Sveučilište u Zagrebu
Fakultet kemijskog inženjerstva i tehnologije
Zavod za industrijsku ekologiju

Kolegij:
PRIMJENA EKOTOKSIKOLOGIJE
1. predavanje

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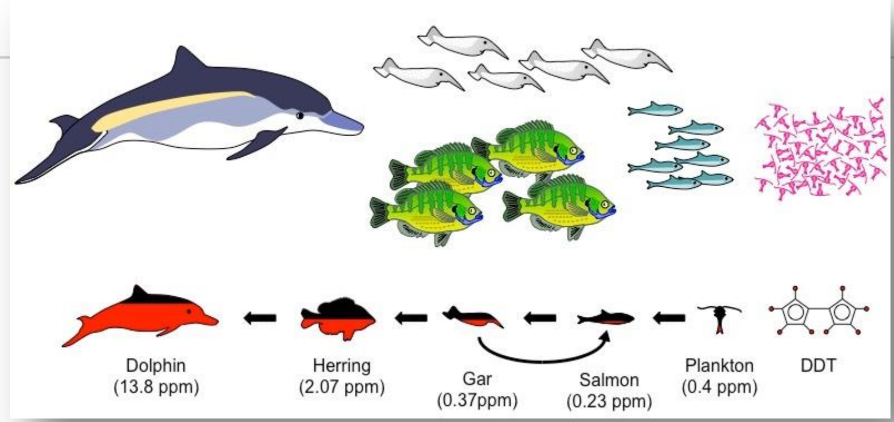
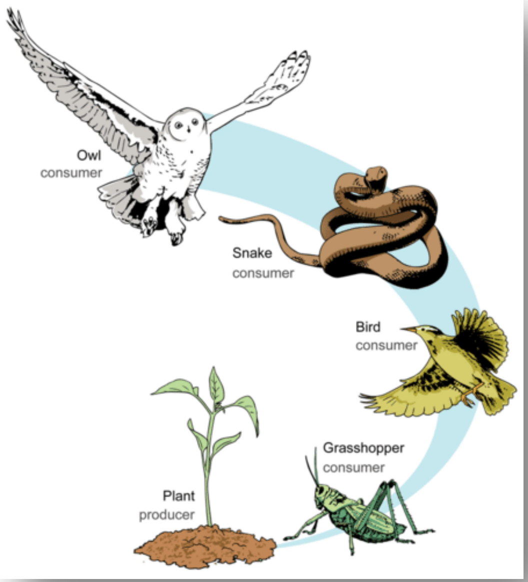
SADRŽAJ



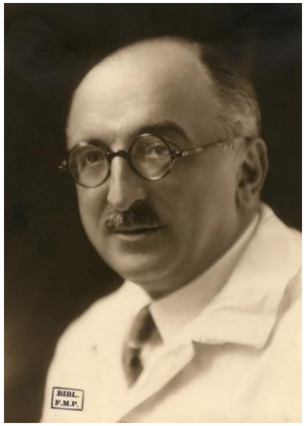
Dolphin
(13.8 ppm)
Herring
(2.07 ppm)
Gar
(0.37ppm)
Salmon
(0.23 ppm)
Plankton
(0.4 ppm)
DDT



Owl
consumer
Snake
consumer
Bird
consumer
Grasshopper
consumer
Plant
producer

EKOTOKSIKOLOGIJA

- Izraz izveden od riječi **ekologija i toksikologija**



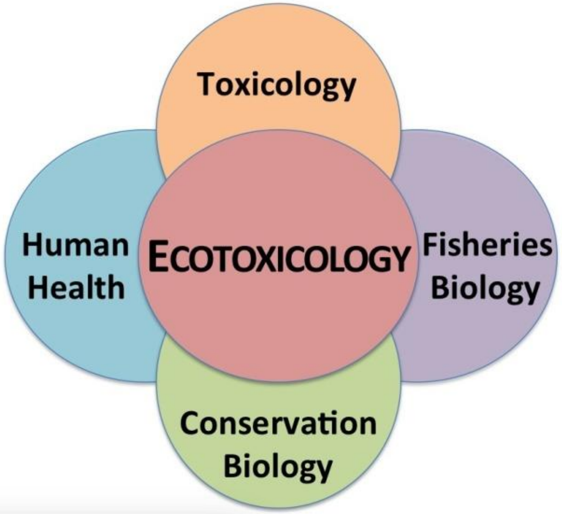
- izraz uvodi Rene Truhaut (1909.-1994.)
- definira pojam ekotoksikologije

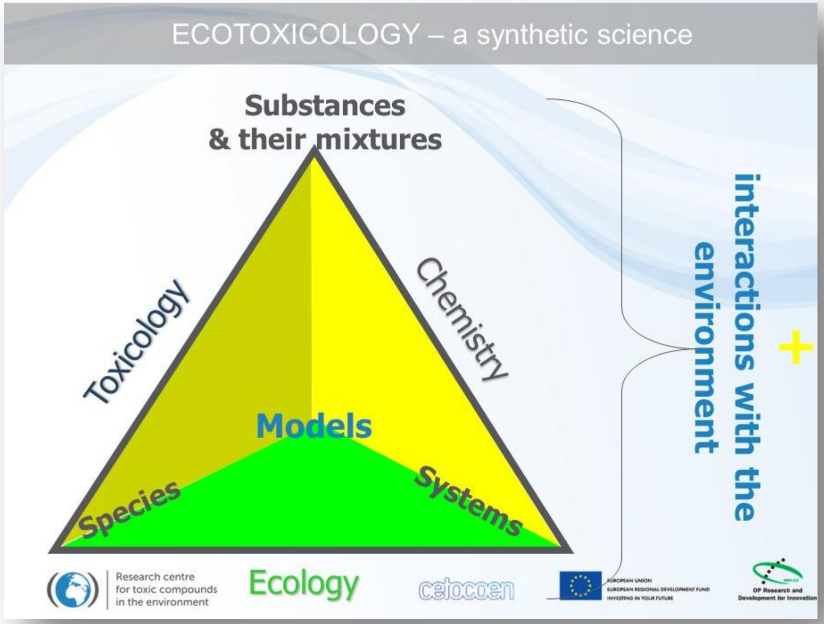
"*Ecotoxicology: objectives, principles and perspectives*"

EKOTOKSIKOLOGIJA – interdisciplinarna znanost koja se bavi istraživanjem učinka nastalih kao posljedica prisutnosti prirodnih ili umjetno stvorenih toksičnih opasnih tvari na sve žive organizme tj. mikroorganizme, biljke, životinje, ljude i sve ostale sastavne dijelove ekosustava (ekologija), u cjelovitom kontekstu

