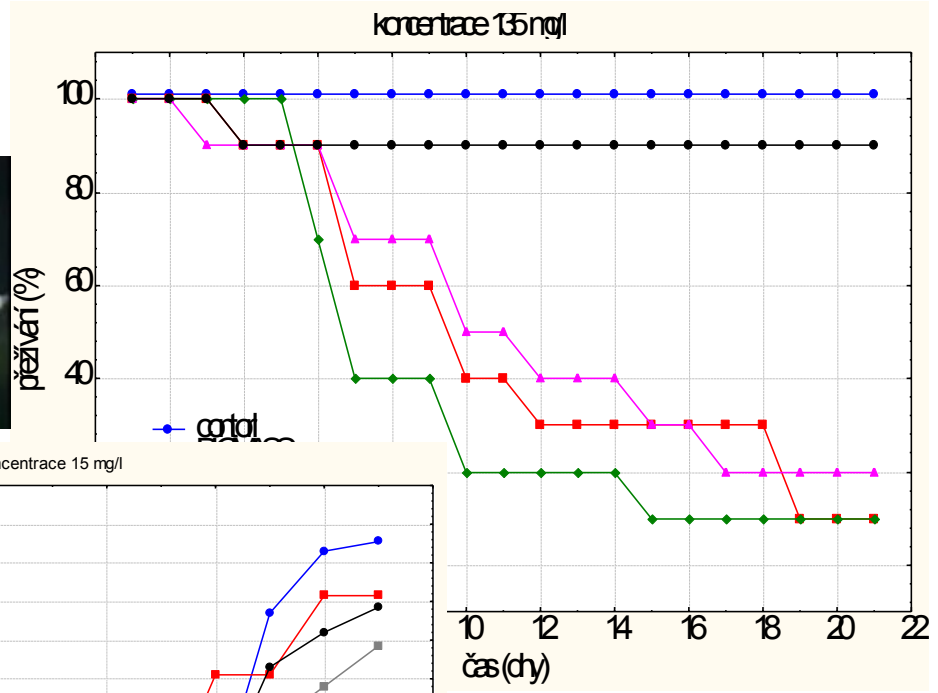


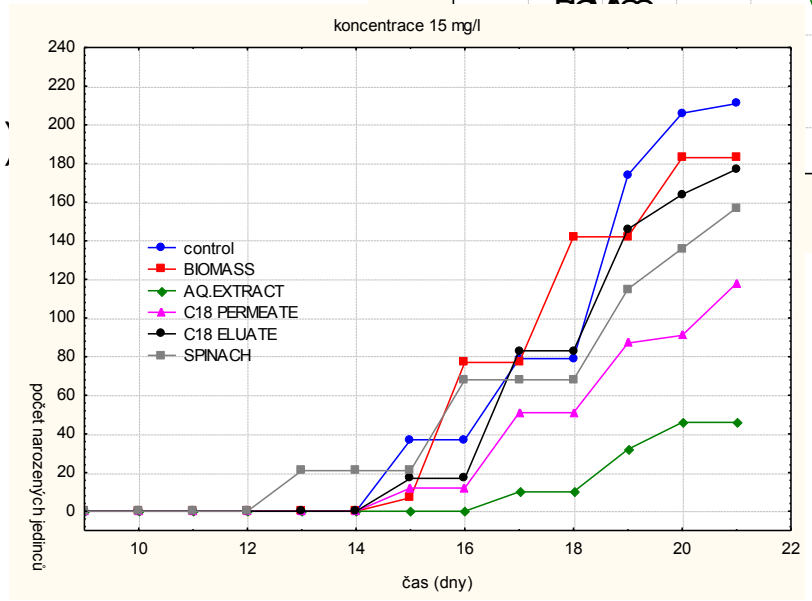
Daphnia magna reproduction test

- 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:

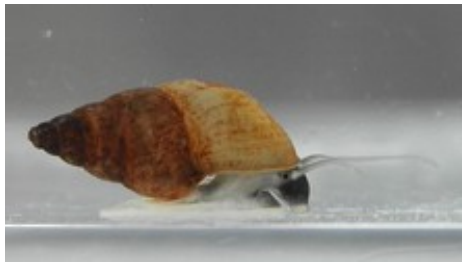
- control: mortality< 10 %;
- reference compound: $K_2Cr_2O_7$ (LC50: mg/L)

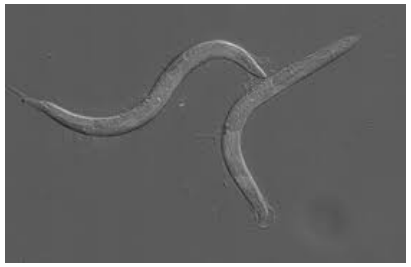




Aquatic bioassays – consumers - invertebrates

other invertebrates used in the bioassays








OECD	Test No. 242: Potamopyrgus antipodarum Reproduction Test	2016
	Test No. 243: Lymnaea stagnalis Reproduction Test	2016
	ISO 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	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

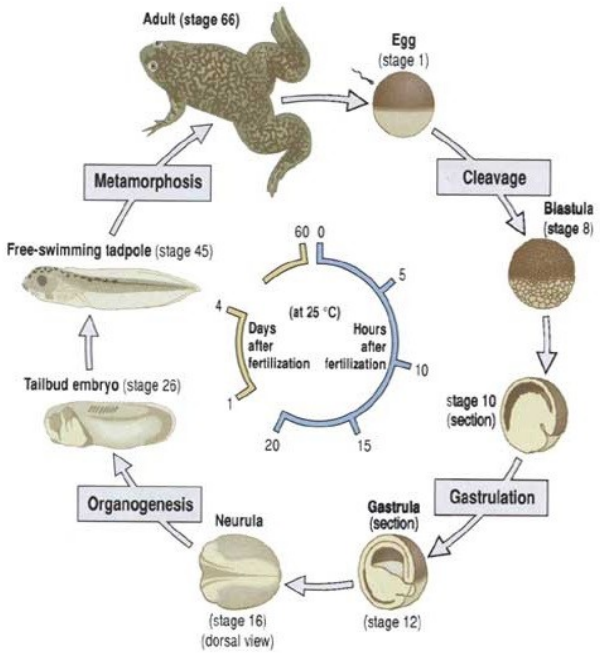
- fertilized eggs are exposed to chemicals for several days
- effects on development = **teratogenicity**
- prolonged to hatching and larvae development
- endpoints: hatching, survival, development, behavior, size

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**



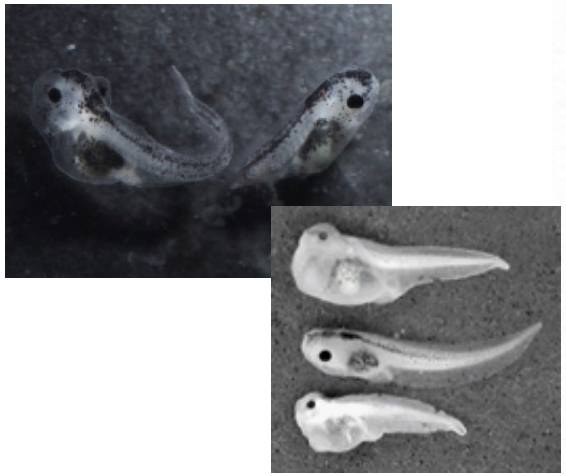
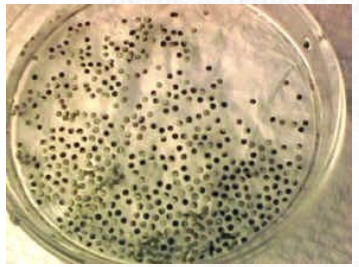


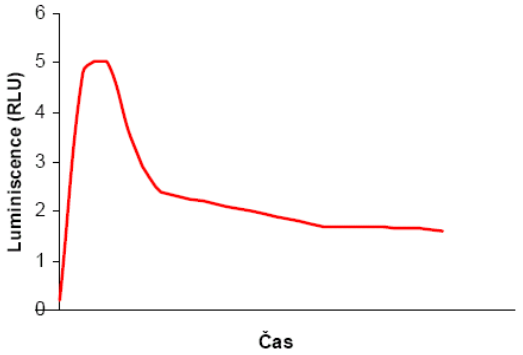
Table 4.11 The Frog Embryo Teratogenesis Assay: Xenopus (FETAX)	
Test type	**96 h static renewal**
Organism	*Xenopus laevis*
Age of parent organism	Adult male: at least 2 years of age Adult female: at least 3 years of age
Size of parent organism	Adult male: 7.5–10 cm in crown-rump length Adult female: 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

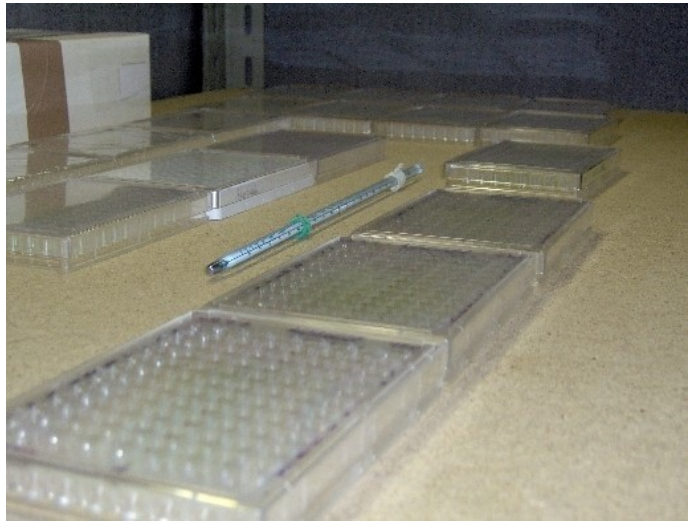


ISO 11348-1:2007/Amd 1:2018	Water quality — Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) — Part 1: Method using freshly prepared bacteria — Amendment 1
ISO 11348-2:2007/Amd 1:2018	Part 2: Method using liquid-dried bacteria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

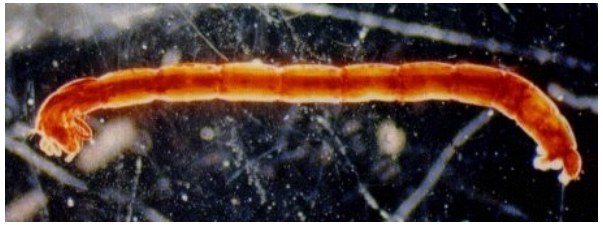
OECD	Test No. 224: Determination of the Inhibition of the Activity of Anaerobic Bacteria	2007
	Test No. 209: Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation)	2010
	Test No. 244: Protozoan Activated Sludge Inhibition Test	2017
ISO	ISO 15522:1999	Water quality — Determination 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

