

Practice No. 8

Toxicity tests on organisms

- For all new chemicals before being released on the market and also for the re-evaluation of the safety of chemicals already used.
- It is necessary to unify them all around the world to avoid excessive use of laboratory organisms and to decrease the price of testing. Once a test is done according to approved methods in one country, it can be accepted after a short evaluation and without further testing also in other countries.
- The rule of three R:
 - Reduction (less laboratory animals)
 - Replacement (replace with evolutionary lower organisms and if possible, by tissue cultures, cells, computer modelling)
 - Refinement (the animal should be stressed and harmed as little as possible, welfare of laboratory animal is absolutely necessary)

Organisation for Economic Co-operation and Development (OECD)

- Chemicals play an important role in daily life. Therefore, their safety for man and the environment has to be considered carefully. OECD works on the development and co-ordination of environment health and safety activities internationally.
- The OECD Guidelines for the Testing of Chemicals are a collection of the most relevant internationally agreed testing methods used by government, industry and independent laboratories to assess the safety of chemical products.
- Testing is divided into several parts:
 - Section 1: Physical Chemical Properties
 - Section 2: Effects on Biotic Systems
 - Section 3: Degradation and Accumulation
 - Section 4: Health Effects
 - Section 5: Other Test Guidelines

Toxicity:

- Is described by main toxicological terms:
 - LD50 (lethal dose which causes death of 50 % of tested animals)
 - LC50 (lethal concentration which causes death of 50 % of tested animals)
 - NOEC (no observed effect concentration – the highest concentration tested in which no adverse effect was detected)
 - LOEC (lowest observed effect concentration – the lowest concentration tested in which an adverse effect was detected)
- Based on the value of LD/LC/LOEC assessed, chemical is classified and described by Hazard statements and Precautionary statements (H/P) and labelled with appropriate symbols in the course of harmonizing classification, labelling and packaging of chemicals ordered by Globally Harmonized System (GHS). In Europe, more specification is given by Regulation (EC) 1272/2008 – CLP (classification, labelling and packaging). So called CLP/GHS system.
- Formerly (till 2017), the risk and safety statements (R/S) and other type of symbols were used to label chemicals. You can still see them on certain packages stored (if still within expiry date), but no more chemicals can be marketed with these old symbols nowadays.

Toxicity tests on mammals

- acute and chronic tests
- tests on lethality, carcinogenicity, genotoxicity, reproduction, mutagenicity, dermal and eye toxicity, inhalation toxicity etc.
- for the treatment of poisonings, the most important is acute toxicity:

Acute toxicity:

If we do not know approximate toxicity from testing on other animals, we start testing with so called limit dose, which is 2000 mg/kg of body weight. If the result is negative (no toxic effect on the organism), we do not continue with further testing.

We apply the dose by a tube directly to stomach and check the animal for 14 days.

Before:

- OECD 401 – Acute oral toxicity:

- not used since 2002, but all the substances which were approved for the use before this date were tested by this method

- 10 animals for each dose (usually 3-5) had to be used

- the result of the test is exact value LD50

- according to LD50, we divide chemicals into several categories, they were described by so called R (risk) sentences (R 28 – very toxic if swallowed, LD50 is less than 25 mg/kg; R 25 – toxic if swallowed, LD50 is between 25 - 200 mg/kg; R 22 – harmful if swallowed – LD 200 – 2000 mg/kg)

Nowadays:

- OECD 423 - Acute Toxic Class Method:

- only 3 animals used (usually females)

- only fixed doses tested: 5, 50, 300 and 2000 mg/kg

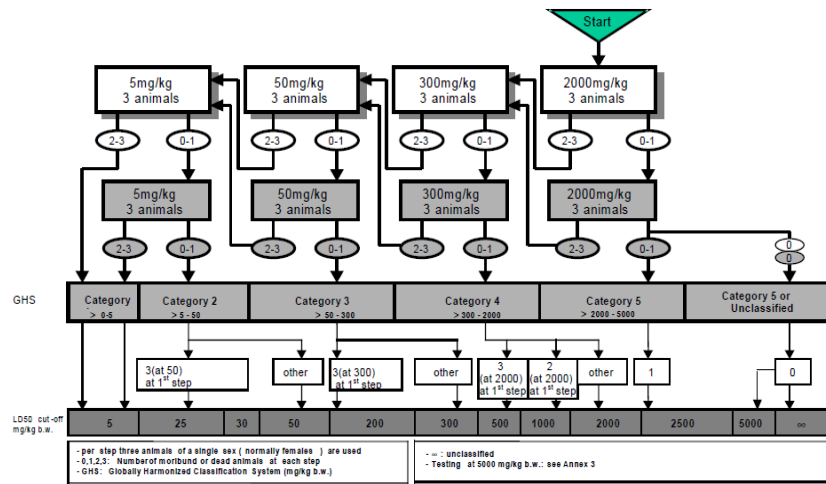
- the experiment goes in several steps (see the scheme bellow)

- one of the fixed doses is chosen (based on information from previous tests, or limit dose), first experiment is done. According to the result, test is repeated with the same dose in males, or the dose might be increased or decreased.