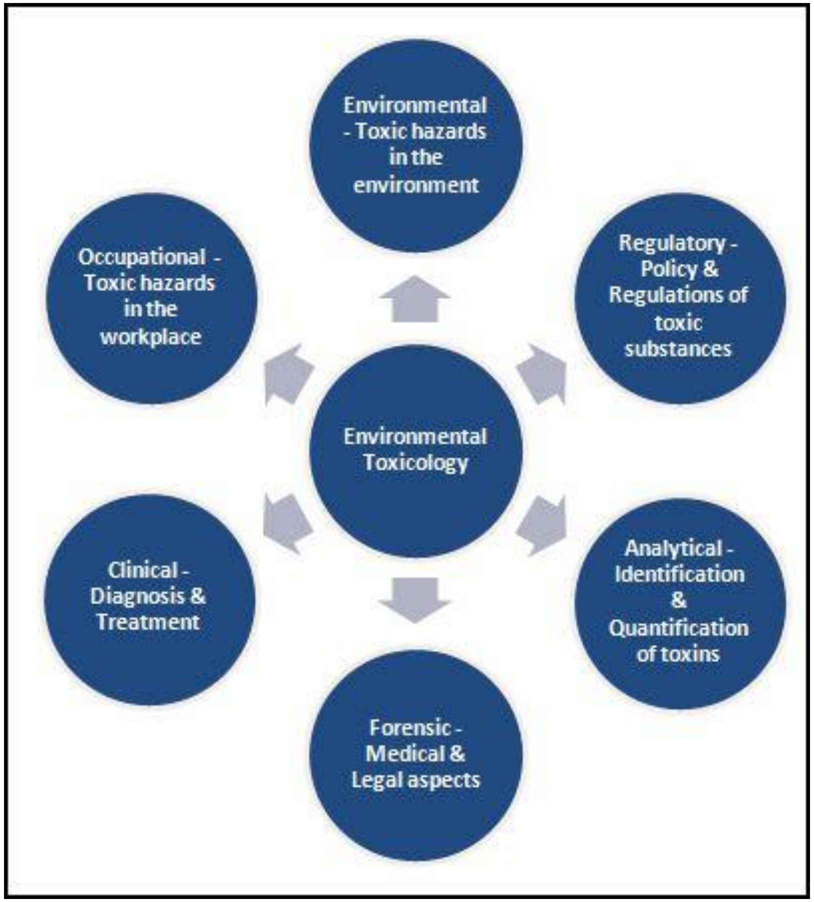
EKOTOKSIKOLOGIJA

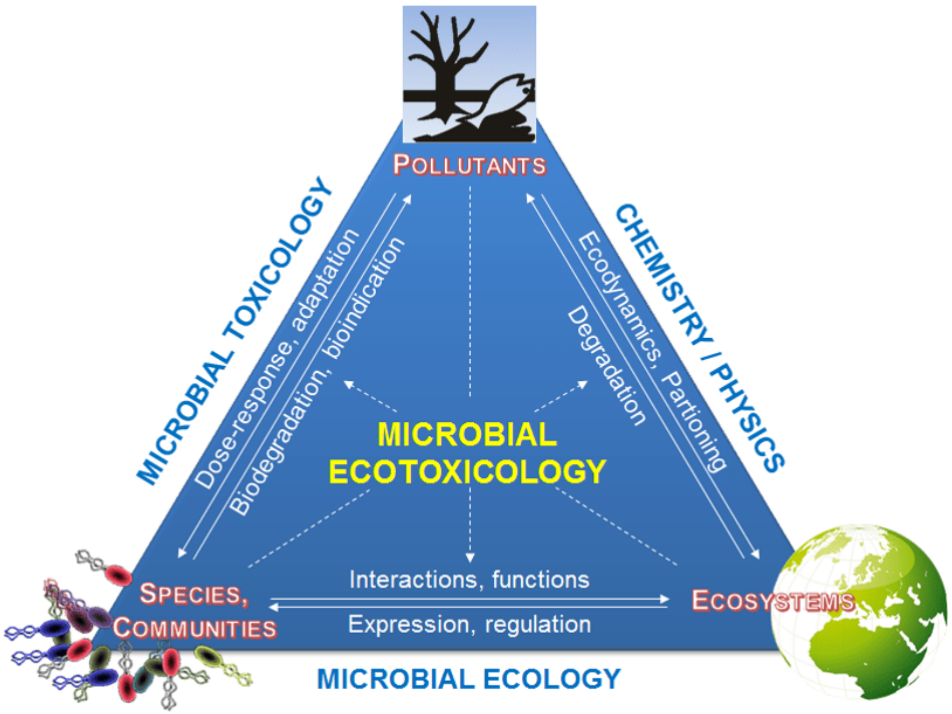
- Interdisciplinarna znanost:
  - Fiziologija
  - Ekologija
  - Toksikologija
  - Patofiziologija
  - Ekofiziologija





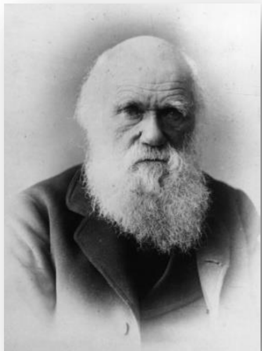
EKOTOKSIKOLOGIJA



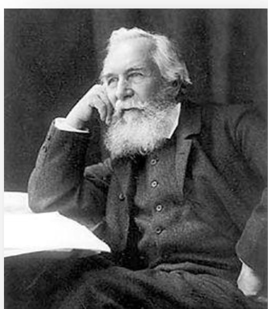


EKOLOGIJA

- grč. “**oikos**” = dom, kuća, nastamba + “**logos**” = znanje, govor



- **Charles Darwin** (1859.) – tvorac suvremene ekologije
- u svom djelu "Porijeklo vrsta" pod pojmom "borba za opstanak“ obuhvatio je sve stalne, uzajamne i promjenjive odnose živih organizama s ostalom živom i neživom prirodom



- **Haeckel** (1866.) – uvodi izraz ekologija
- **znanost o interakcijama između organizama i njihovog okoliša, organskog i anorganskog**

TOKSIKOLOGIJA

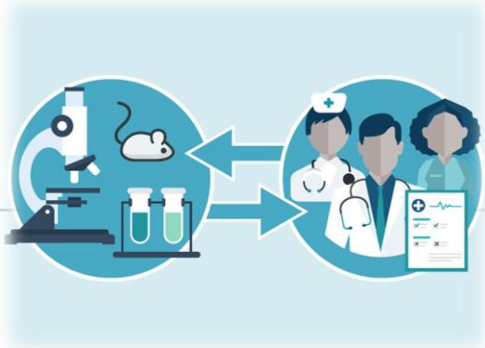
- prvi zapisi vezani uz toksikologiju potječu iz prahistorijskog vremena – različiti spojevi i njihove smjese korišteni kao tonici i otrovi (arsen, živa)
- Prvi istraživači toksikologije liječnici i alkemičari
- Švicarski liječnik Paracelsus (1493.-1541.) – doza-učinak- tvorac suvremene toksikologije

  "Sve su tvari otrovi i ništa nije bez otrova. Samo doza određuje da neka tvar nije otrov."

- 1962. pobuđena svijest javnosti o toksičnom djelovanju kemijskih spojeva na okoliš – Rachel Carson "**Silent Spring**" (nestanak ptica s dijelova rijeka kao posljedica djelovanja insekticida i pesticida)

TOKSIKOLOGIJA – znanost koja se bavi različitim oblicima štetnih / opasnih tvari na žive organizme ili određeni biološki sustav

TOKSIKOLOGIJA



- Najčešće se **bavi kemijskim tvarima** koje se koriste u :

  - medicini (dijagnostička, prevencijska, terapijska)

  - prehrambenoj industriji (izravni i neizravni dodatci hrani)

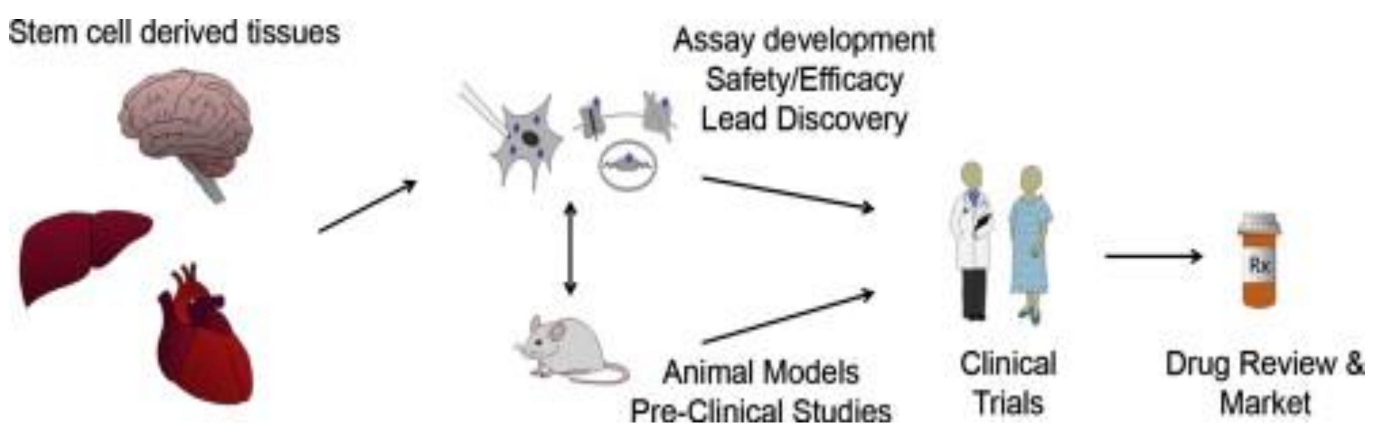
  - kemijskoj industriji

- **Podjela toksikologije**:

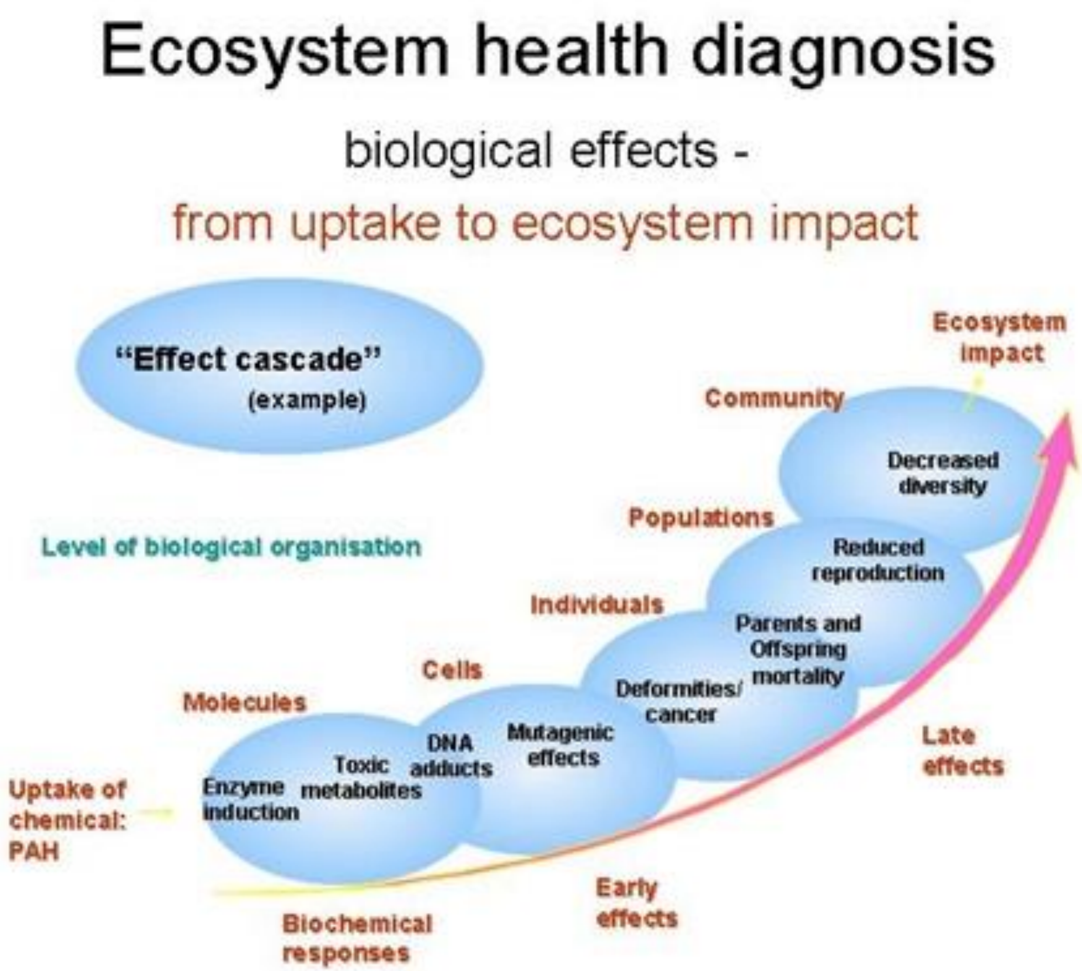
  (1) PRISTUPU (deskriptivna toksikologija, analitička i molekularna toksikologija)

  (2) PODRUČJU (forenzička, klinička i **ekotoksikologija**)

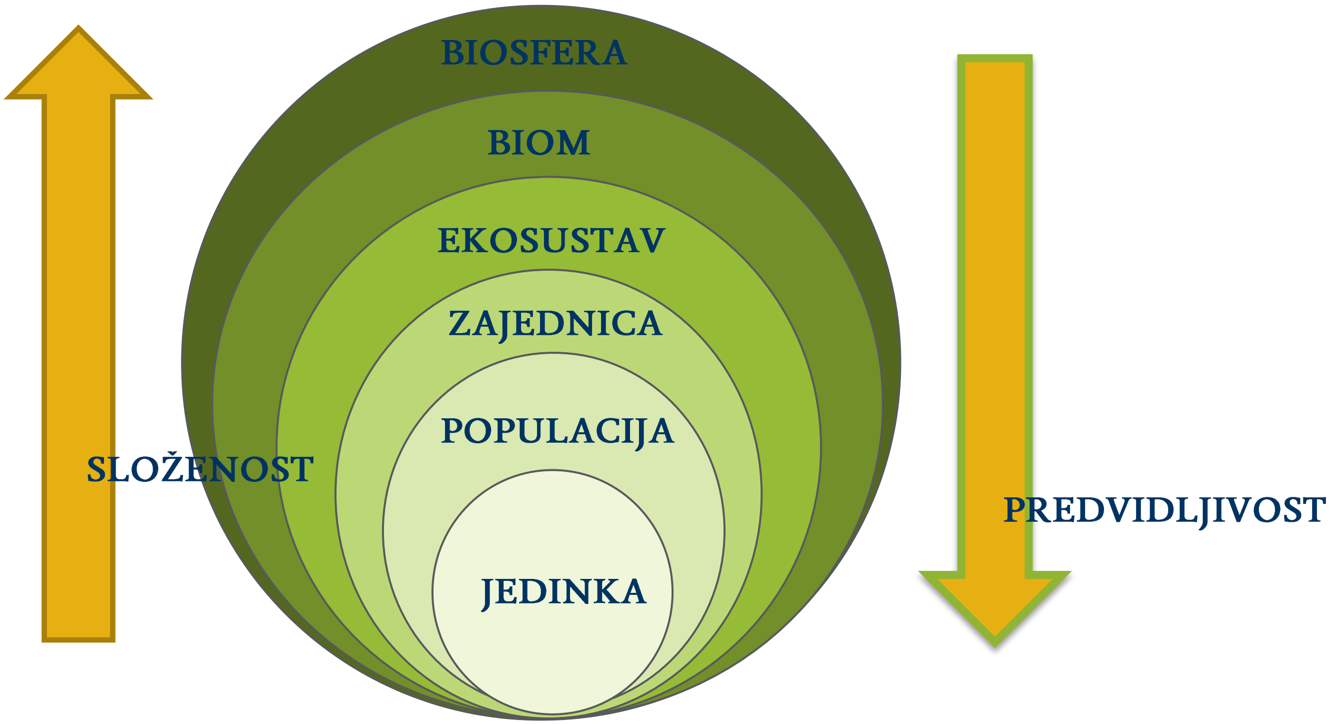
  (3) PROFESIJI (mehanistička, deskriptivna)



HIJERARHIJA U PRIRODI

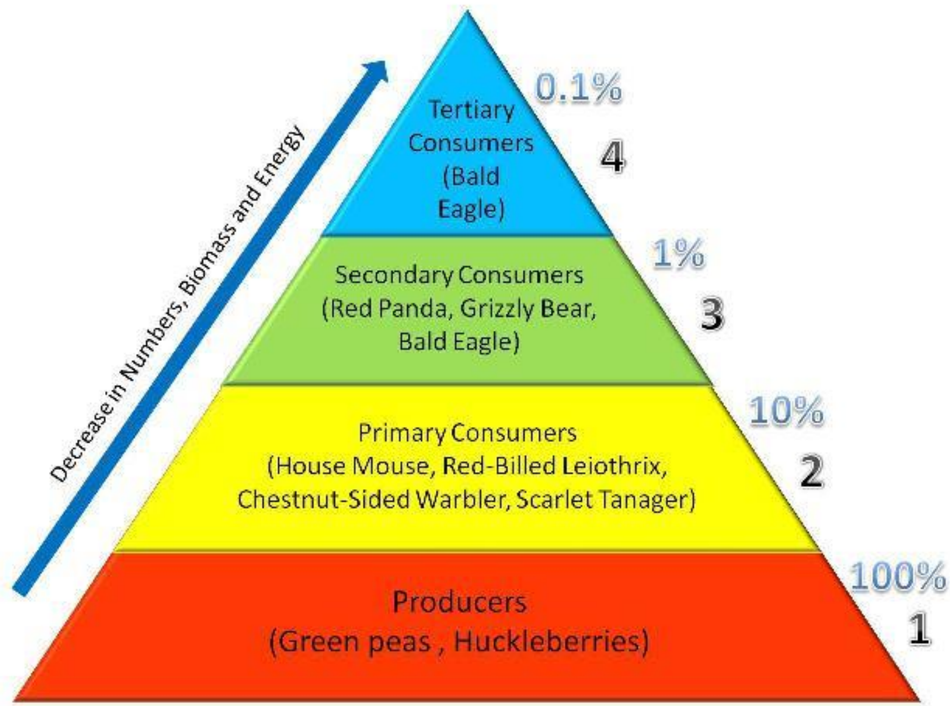


EKOLOŠKE ORGANIZACIJSKE JEDINICE (hijerarhija biotičkih sustava)