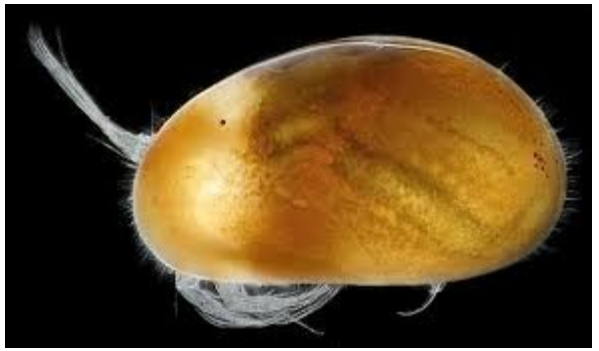
- sediment = aquatic equivalent of the soil ➔ very **heterogenic matrix**, contains **solid phase** (mineral particles and organic matter) and **pore water**
- sediment is often potential long-term reservoir / source of contaminants
- **distribution of contaminatns between water and sediment (sorption)**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

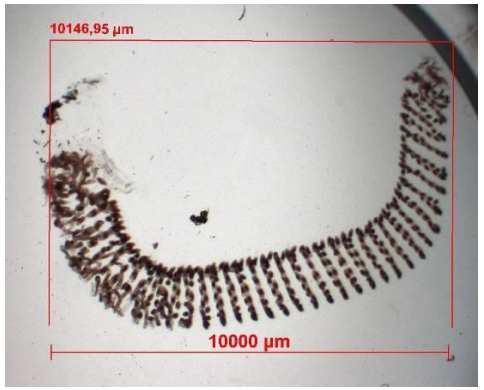
- same as Daphnia acute test, just with Chironomus larvae

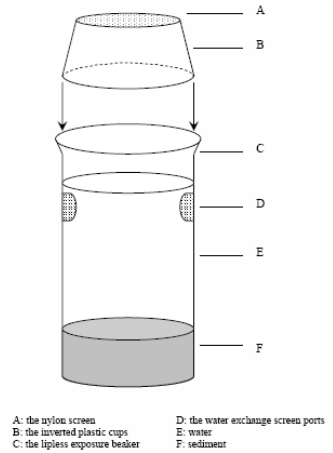
OECD 218+219

- 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
-

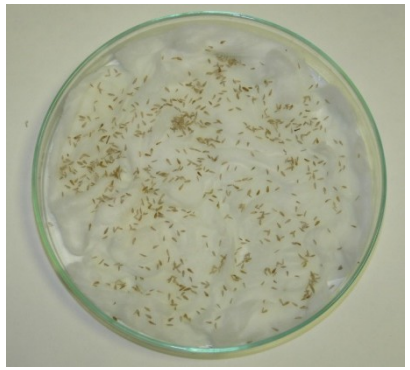


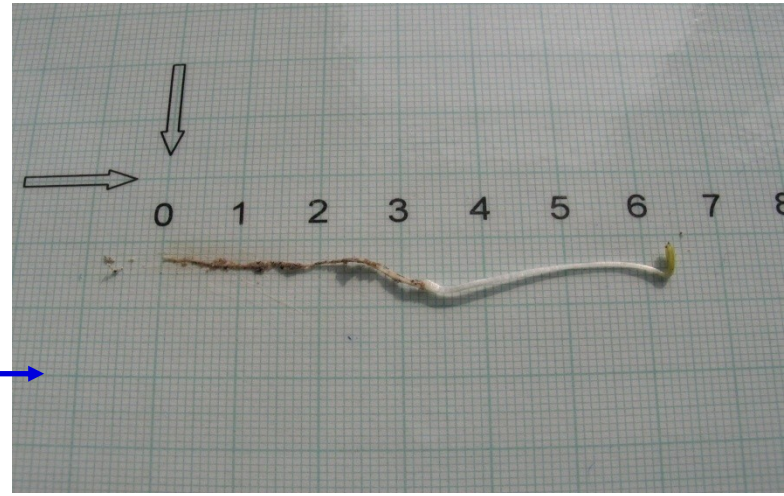


Soil bioassays – producents - plants

Root elongation inhibition

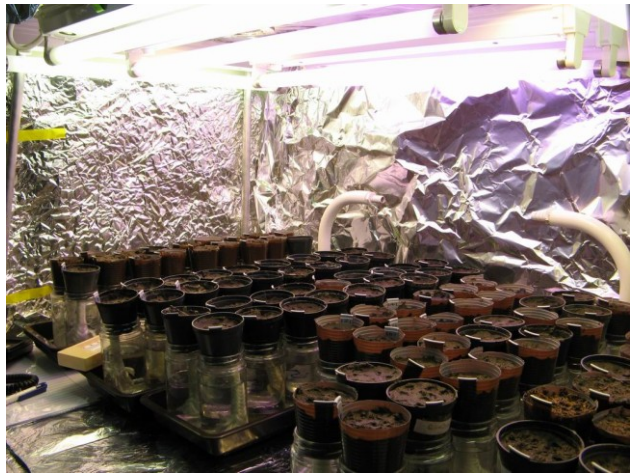
- preparation of seeds to 2 mm
- 15 seeds to 200-300 g soil
- 3-5 d; soil pH; dark; 24°C
- root length

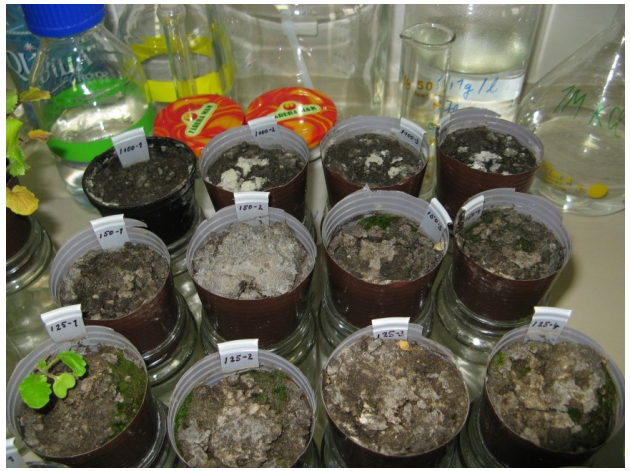




Soil bioassays – producents - plants

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

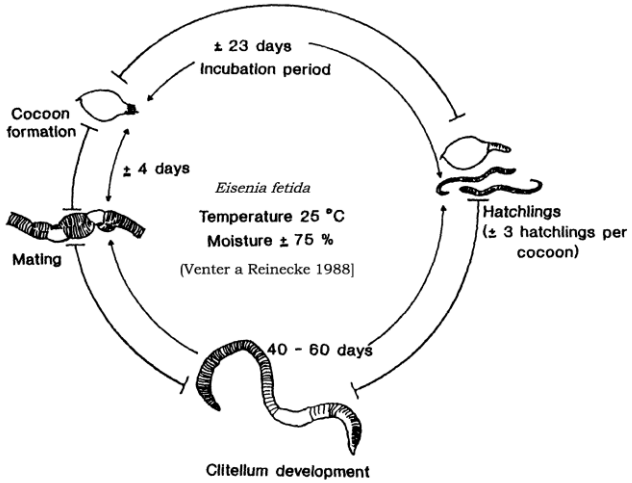
Earthworms

- 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

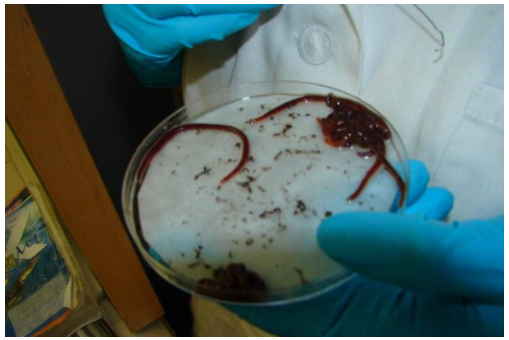
Earthworms - acute

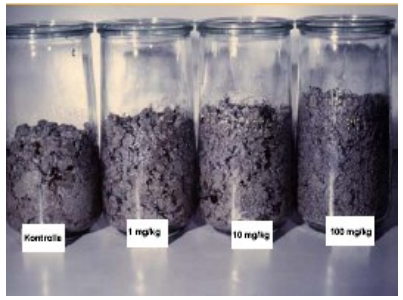
- Eisenia fetida / Eisenia andrei
- culcure in mixture manure/soil/garden-substrate/peat
- 7,14 days
- 500 g soil; 50-60 % water holding capacity
- artificial soil or LUFA 2.2 or other



Soil bioassays – consumers - invertebrates

Earthworms - acute



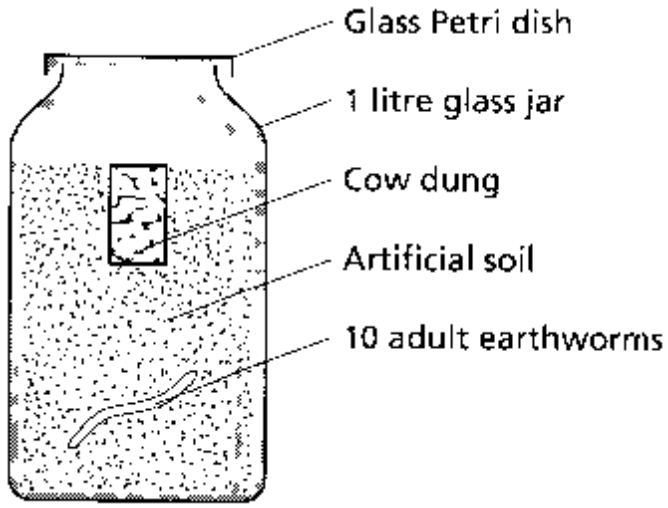


Test No. 207: Earthworm, Acute Toxicity Tests		1984
ISO 11268-1:2012	Soil quality — Effects of pollutants on earthworms — Part 1: Determination of acute toxicity to Eisenia fetida/Eisenia andrei	

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

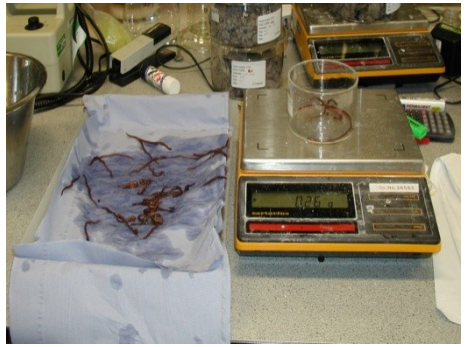
WHC measurement

Water added

Soil weighted to jars

10 adults from culture

Washed



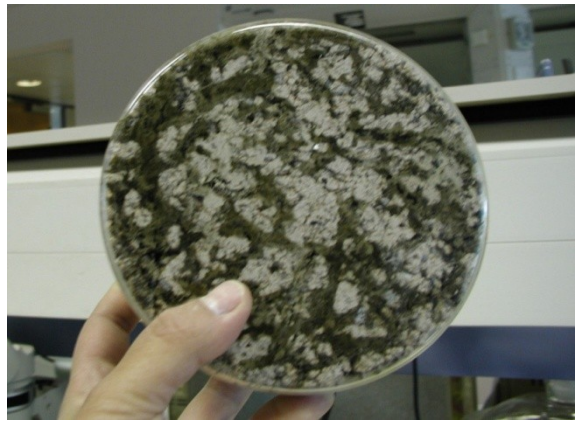
Weighting worms

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

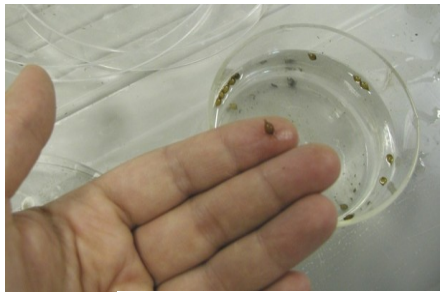
Water bath, increasing temperature 40ºC - 60ºC