- according to the results in last step, substance is classified into one of the 5 categories (0-5, 5-50, 50-300, 300-2000 and 2000-5000 mg/kg) according to Globally Harmonized System (GHS) and H statements is assigned:

- H300: Fatal if swallowed (0-50 mg/kg); H301: Toxic if swallowed (50-300 mg/kg); H302: Harmful if swallowed (300-2000 mg/kg).

ANNEX 1d: TEST PROCEDURE WITH A STARTING DOSE OF 2000 MG/KG BODY WEIGHT



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Toxicity tests on fish

- Fish used in the tests:
 - e.g. zebrafish (*Danio rerio*), common carp (*Cyprinus carpio*), medaka (*Oryzias latipes*), guppy (*Poecilia reticulata*), rainbow trout (*Onchorhynchus mykiss*)
- Limit test is done with the concentration 100 mg/l of water
- Types of test based on testing solution changes:
 - Static test (only one solution during whole 96 hours) - usually impossible to perform it due to cummulation of metabolism products and due to decrease in tested substance concentration
 - Semistatic test (change of solution every 48, 24, 12 or less hours according to the situation and stability of the substance) – fish are moved to other aquariums with newly prepared tested solutions
 - Flow through test – continuous change of tested solution
- Test with standard ($K_2Cr_2O_7$) together with test with the substance itself is necessary to check sensitivity of tested fish and avoid using more or less sensitive fish than is average

- Validation of the tests:
 - Mortality of fish in control tank should not exceed 10 % in adults, 25 % in embryos
 - Oxygen saturation in water has to be higher than 60 %
 - Concentration of tested substance should not decrease under 80 % of original concentration
 - Temperature suitable for the species, fluctuation max. $\pm 1^{\circ}\text{C}$
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OECD 203 - Fish, Acute Toxicity Test:

- 7-10 fish into each concentration
- Duration is 96 hours
- Result is 96hLC50 value

OECD 210 - Fish, Early-Life Stage Toxicity Test + OECD 212 - Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages

- Fertilised embryos are used
- Lasts:
 - 31 days for carp – OECD 210, so called embryolarval test
 - 6-7 days for carp – OECD 212, so called embryonal test
- Results are NOEC and LOEC

OECD 215 - Fish, Juvenile Growth Test

- Weight gain is evaluated
- Duration of the test is 28 days
- We use mainly rainbow trout and zebrafish
- Results are NOEC and LOEC values

Classification of substances according to their toxicity for fish:

- H400: Very toxic to aquatic life (LC50 is less than 1 mg/l)
- H401: Toxic to aquatic life (LC50 is between 1-10 mg/l)
- H402: Harmful to aquatic life (LC50 is between 10-100 mg/l)

- H410: Very toxic to aquatic life with long-lasting effects
- H411: Toxic to aquatic life with long-lasting effects
- H412: Harmful to aquatic life with long-lasting effects
- H413: May cause long-lasting harmful effects to aquatic life

- Formerly R50 (equals H400), R51 (H401), R52 (H402) and R53 (H413)

Toxicity tests on birds

OECD 205 - Avian Dietary Toxicity Test:

- Oral toxicity
- 5 days of feeding (commercial feed + tested substance), then 3 days of observation
- LC50 in mg/kg of feed

OECD 206 – Avian Reproduction Test:

- Duration 20 weeks
- NOEC and LOEC

Classification:

- no H (R) sentences for birds

Toxicity tests on honey bees

- Testing of pesticides mainly

OECD 213 - Honeybees, Acute Oral Toxicity Test:

- oral LD50 in micrograms/1 bee
- tested substances is given orally in sugar solution

OECD 214 Honeybees, Acute Contact Toxicity Test:

- dermal LD50 in micrograms/1 bee
- tested substance is applied on the dorsum dissolved in acetone, control group obtains only acetone

Classification:

LD50 < 2 micrograms/bee – very toxic (formerly R57)

2-11 micrograms/bee slightly toxic

> 11 micrograms/bee relatively non-toxic

No H classification, bees have their own now:

- Commission Regulation (EU) No 547/2011 on plant protection products, statement **SPe 8 (dangerous for bees)**

Rules for their application: To protect bees and pollinating insects do not apply to crop plants when in flower./ Do not use where bees are actively foraging./ Remove or cover beehives during application and for (*state time*) after treatment./ Do not apply when flowering weeds are present./ Remove weeds before flowering.

Classification can be based also on hectare dose of pesticide: calculation of so called Risk factor (RF).

RF = maximum hectare dose in grams/ LD50 in micrograms per bee

RF > 2500 very toxic

RF 50-2500 slightly toxic

RF < 50 relatively non-toxic