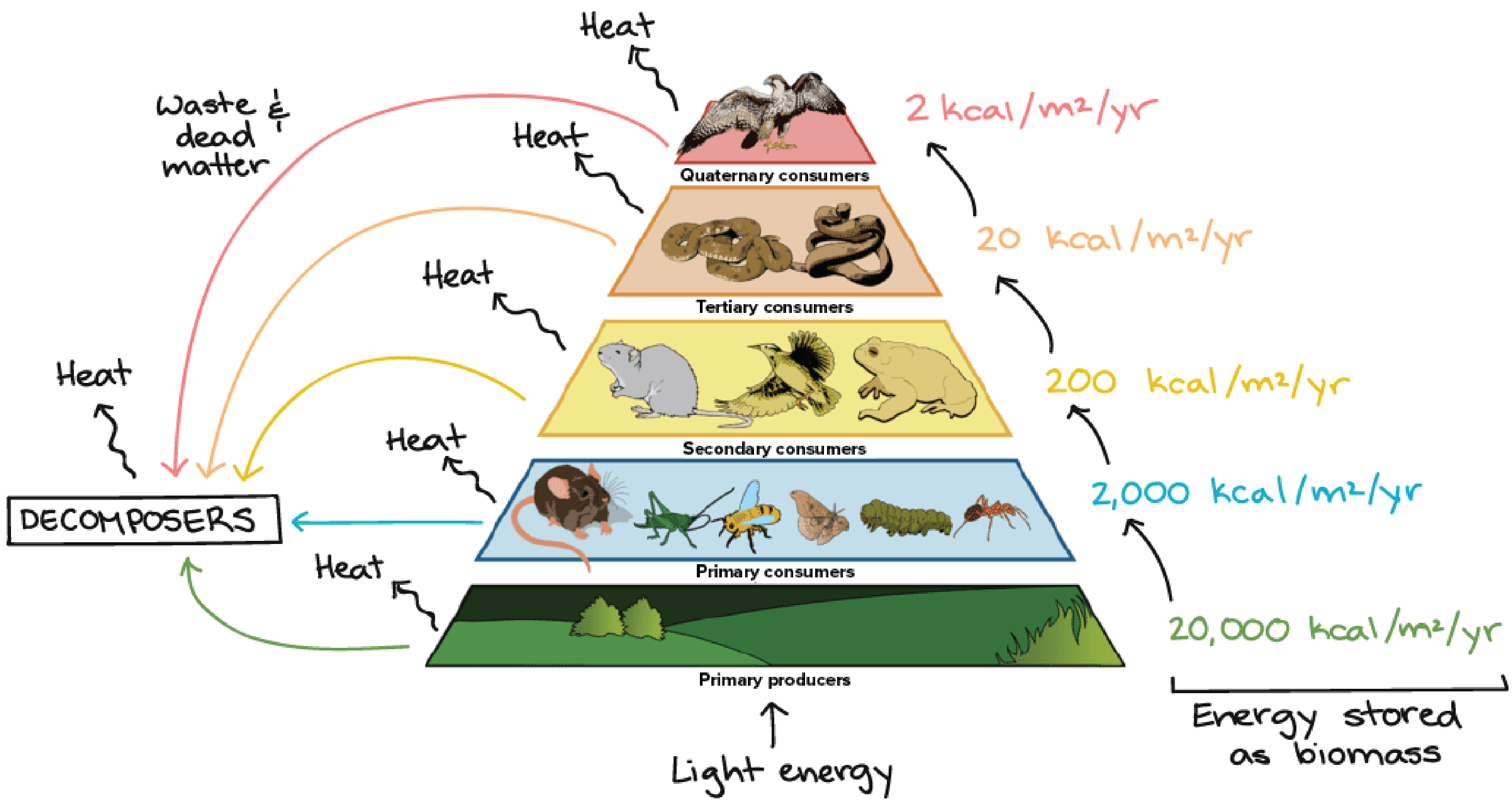


EKOLOŠKA PIRAMIDA

- EKOLOŠKA PIRAMIDA BROJEVA - duž hranidbenog lanca raste veličina tijela jer svaki predator jede plijen koji je nešto manji od njega. Sve veće životinje trebaju sve veći prostor da bi pronašle hranu, pa je njihov broj duž hranidbenog lanca sve manji
- PIRAMIDA ENERGETSKIH TRANSFORMACIJA - na svakoj višoj trofičkoj razini ima sve **manje** raspoložive energije
- PIRAMIDA BIOMASE



EKOLOŠKA PIRAMIDA



EKOLOŠKA PIRAMIDA

BIOMAGNIFIKACIJA

BIOAKUMULACIJA

BIORASPOLOŽIVOST

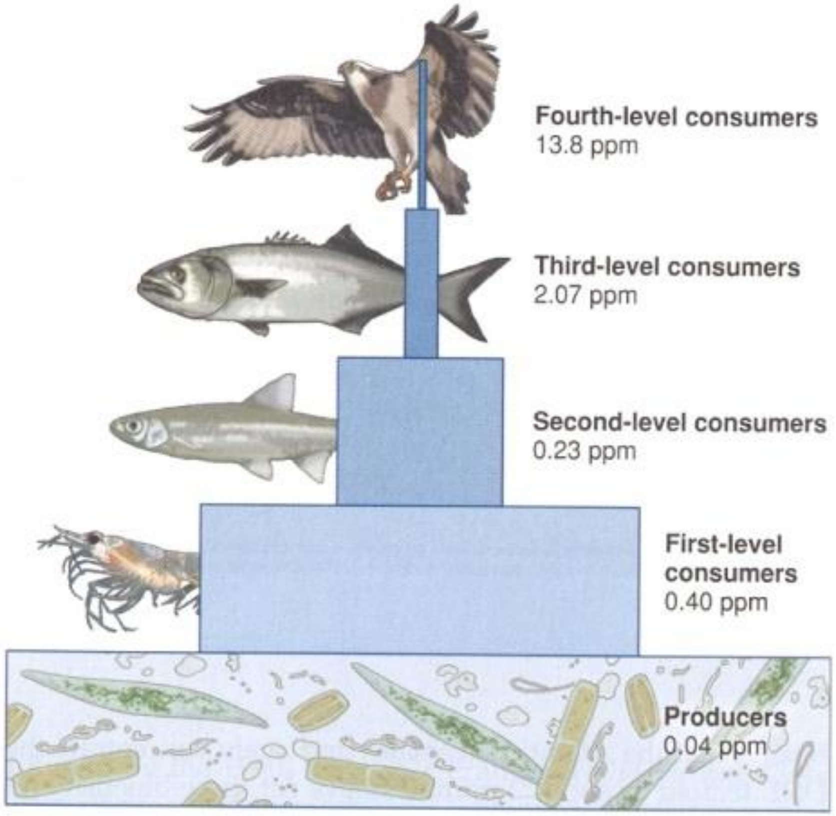
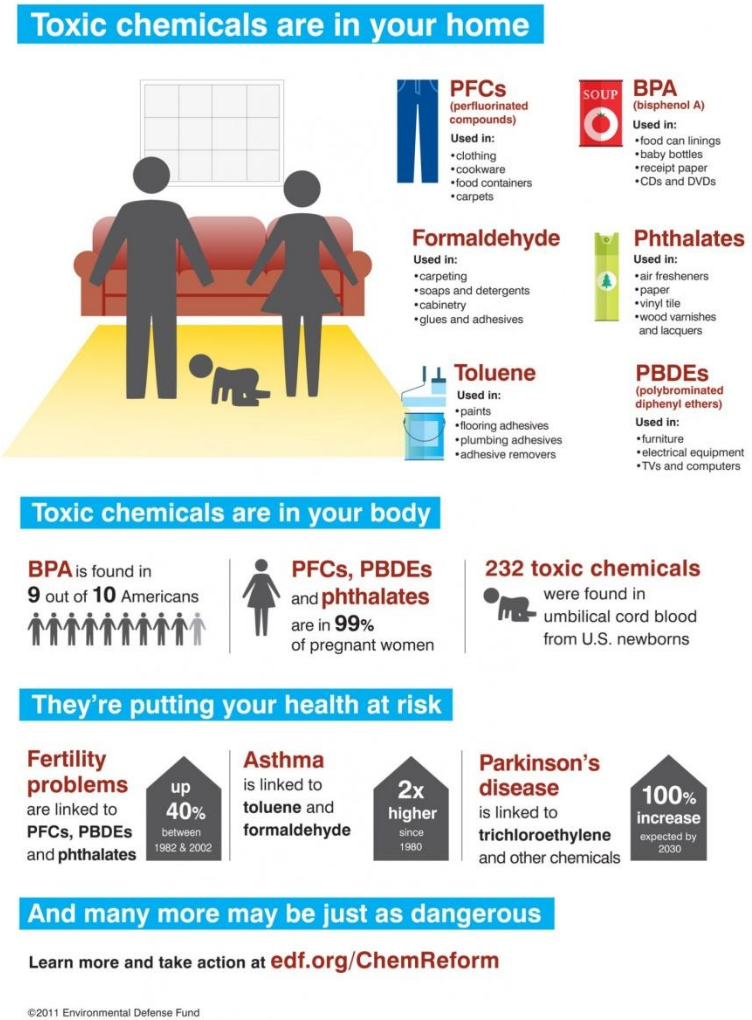


FIGURE 5.16 ***Biomagnification*** *The concentration of DDE increases from 0.04 ppm in primary producers to 13.8 ppm as it moves up the food chain. These large increases are possible because the concentration increases by about a factor of 10 at each step. For example, the concentration increases about ninefold (2.07 ppm/0.23 ppm) from silversides (second-level consumers) to bluefish (third-level consumers).*

ŠTETNI UČINCI KAO MJERILO OTROVNOSTI



ŠTETNI UČINCI KAO MJERILO OTROVNOSTI

VRSTE ŠTETNIH UČINAKA

- CLP Uredba ili Uredba (EZ) br. 1272/2008 – opasne tvari njihove smjese se razvrstavaju na temelju njihovih karakteristika opasnosti koje proizlaze iz njihovih svojstava opasnosti za zdravlje ljudi i svojstava opasnosti za okoliš
- Vrste / razredi opasnosti:

1. AKUTNA TOKSIČNOST
2. SUBKRONIČNA I KRONIČNA TOKSIČNOST
3. MUTAGENOST
4. KANCEROGENOST
5. GENOTOKSIČNOST
6. TETRATOGENOST
7. REPRODUKTIVNA TOKSIČNOST
8. EKOTOKSIČNOST
9. OSTALI ŠTETNI UČINCI (npr. utjecaj na endokrilne funkcije, reproduktivne organe)