After 20 min juveniles appear

Collecting and counting juveniles

Sieving the soil

Hand sorting of cocoons

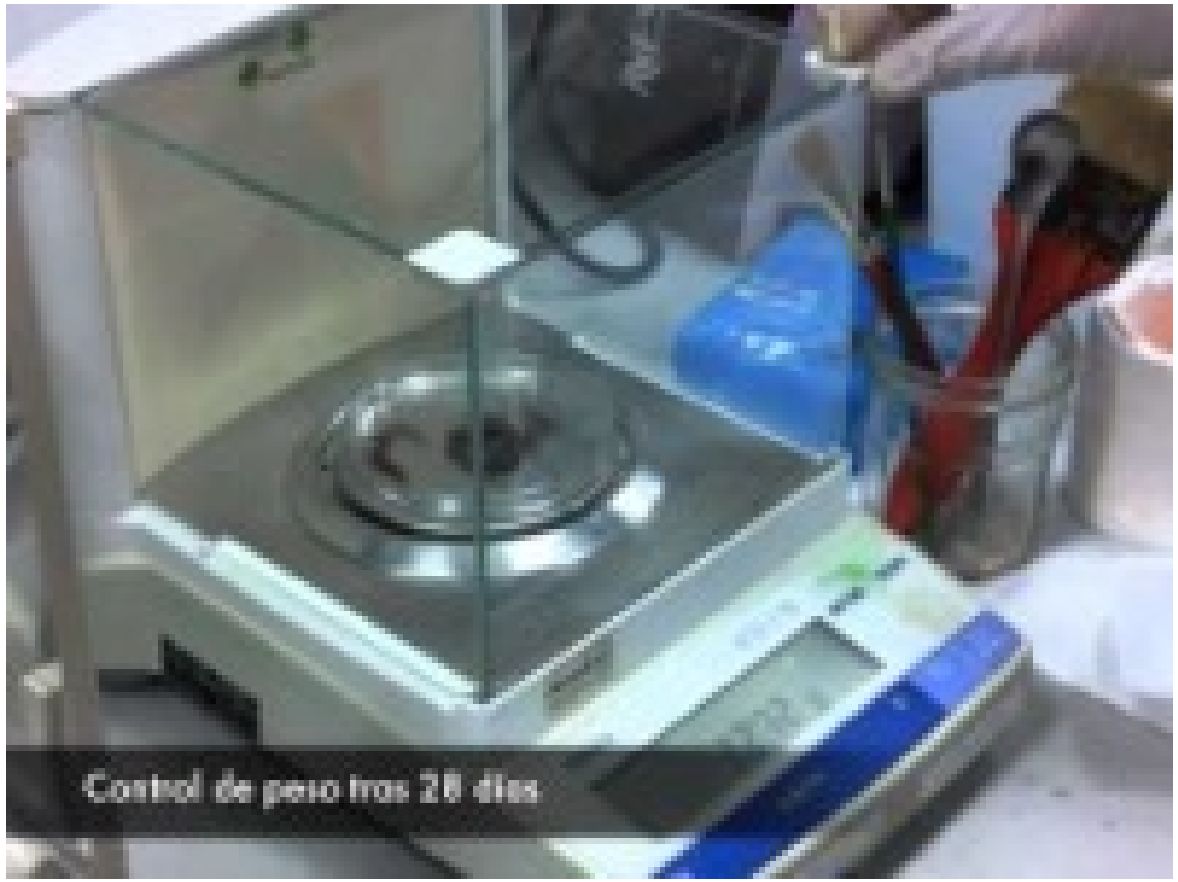


Counting

Soil bioassays – consumers - invertebrates

Earthworms - reproduction

- …

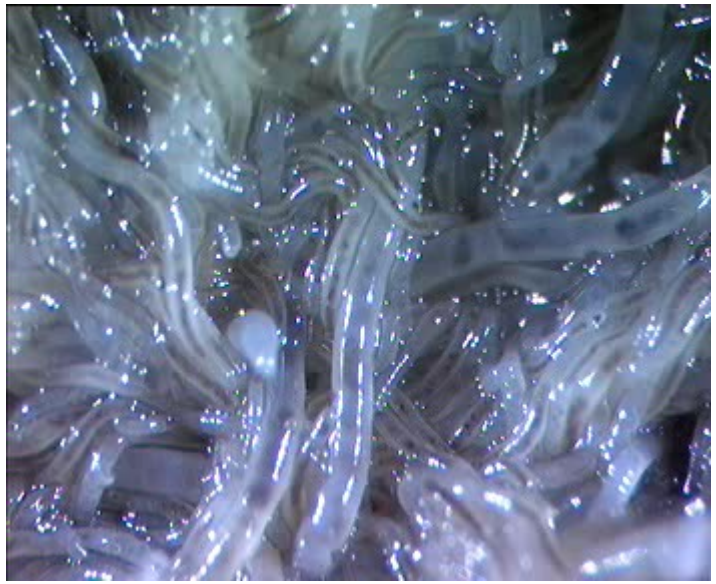


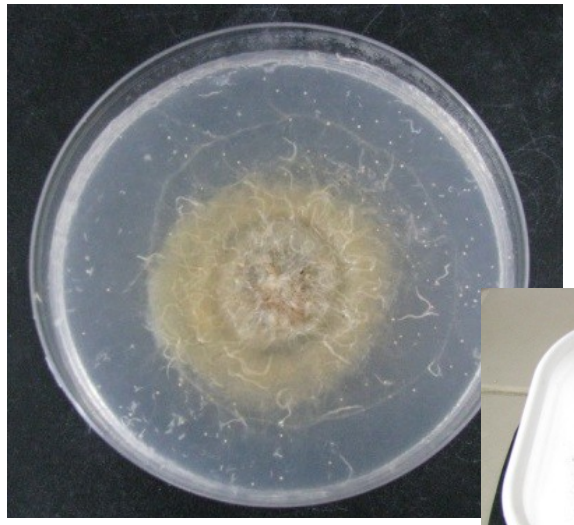
Test No. 222: Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)		2016
ISO 11268-2:2012	Soil quality — Effects of pollutants on earthworms — Part 2: Determination of effects on reproduction of Eisenia fetida/Eisenia andrei	

Soil bioassays – consumers - invertebrates

Enchytraeids

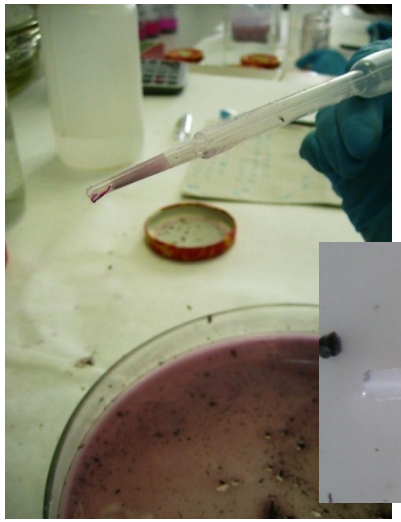
- Enchytraeus albidus or Enchytraeus crypticus
- 28 d; 20-30 g soil; 50-60% water holding capacity
- artificial soil or LUFA 2.2 or other