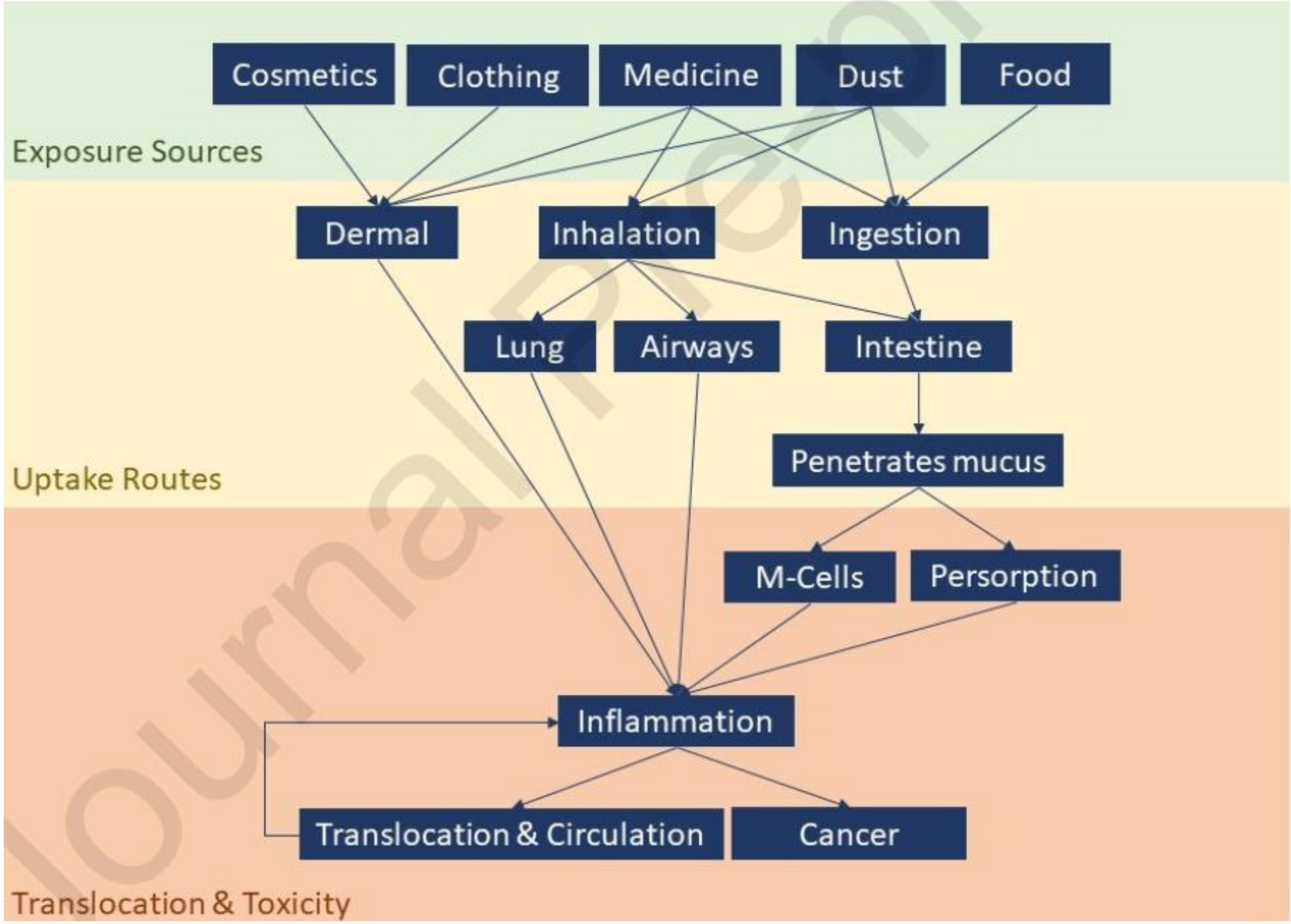
VRSTE ŠTETNIH UČINAKA

Table 11.3 Hazard Class and Categories, Pictogram, Signal Word, Hazard Statement Code, and Description of Selected Hazard Classes for GHS Environmental Hazards

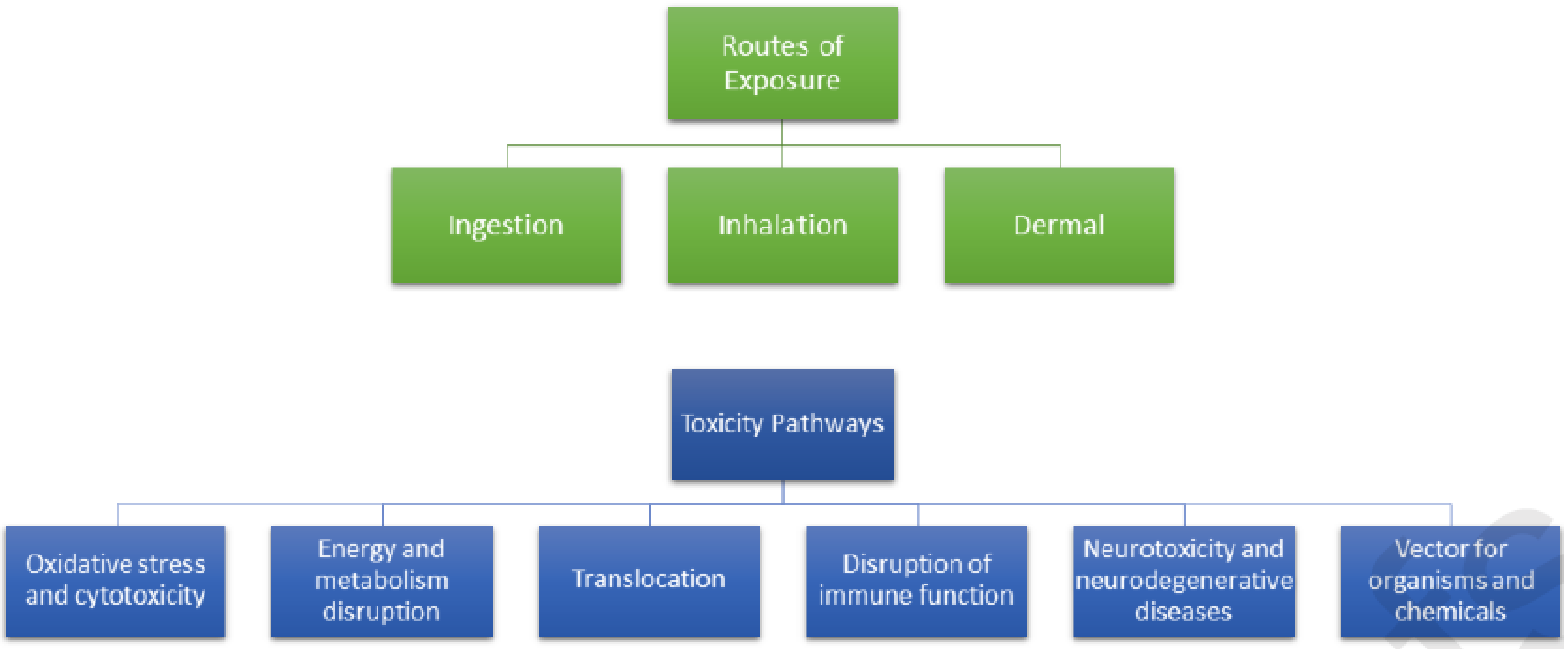
Hazard class	Hazard categories	Pictogram	Signal word	Hazard statement code	Description of hazard statement
Hazardous to aquatic environment short term (acute)	Acute 1		Warning	H400	Very toxic to aquatic life
Hazardous to aquatic environment long term (chronic)	Chronic 1		Warning	H410	Very toxic to aquatic life with long lasting effects
Hazardous to aquatic environment long term (chronic)	Chronic 2		None	H411	Toxic to aquatic life with long lasting effects

Modified from UN (2013).

ŠTETNI UČINCI - mikroplastika



ŠTETNI UČINCI - mikroplastika



AKUTNA TOKSIČNOST

- Obuhvaća jednokratni unos visoke doze štetne tvari u organizam i posljedice koje se pri tome pojavljuju
- Obuhvaća i višekratne doze dane u roku 24 sata ili četverosatne izloženosti udisanjem

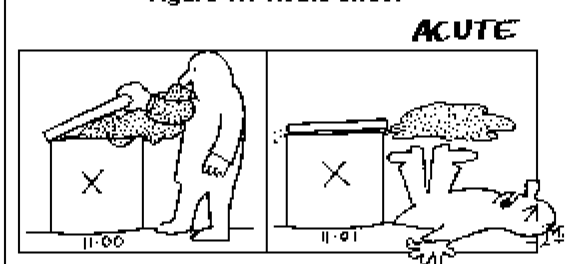
- **Akutna toksičnost** dijeli se na:

  (1) akutna oralna toksičnost

  (2) akutna dermalna toksičnost

  (3) akutna inhalacijska toksičnost

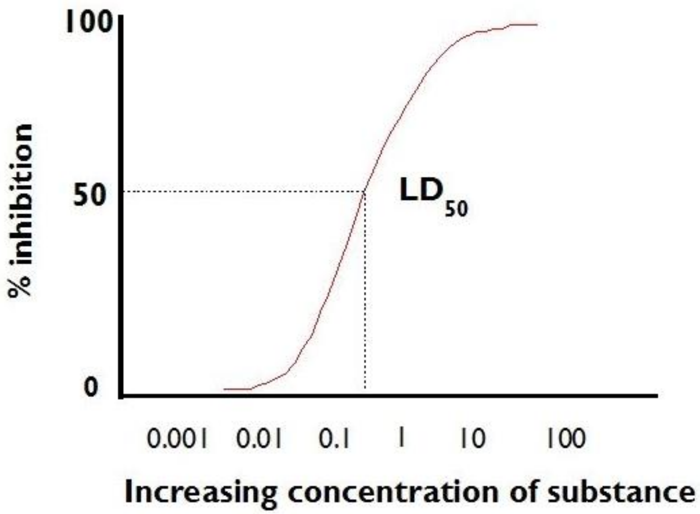
Figure 17. Acute effect

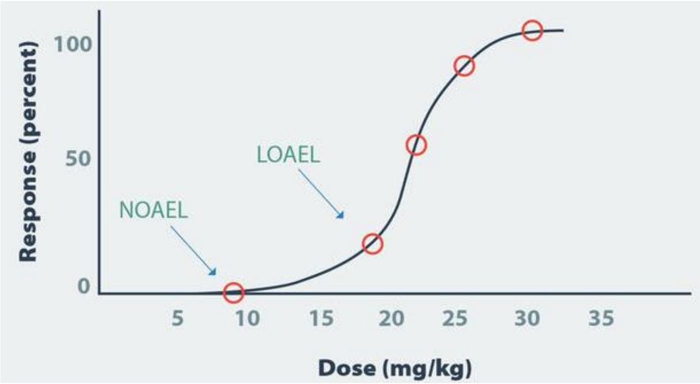


- Akutna trovanja - povezana s **nesrećama** (radno mjesto, katastrofe, kemijske nesreće), **namjernim uzimanjem** ili **ratnim djelovanjima** (uporaba kemijskog oružja)

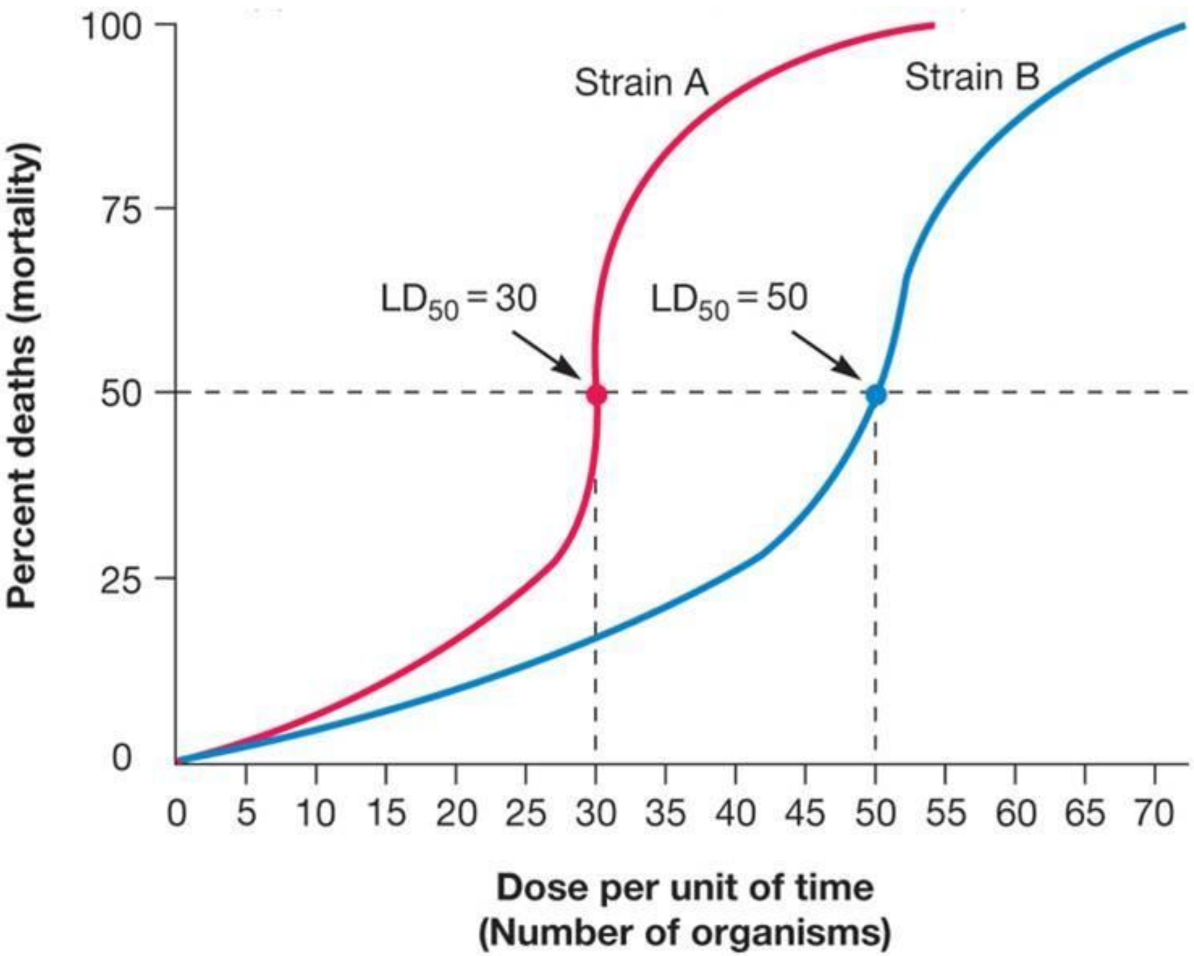
AKUTNA TOKSIČNOST

- Akutna toksičnost se izražava **dozom otrova** koja izaziva smrt kod 50 % organizama – **"lethal dose"** LD_{50} i najčešće se izražava u **mg/kg tjelesne težine**
- Ta doza se odnosi samo na **jednokratnu dozu unesenu tijekom 24 sata**, ili na **višekratnu dozu s unosom unutar 24 sata**, ako se istražuje slabo toksična ili djelomično toksična tvar
- Uz prikazivanje podataka za LD_{50} uvijek je potrebno navesti na koje se organizme ona odnosi i koji je put unosa opasne tvari u organizam
- EC_{50} – ("effect concentration") – koncentracija tvari koja rezultira 50 % štetnim učinkom na populaciju
- LOEC ("lowest observable effect concentration") - najniža koncentracija koja izaziva vidljivi štetni učinak
- NOEC ("no observable effect concentration") – vrijednost koncentracije tvari koja ne izaziva vidljiv štetni učinak





AKUTNA TOKSIČNOST



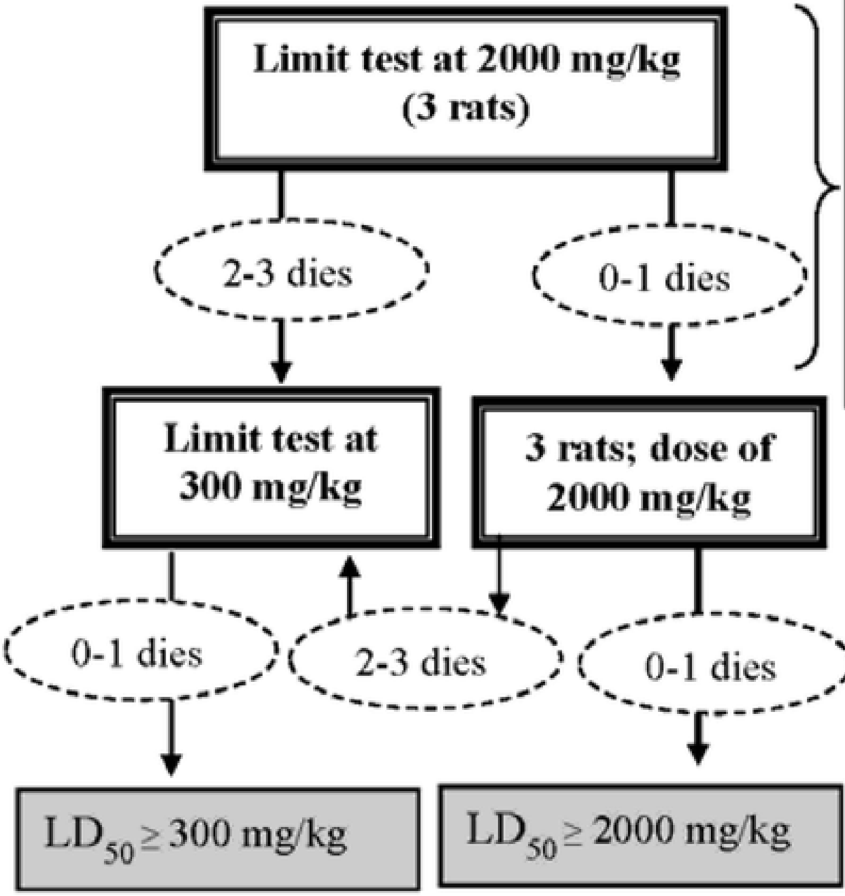
SER = MLD_A/MLD_B

(omjer selektivne toksičnosti)

MLD – srednja vrijednost letalne doze ili koncentracije

Ovaj omjer govori koliko je ista tvar štetnija ili manje štetna za vrstu A u odnosu na vrstu B

AKUTNA TOKSIČNOST



Step 1: 3 rats orally received limit dose of 2000 mg/kg.