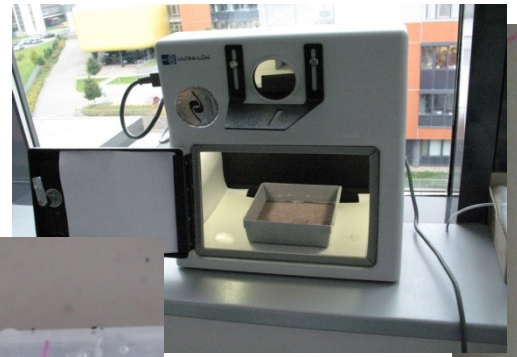


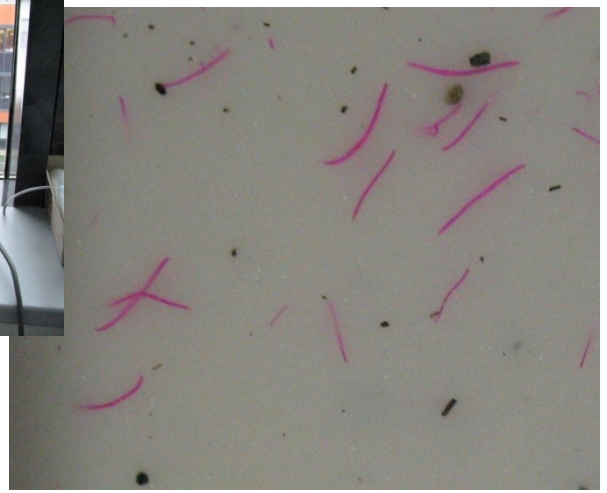


Soil bioassays – consumers - invertebrates

Enchytraeids

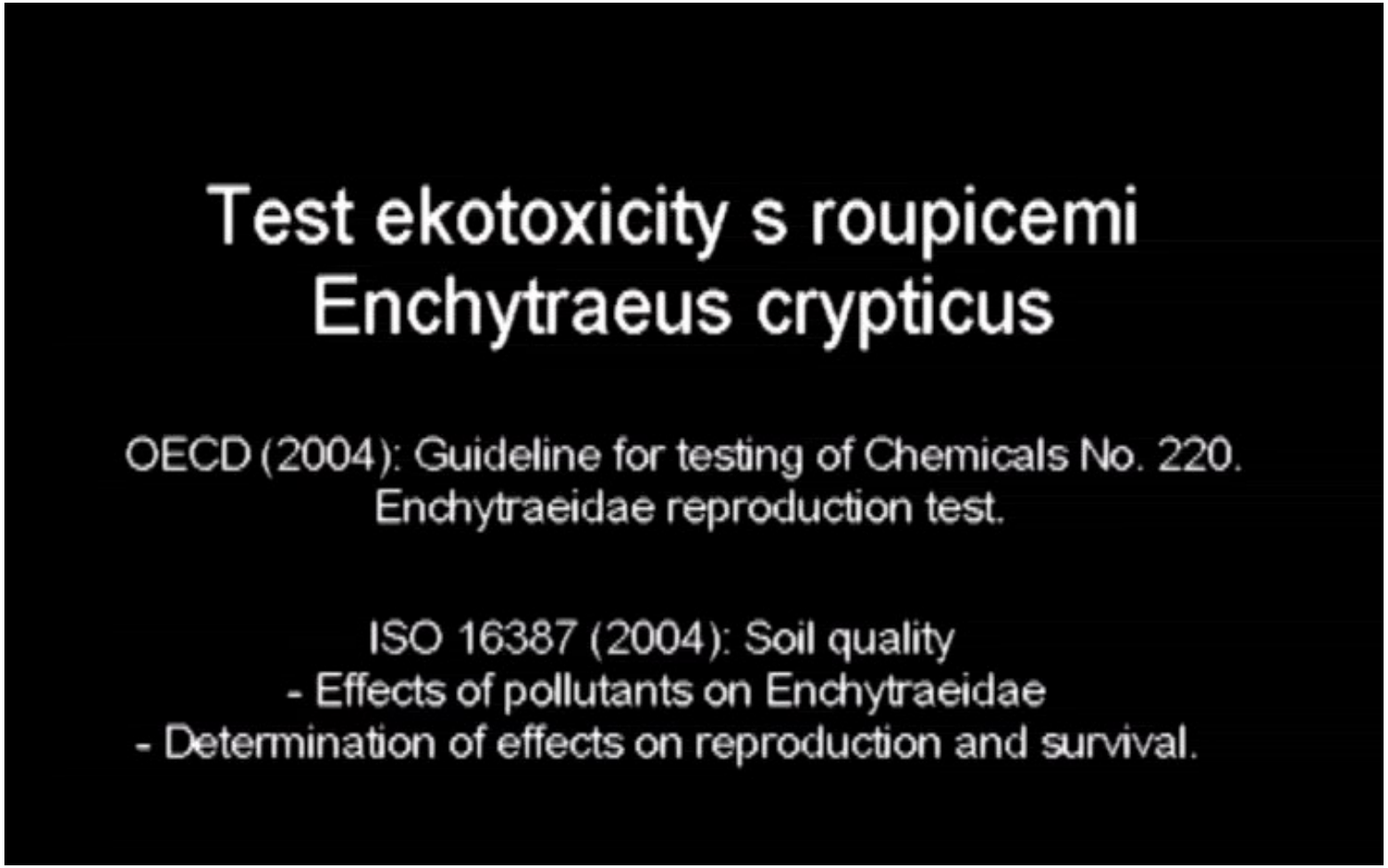






Soil bioassays – consumers - invertebrates

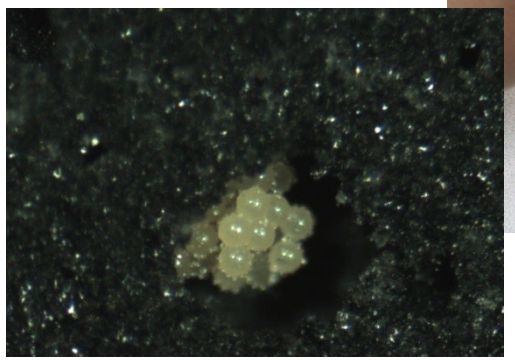
Enchytraeids



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



Test No. 232: Collembolan Reproduction Test in Soil		2016
ISO 11267:2014	Soil quality — Inhibition of reproduction of Collembola (Folsomia candida) by soil contaminants	

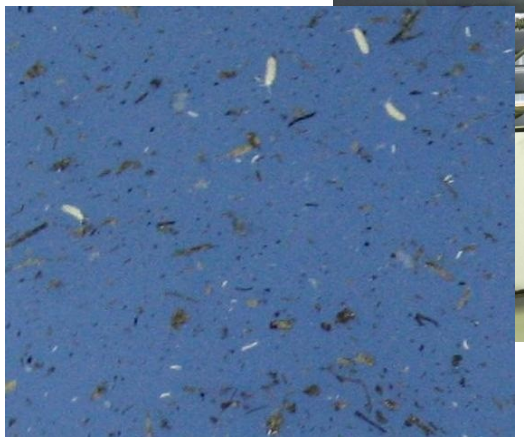
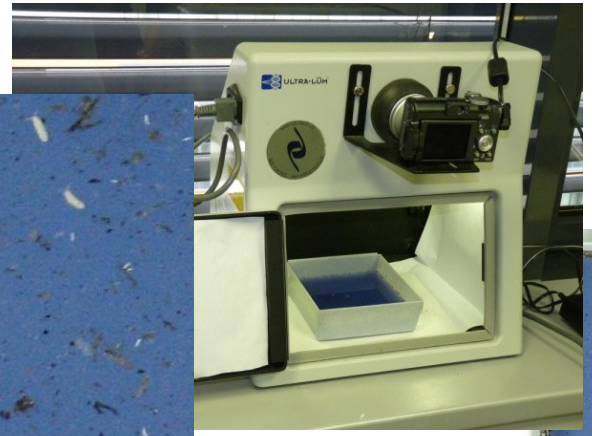
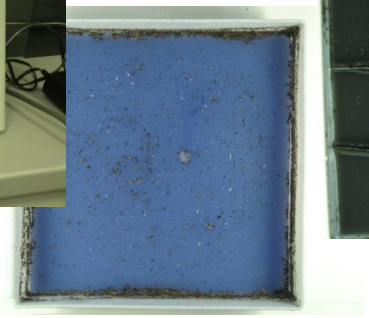
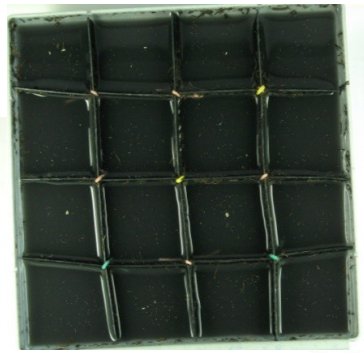
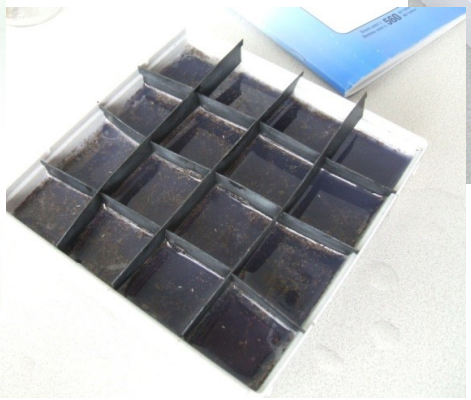
Soil bioassays – consumers - invertebrates

Springtails





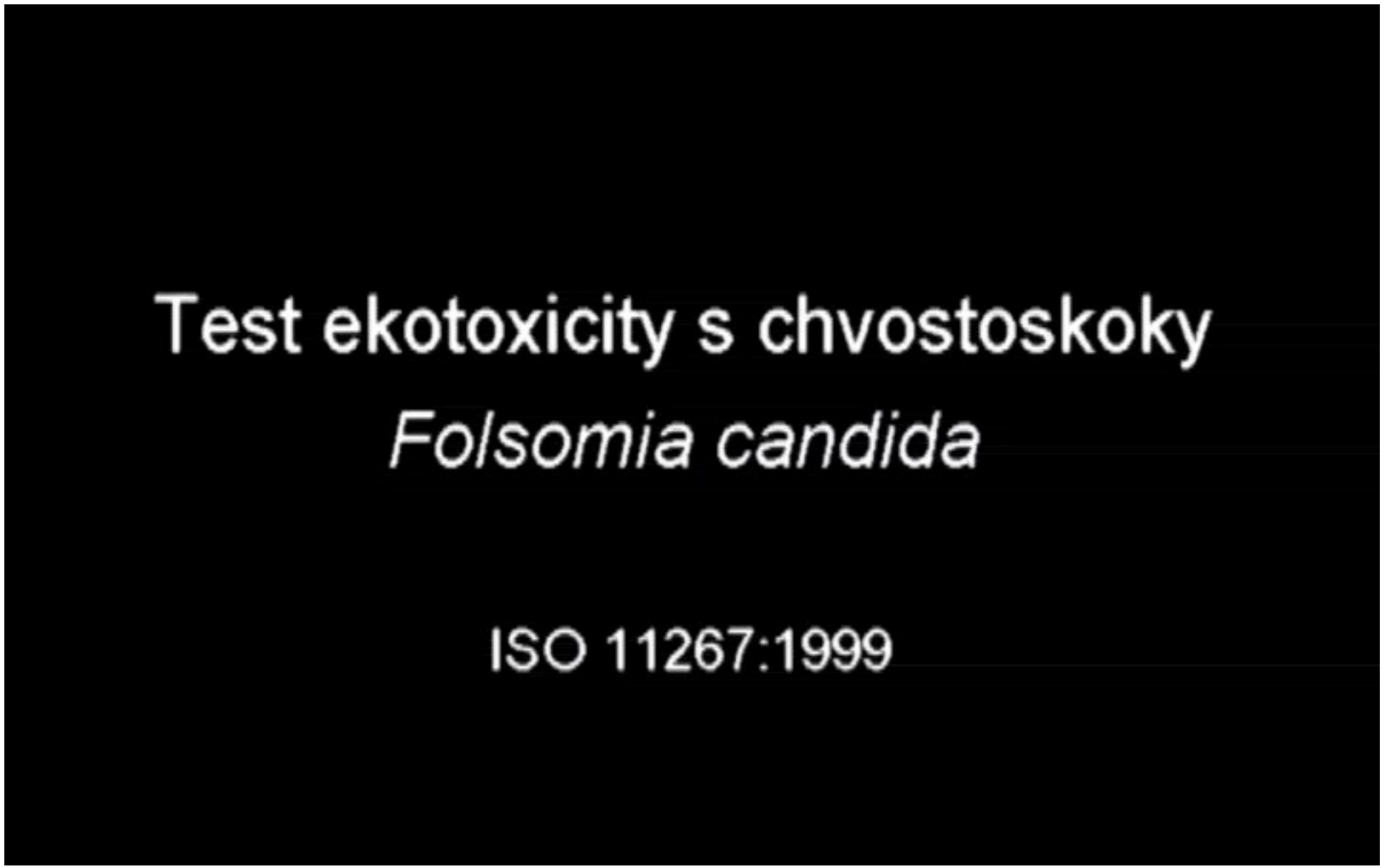


Test No. 232: Collembolan Reproduction Test in Soil		2016
ISO 11267:2014	Soil quality — Inhibition of reproduction of Collembola (Folsomia candida) by soil contaminants	