- If 2-3 animals die, LD_{50} should be below 2000 mg/kg; hence, a further limit test will be carried out at 300 mg/kg.
- If none or only one animal dies (0-1), step 2 will be conducted.

Step 2: 3 rats will be treated with a limit dose of 2000 mg/kg.

- If 2-3 animal die, LD_{50} should be below 2000 mg/kg; hence, a further limit test will be carried out at 300 mg/kg.
- If none or only one animal dies, LD_{50} will be considered higher than 2000 mg/kg.

Klasifikacija toksičnosti prema WHO

Class	Description	LD_{50} for the Rat (mg/kg Body Weight)			
		Oral		Dermal	
		Solids	Liquids	Solids	Liquids
Ia	Extremely hazardous	≤5	≤20	≤10	≤40
Ib	Highly hazardous	5–50	20–200	10–100	40–400
II	Moderately hazardous	50–500	200–2,000	100–1,000	400–4,000
III	Slightly hazardous	>500	>2,000	>1,000	>4,000

doi:10.1371/journal.pmed.1000357.t001

AKUTNA TOKSIČNOST

Toxicity Classes

LD_{50} (rat,oral)	Correlation to Ingestion by 150 lb Adult Human	Toxicity
<1mg/kg	a taste to a drop	extremely
1-50 mg/kg	to a teaspoon	highly
50-500 mg/kg	to an ounce	moderately
500-5000 mg/kg	to a pint	slightly
5-15 g/kg	to a quart	practically non-toxic
Over 15g/kg	more than 1 quart	relatively harmless

Chapter 8 10

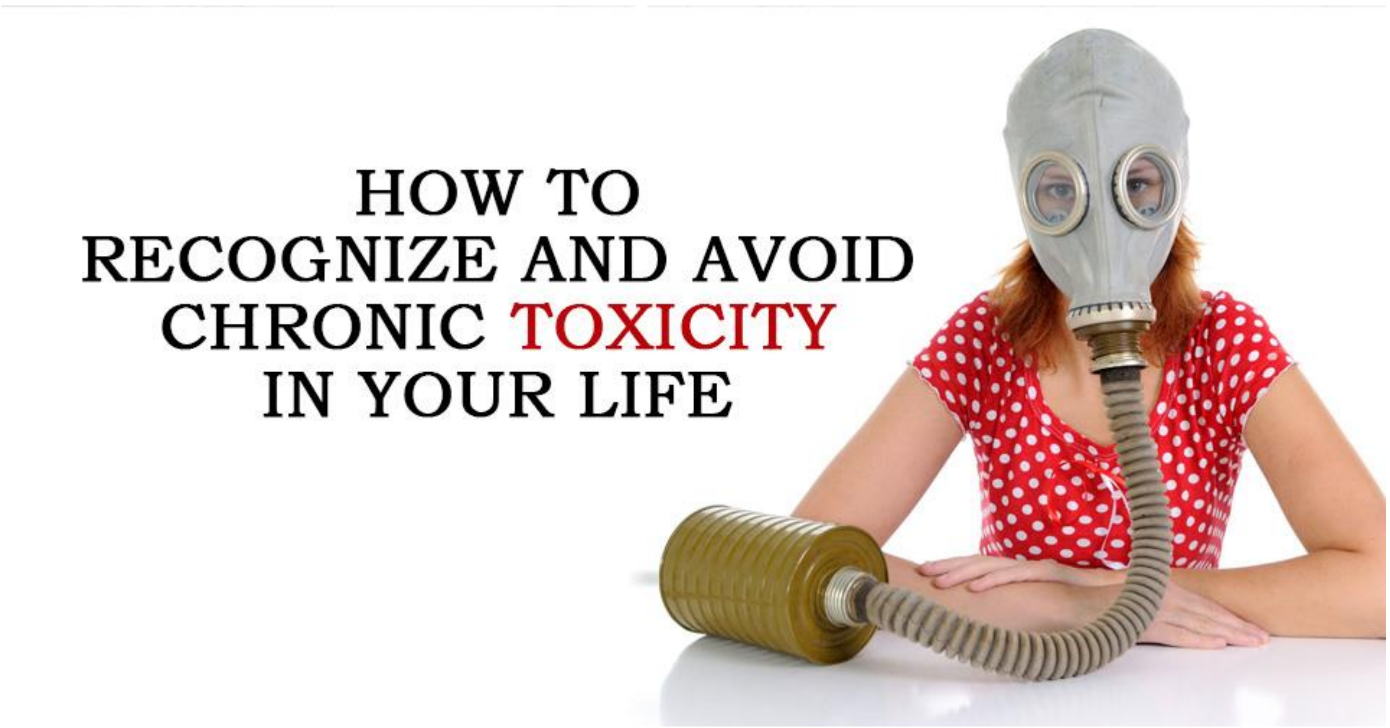
AKUTNA TOKSIČNOST

Table 1. LD_{50} value of atrazine on various test species

Type	mode	Species	Amount	Units
LD_{50}	oral	Rat	672	$mg\ kg^{-1}$
LD_{50}	intraperitoneal	Rat	235	$mg\ kg^{-1}$
LD_{50}	oral	Mouse	850	$mg\ kg^{-1}$
LD_{50}	oral	Rabbit	750	$mg\ kg^{-1}$
LD_{50}	skin	Rabbit	7500	$mg\ kg^{-1}$
LD_{50}	oral	Humane	1000	$mg\ kg^{-1}$
LD_{50}	inhalation	Rat	5200	$mg\ m^{-3}\ 4hr^{-1}$
LD_{50}	intraperitoneal	Mouse	626	$mg\ kg^{-1}$

- Vrijednosti akutne toksičnosti izražavaju se kao (**približne**) vrijednosti LD_{50} (oralno, dermalno) ili LC_{50} (udisanje) ili kao **procijenjene** vrijednosti akutne toksičnosti ATE (“Acute Toxicity Esitamtes”)

SUBKORNIČNA I KRONIČNA TOKSIČNOST



SUBKORNIČNA I KRONIČNA TOKSIČNOST

- Svojstvo štetne tvari koja se javlja tijekom dužeg unosa otrova u organizam uz više ili manje redovitu učestalost unosa i uz različite doze
- Doze znatno manje nego pri akutnom unosu
- Pojedinačna doza – mala, ne izaziva nikakve učinke na organizam, ali može umanjiti sposobnost jedinke da izbjegne predatora, pronađe hranu ili se razmnožava
- Subkroničnim i kroničnim unosom – povećava se razina stresa i onemogućava optimalno funkcioniranje jedinke
- Ispitivanja se provode na **životinjama**, ali i epidemiološkim istraživanjima na **skupinama ljudi** koji su na radnom mjestu ili u okolišu dugotrajno izloženi otrovu
- Štetni učinci na središnji i periferni živčani sustav, srce, pluća, probavni sustav, bubreg, jetra

EKOTOKSIČNOST

- Štetni učinak kemijskih tvari na živa bića i okoliš
- Kemikalije opasne za okoliš jesu tvari i pripravci koji zbog svojih svojstava, količine i unošenja u okoliš mogu biti štetni za zdravlje ljudi, biljni i životinjski svijet, odnosno biološku i krajobraznu raznolikost
- Svaki poremećaj u malom dijelu okoliša ima odraz na čitav ekosustav
- Primjer: pesticid DDT – široka primjena nakon II. Svjetskog rata – ekosustav onečišćen – nalazio se i u masnom tkivu Eskima koji ga nisu koristili, izumiranje ptica, (ljuske jajeta meka, razbijale pod težinom ptica), smanjenje ptica – porast broja kukaca
- Štetne tvari u okolišu s obzirom na ekotoskičnost – primarne i sekundarne
- Primarne – izazivaju neposrednu štetu (otrovi), a sekundarne – štetne učinke koji su posljedica kemijskih promjena nastalih u okolišu