Soil bioassays – consumers - invertebrates

Springtails

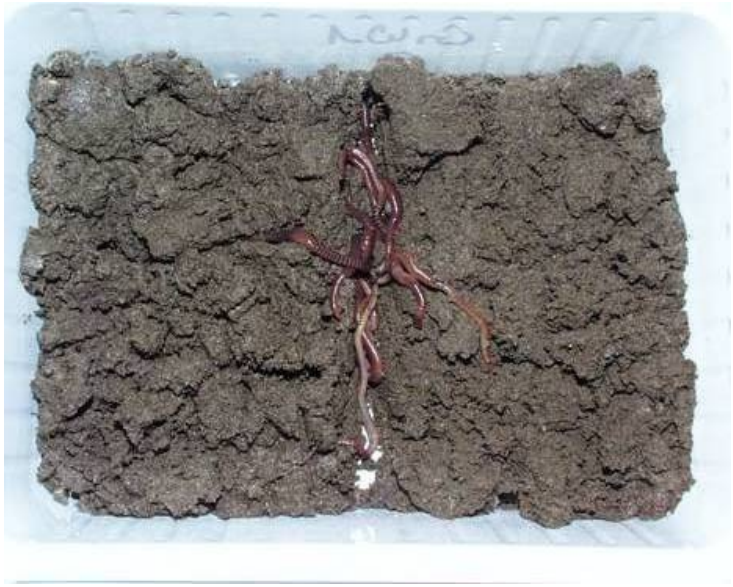


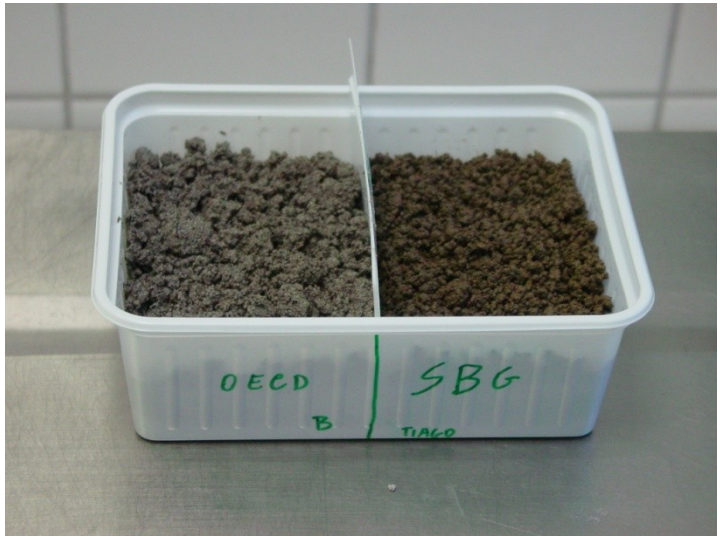
Test No. 232: Collembolan Reproduction Test in Soil		2016
ISO 11267:2014	Soil quality — Inhibition of reproduction of Collembola (Folsomia candida) by soil contaminants	

Soil bioassays – consumers - invertebrates

Avoidance tests

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





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)

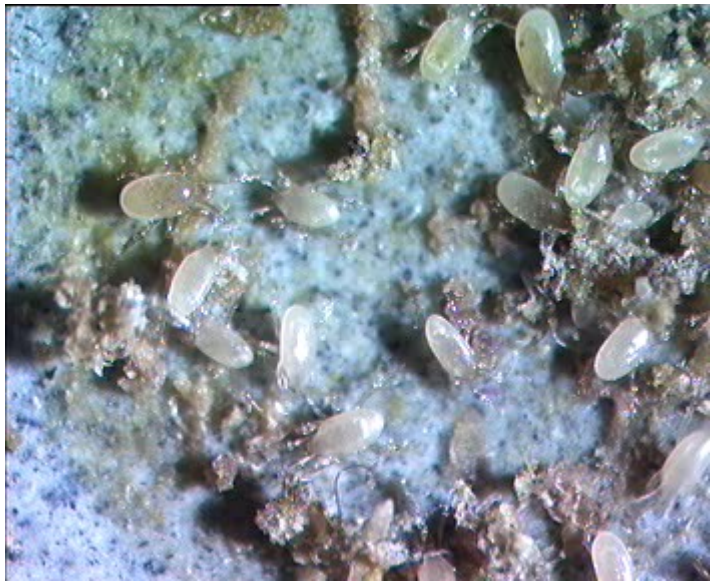
Soil bioassays – consumers - invertebrates

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

- ...

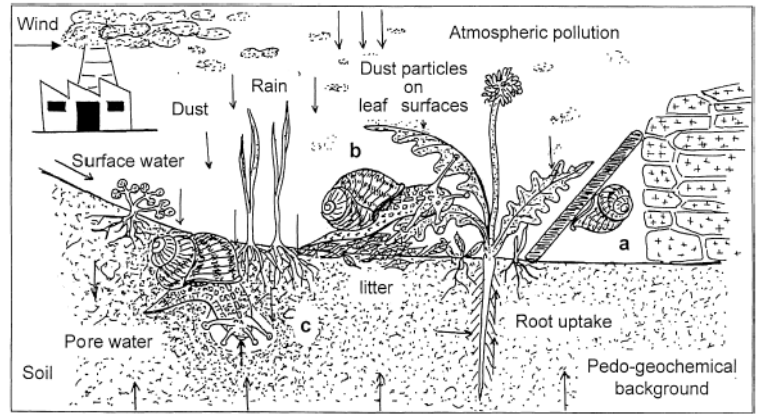
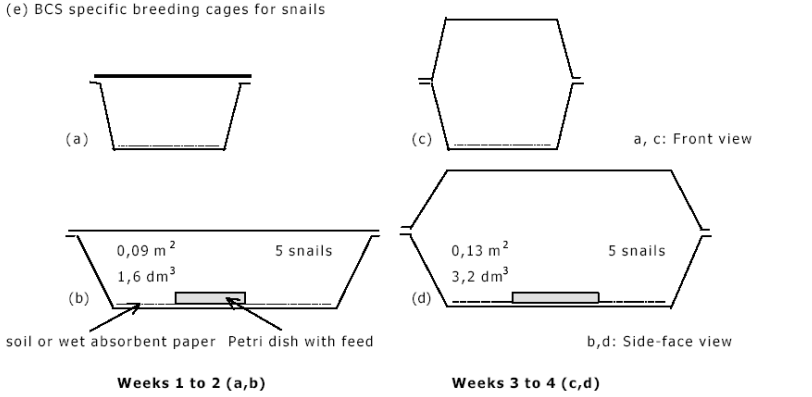


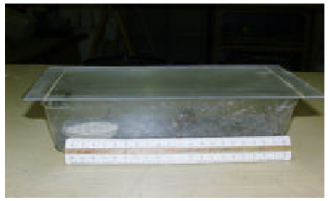
Fig. 6. Possible routes of direct and indirect exposure of land snails to contaminants: (a) contamination by breathing air; (b) contamination at soil surface by eating plants, soil contact, breathing air, rain and surface water contact; (c) contamination in the soil by swallowing soil particles, soil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



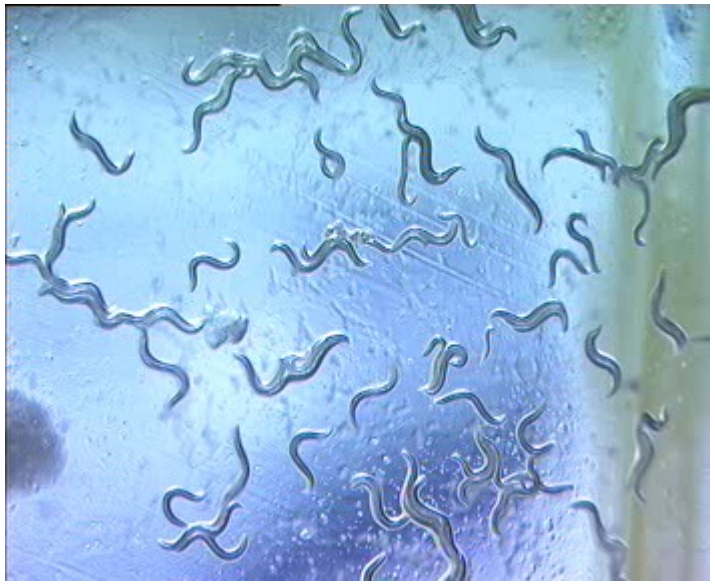


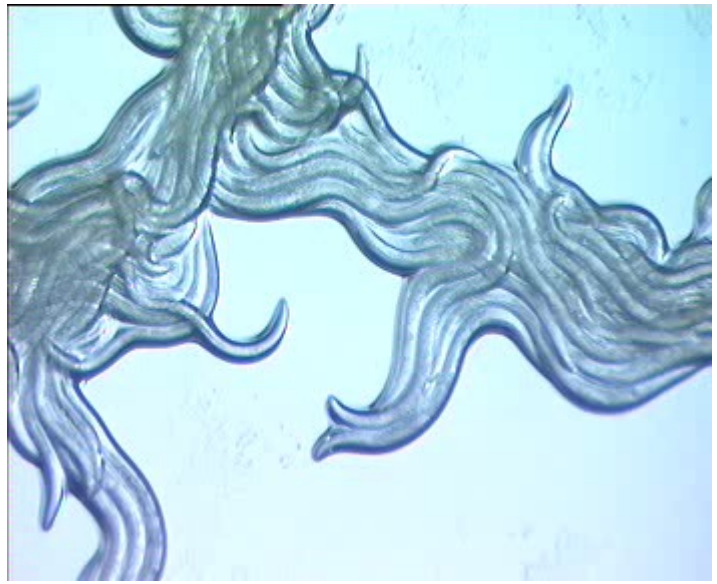
(e)

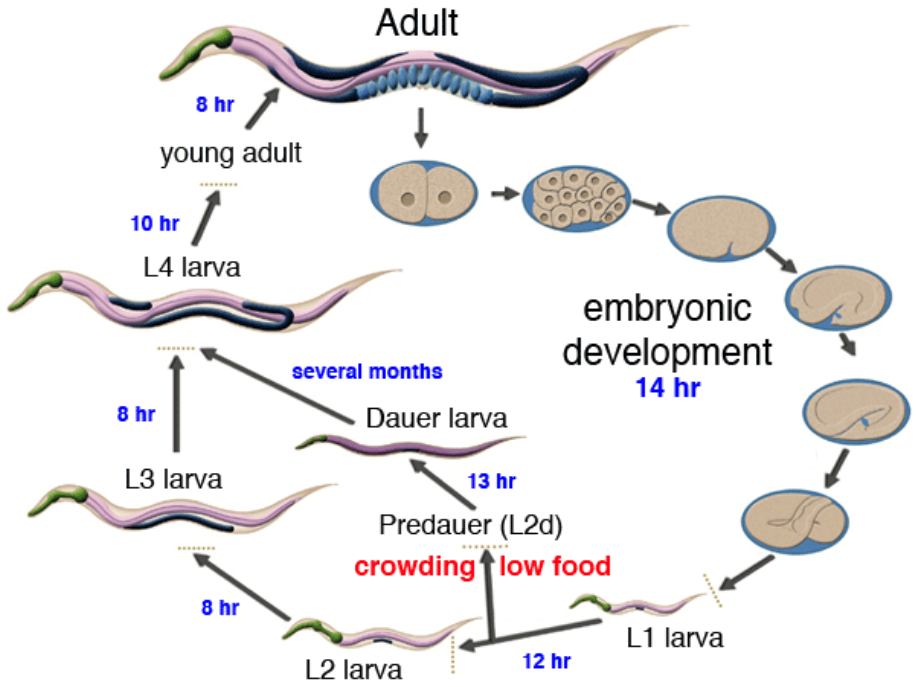
Soil bioassays – consumers - invertebrates

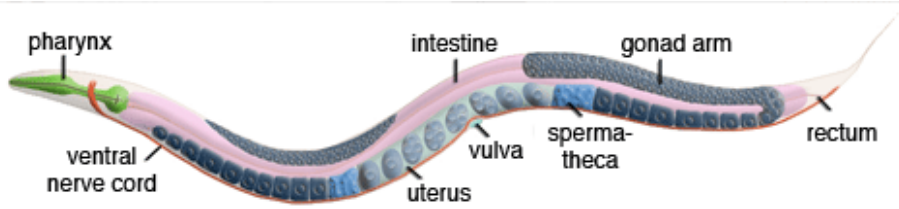
Nematodes

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





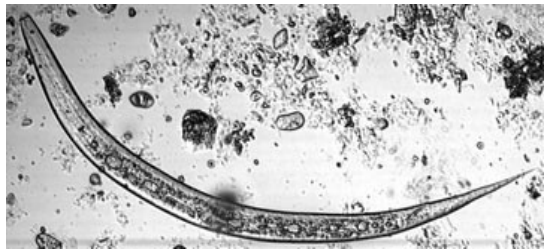


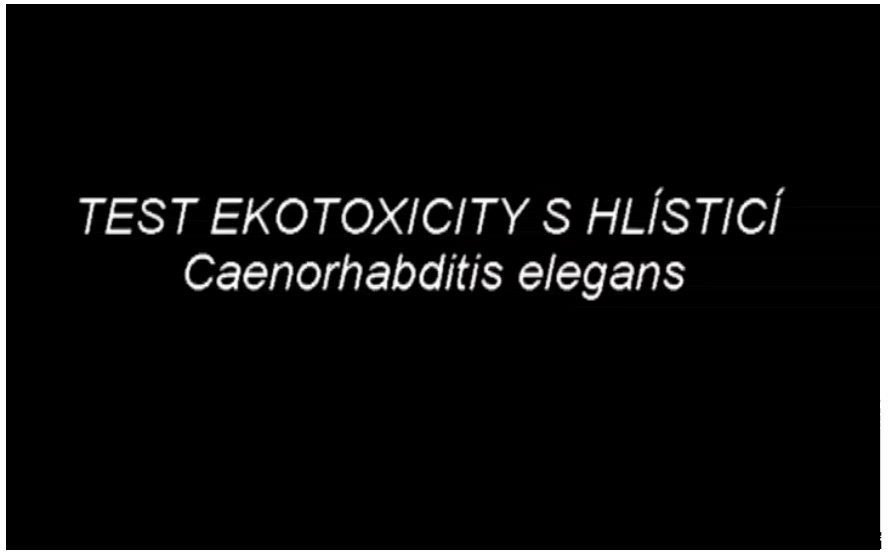


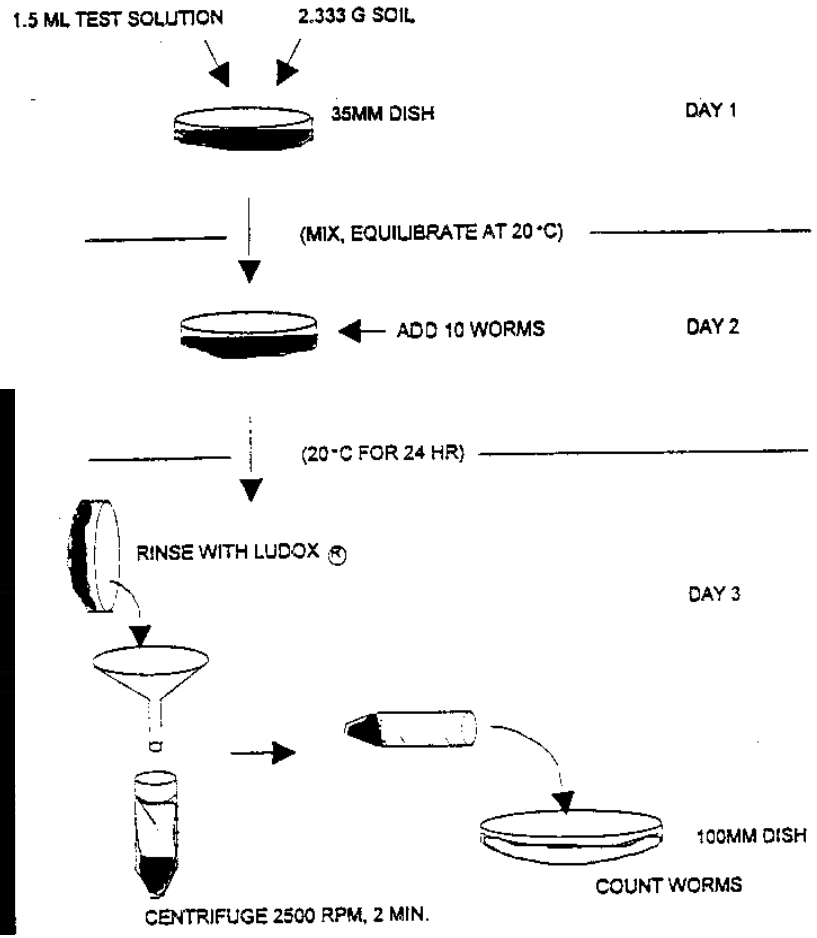
Soil bioassays – consumers - invertebrates

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

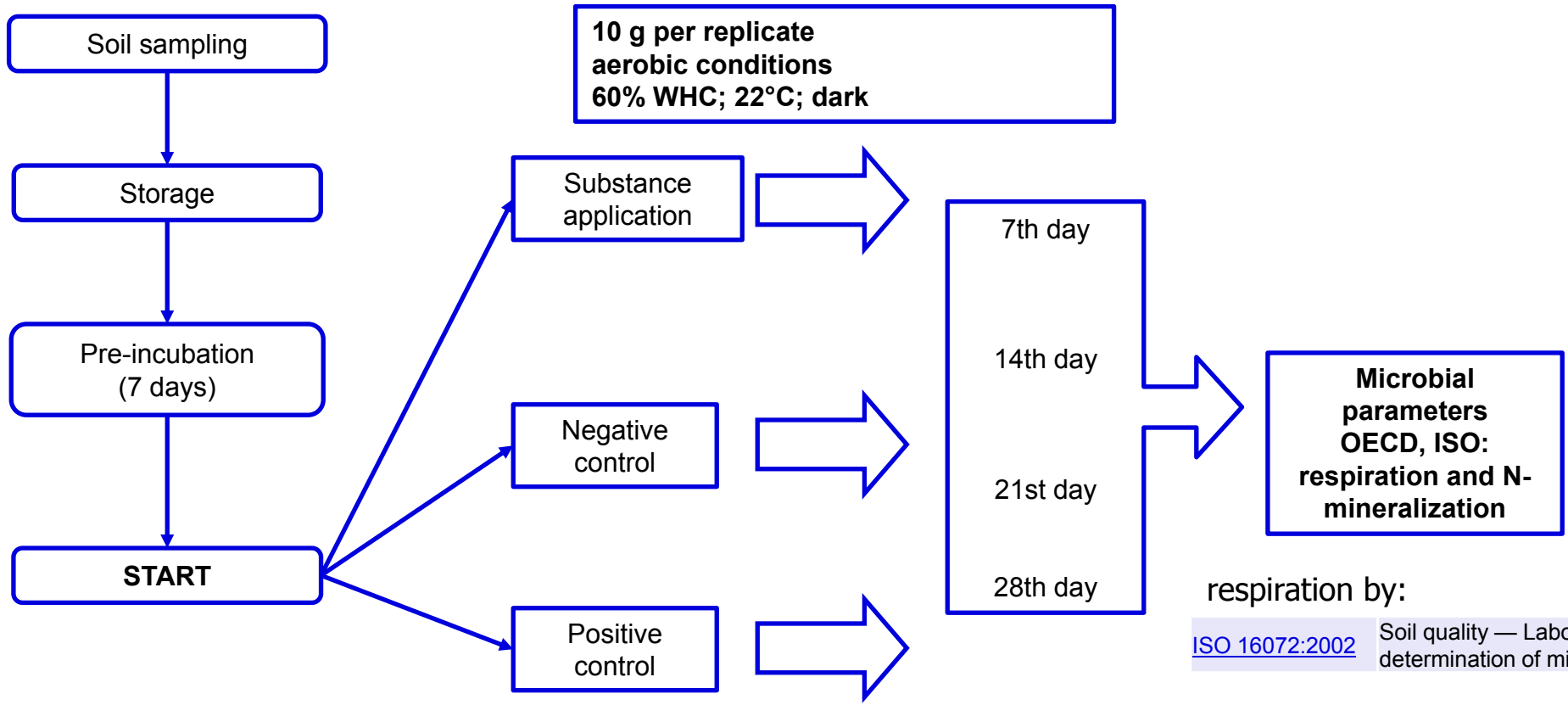
Whole community testing for microbial activities

- 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

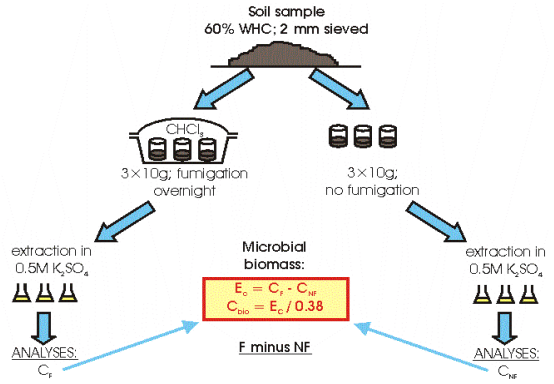


Test No. 216: Soil Microorganisms: Nitrogen Transformation Test		2000
Test No. 217: Soil Microorganisms: Carbon Transformation Test		2000
ISO 14238:2012	Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes	

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

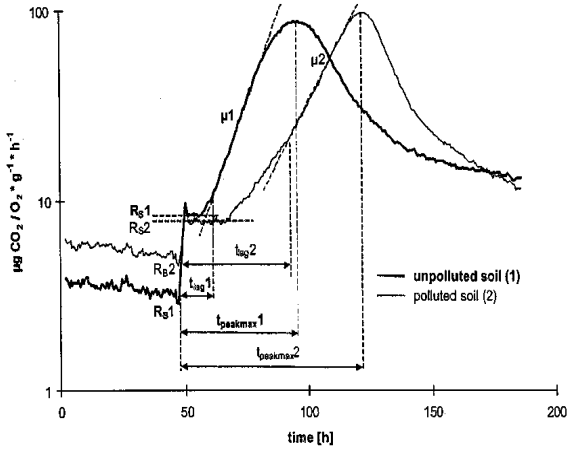
Whole community testing for microbial activities – quick tests

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

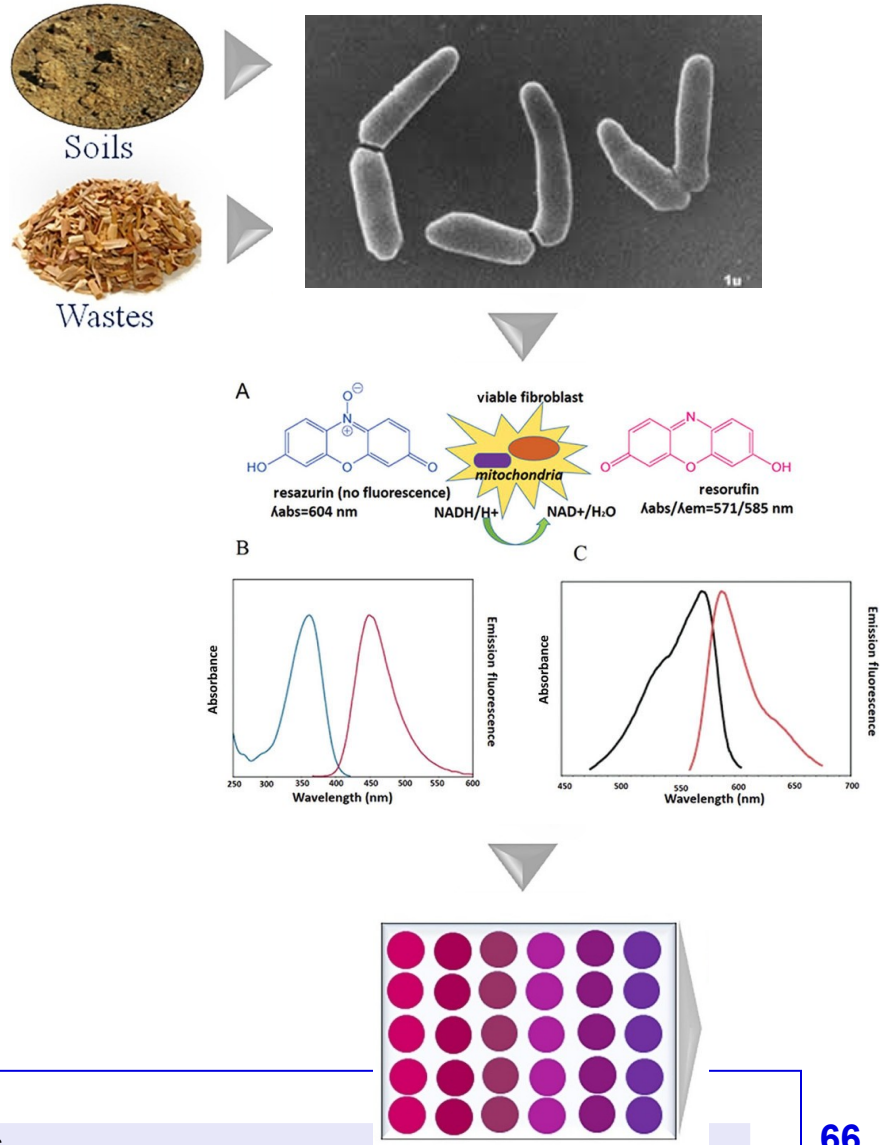


ISO 15685:2012	Soil quality — Determination of potential nitrification and inhibition of nitrification — Rapid test by ammonium oxidation
ISO 17155:2012	Soil quality — Determination of abundance and activity of soil microflora using respiration curves

Soil bioassays – destruents – microorganisms

Arthrobacter globiformis test

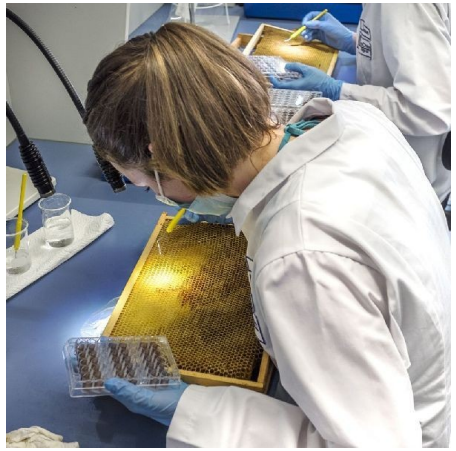
- introduced bacteria species NOT whole community
- contaminated soil or other solid materials (waste) or soil spiked with tested chemical(s)
- A. globiformis added and dehydrogenase activity measured



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

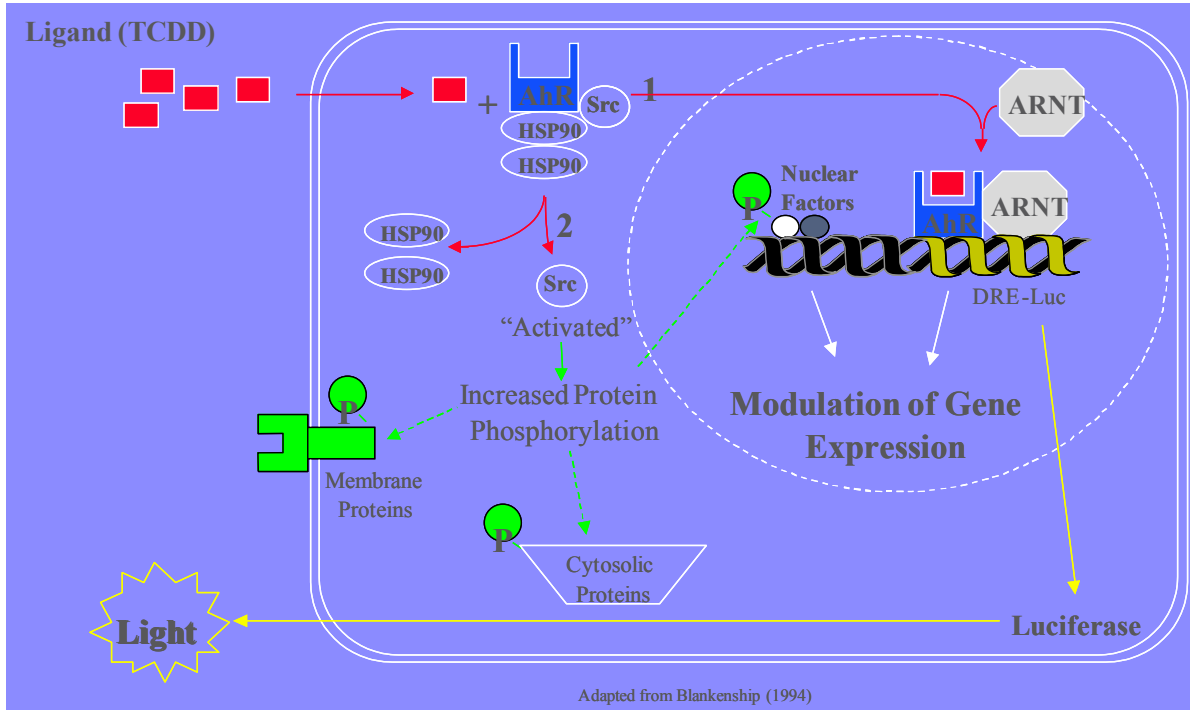
Test type	Avian reproduction
Organisms	Ring-necked pheasant (*Phasianus colchicus*), bobwhite (*Colinus virginianus*), Japanese quail (*Coturnix japonica*), chicken (*Tympanuchus cupido*), mallard (*Anas platyrhynchos*), black duck (*Anas rubripes*), screech owl (*Otus asio*), American kestrel, ring 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

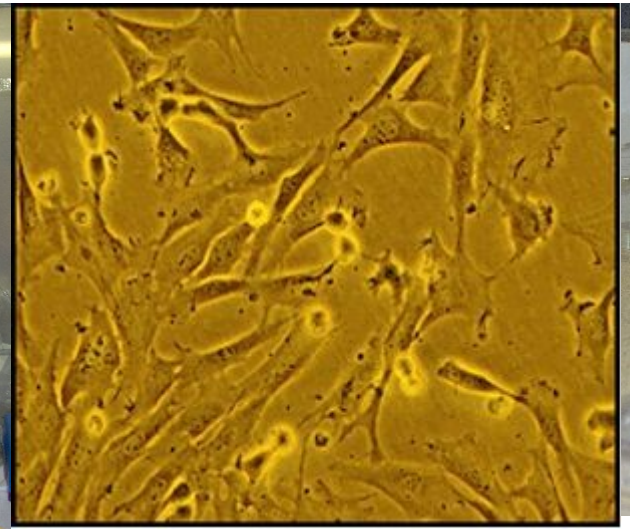
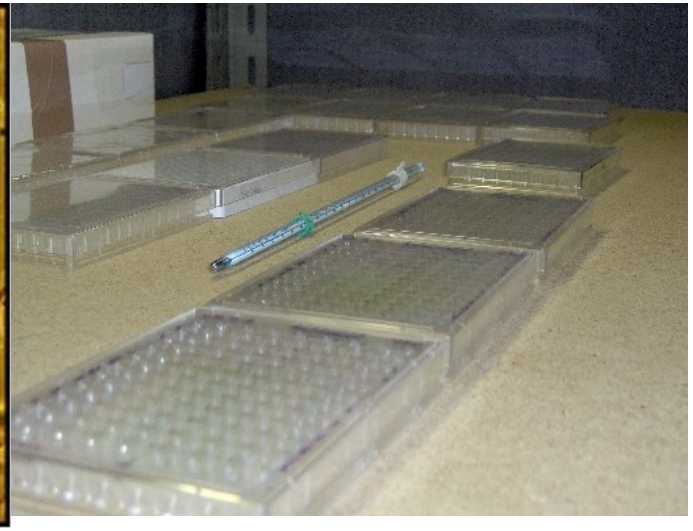
- special cell lines
- specific compound ➔ specific cellular or intracellular reporter ➔ reporter gene
- dioxin-like toxicity (AhR), estrogenicity (ER), thyroid-hormone-like toxicity ...

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

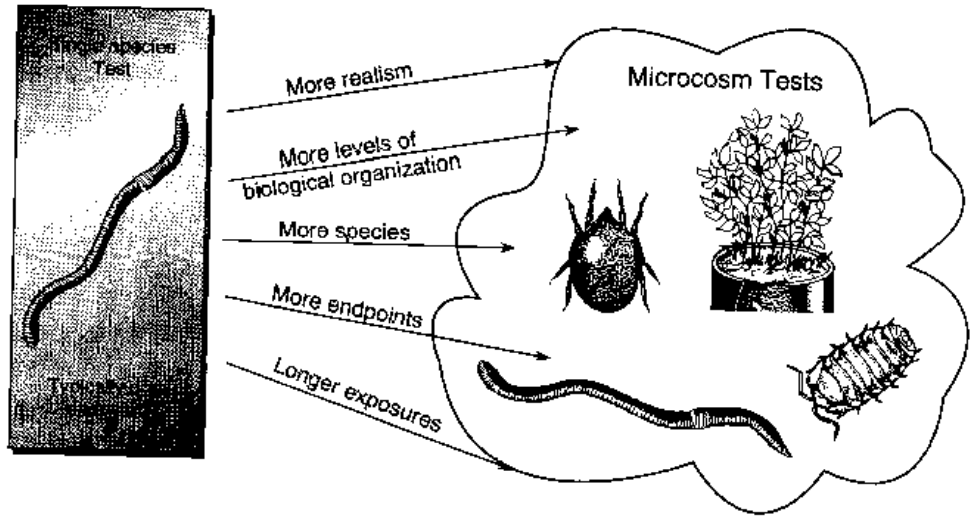


Figure 1 Microcosm tests can be defined by how they differ from single-species tests. Most importantly, there are more species. This usually also means more levels of biological organization (population and community effects) and more toxicity endpoints. They may also provide more realism and allow longer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

Microcosms / mesocosms

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

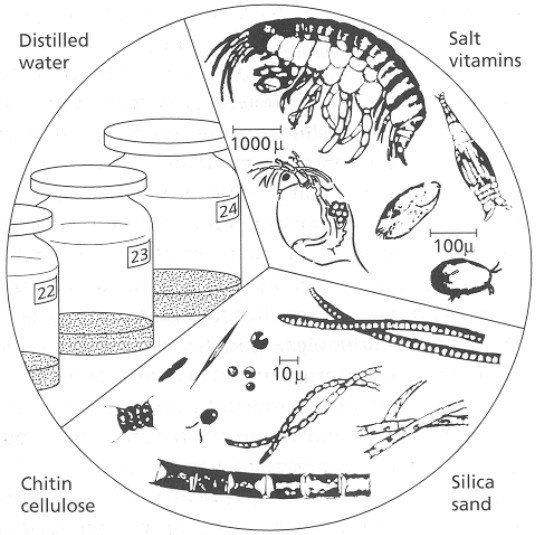


Fig. 5.2 Components of a standardized aquatic microcosm.

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

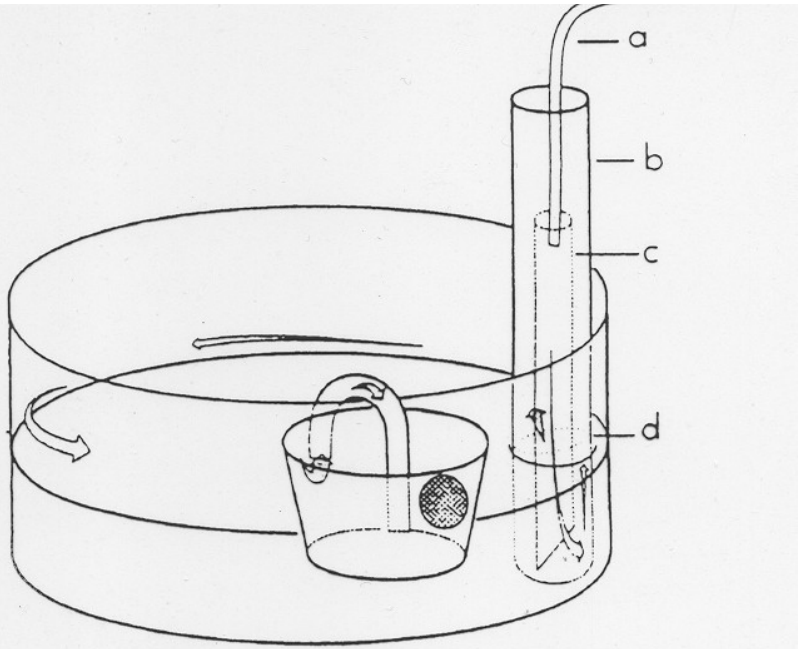


Figure 7. Flow-through exposure chamber for flow-through tests with polychaetes. The exposure chamber is a glass crystallizing dish with an inflow of water over the sediment surface. Arrows show flow of water into the test tube (b) through silicone tubing (a), which has a piece of glass tubing (c) attached 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.)

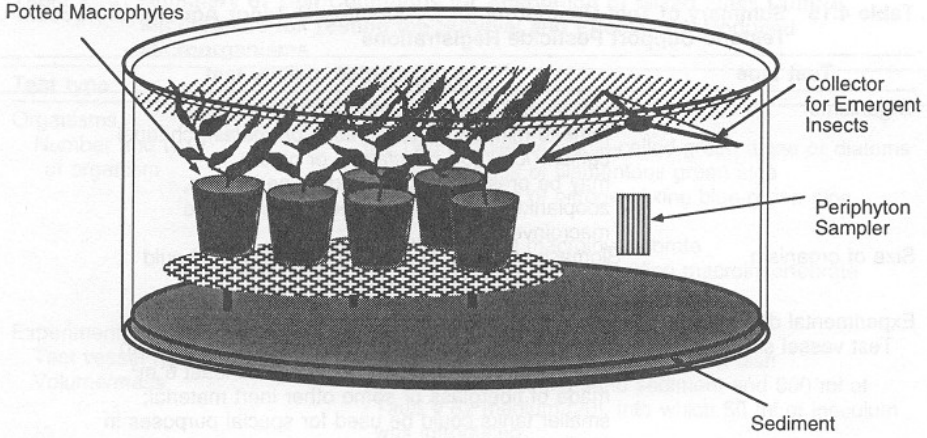


Figure 4.2 FIFRA microcosm experimental unit. An example of a microcosm experimental unit designed to test the effects of a herbicide on an aquatic environment. This particular setup does not include fish since the predatory effects would tend to hide lower trophic level effects upon the invertebrate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

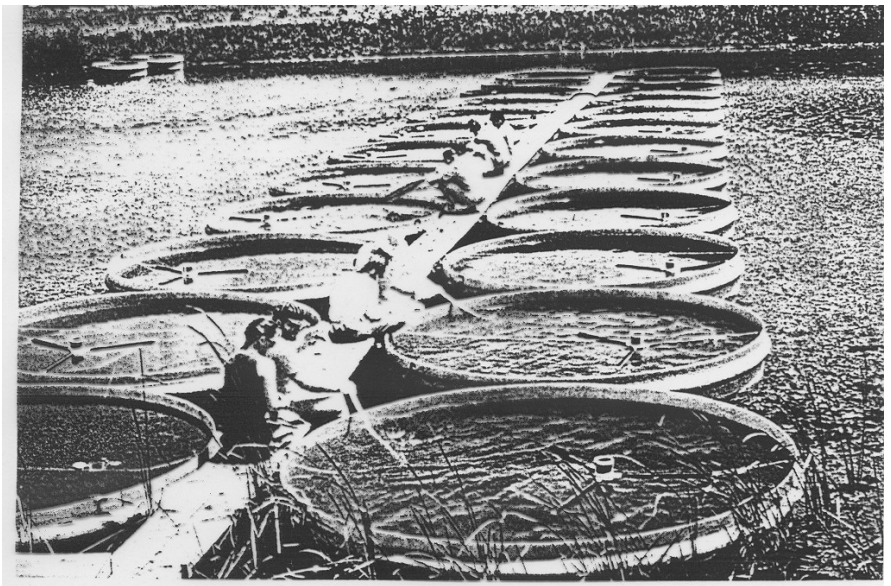


Figure 6. Photograph of outdoor microcosm test systems (10,000-L tanks) located at the University of Kansas. These fiberglass tanks have been used by Springborn Laboratories, Wareham, MA. to evaluate the impact of pesticides on aquatic communities.

Microcosms / mesocosms

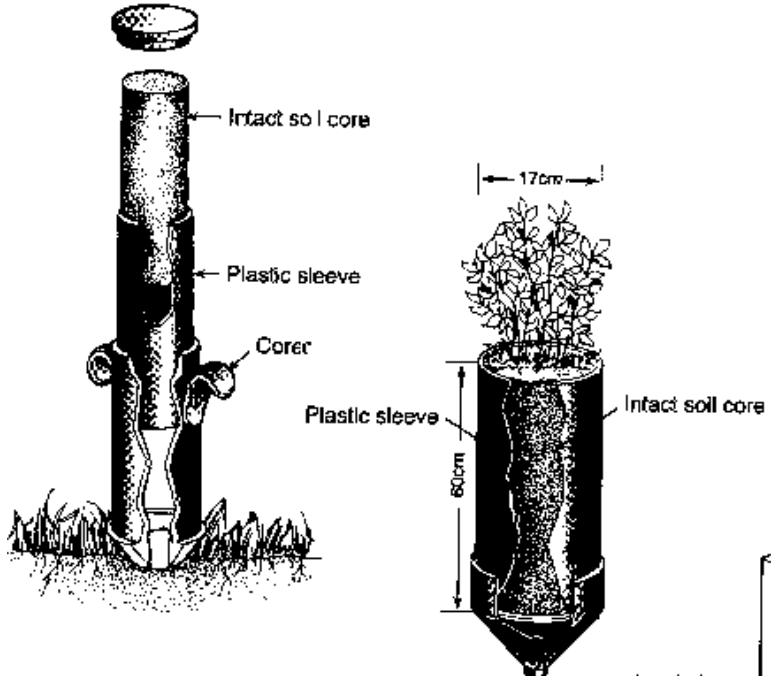
- **Soil:** usually core/column (made of plastic, wood, metal) of soil with vegetation, contains standard soil or real soil; can be outside (open system) or in the climate chamber (also gas monitoring)

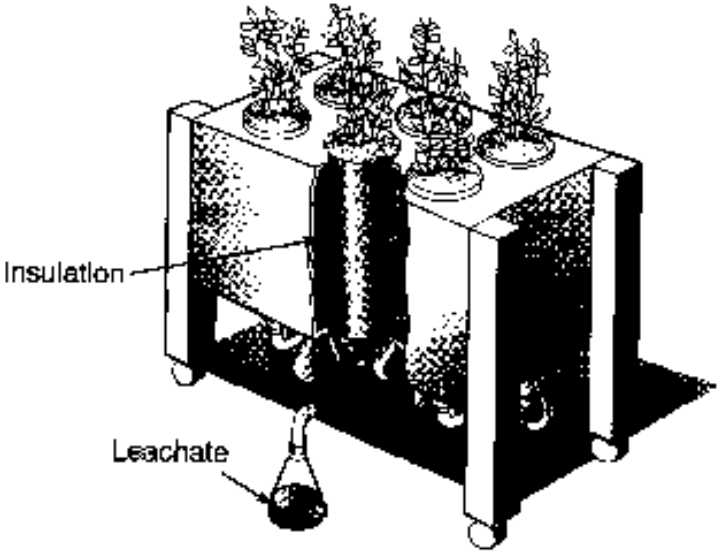
Table 9.1 Classification of Various Semi-Field Tests

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