EKOTOKSIČNOST

USGS
science for a changing world

Prepared by the USGS National Wildlife Health Center

Lead Poisoning in Wild Birds

Introduction

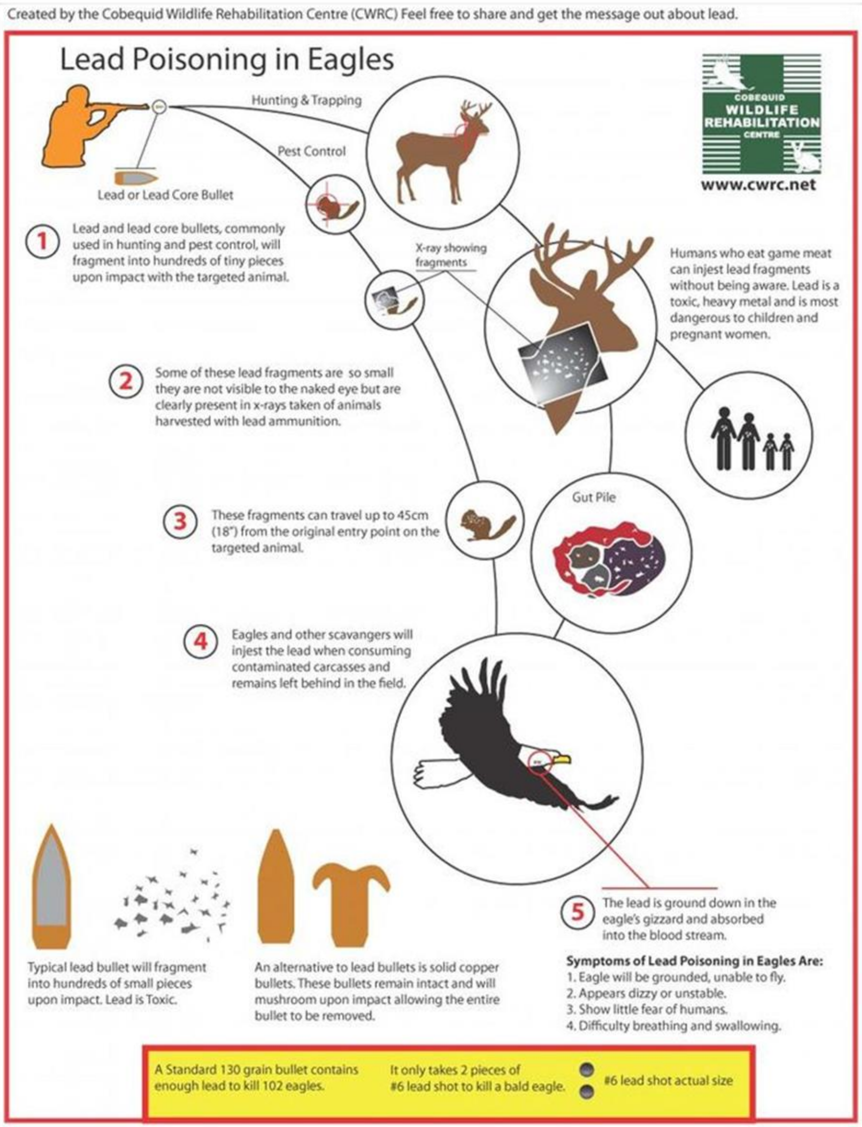
Lead in its various forms has been used for thousands of years, originally in cooking utensils and glazes and more recently in many industrial and commercial applications. However, lead is a potent, potentially deadly toxin that damages many organs in the body and can affect all animals, including humans. By the mid 1990s, lead had been removed from many products in the United States, such as paint and fuel, but it is still commonly used in ammunition for hunting upland game birds, small mammals, and large game animals, as well as in fishing tackle. Wild birds, such as mourning doves, bald eagles, California condors, and loons, can die from the ingestion of one lead shot, bullet fragment, or sinker. According to a recent study on loon mortality, nearly half of adult loons found sick or dead during the breeding season in New England were diagnosed with confirmed or suspected lead poisoning from ingestion of lead fishing weights. Recent regulations in some states have restricted the use of lead ammunition on certain upland game hunting areas, as well as lead fishing tackle in areas

Radiograph showing scattered lead fragments from a lead rifle bullet in the thoracic region of a mule deer (The Peregrine Fund).

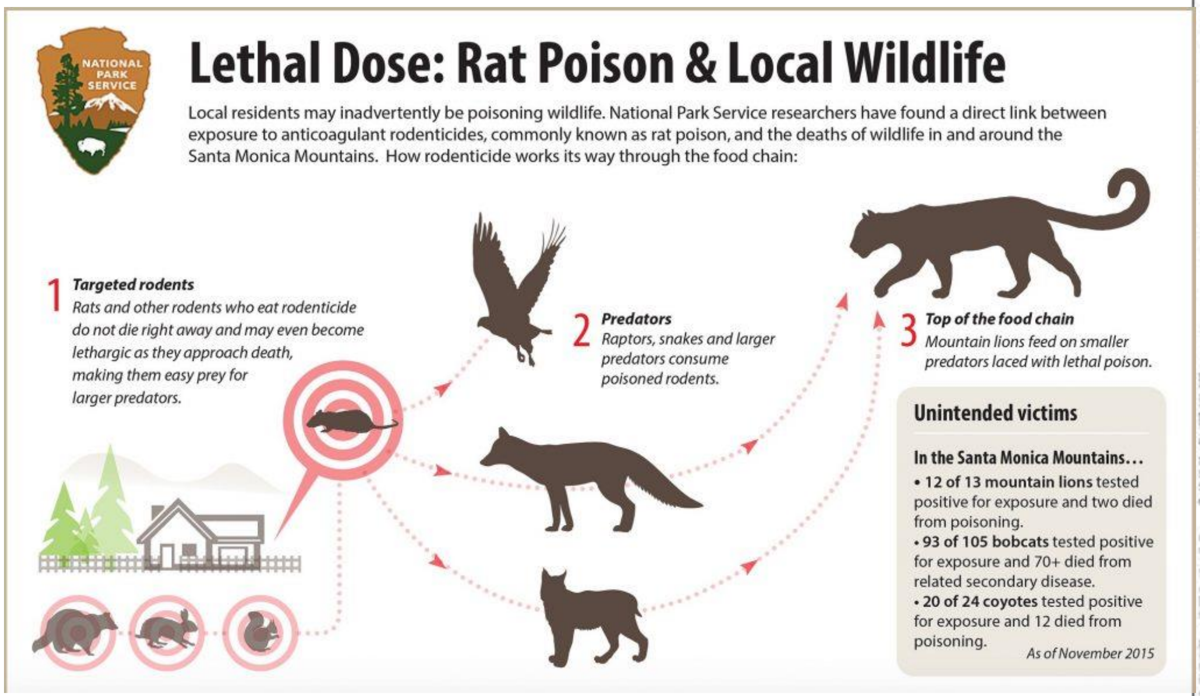
Lead Ammunition Poisoning of Avian Predators and Scavengers

Lead ingestion and poisoning from ammunition sources has been

EKOTOKSIČNOST



EKOTOKSIČNOST



Bigger beasts are ingesting rodent poison, scientists say

Toxin found in two dead mountain lions in Simi

Anticoagulant appears to move up food chain

By Stephanie Hoops
shoops@VenturaCountyStar.com

The National Park Service has confirmed that rodent poisons are killing area mountain lions.

Two of the cats were found dead in the Simi Hills before Christmas, and lab tests have come back confirming they both died of anticoagulant poisoning from ingesting bromadiolone and brodifacoum, active ingredients in household rat and mice poisons.

"We know both the lions that died had killed coyotes in the month before they died."
Seth Riley, National Park Service wildlife ecologist

The mountain lions were a female named P4 and a male named P3. They died in the Simi Valley hills having suffered from internal bleeding.

In the past year, other animals in the local mountains also have shown signs of anticoagulant poisoning. In April, six bobcats turned up dead with the poison in their systems. The poison couldn't be pinpointed as the cause, however, because there was an interaction among the poison, mange and a weakened immune system.

With the mountain lions, it's a different story. The poison has been identified as the sole cause of death. Necropsies showed that even though the lions also had mange, it was not enough to kill them.

"At this point," said Ray Sauvajot, chief ecologist for the Santa Monica Mountains National Recreation Area, "it is a more serious problem than we'd ever anticipated and more widespread in that it's affecting more than just bobcats and coyotes. I guess ultimately this is something that hasn't been reported before, and we need to make sure people understand the implications of using these poisons."

Officials at the National Park Service do not know for sure but suspect the lions ingested the poison by eating coyotes that had themselves eaten poisoned rats or mice. In other words, the poisons may be working their way from back yards to the food chain.

The National Park Service has not identified the users of the chemicals that killed P3 and P4.

See POISON on B3

Poisoned wildlife

The National Parks Service has found two mountain lions in the Simi Hills that died of anticoagulant poisoning from ingesting bromadiolone and brodifacoum, active ingredients in household rat and mice poisons.

Anti-coagulant rodenticides
Rodent poisons using anti-coagulants kill by preventing normal blood clotting and results in fatal hemorrhaging.

Exposure symptoms
- Nosebleeds
- Bleeding gums
- Blood in urine and feces
- Bruises because of ruptured blood vessels
- Skin damage

Poison food chain

Rats and vermin eat poison. Coyotes eat the rats. Mountain lions eat the coyotes.

Mountain lion
Also known as: Puma, cougar.
Range: North and South America.
Size: Average 6-8 feet.
Weight: 100-150 pounds.
Life span: 10-15 years.

Mountain lion area

TESTOVI EKOTOKSIČNOSTI

TESTOVI EKOTOKSIČNOSTI

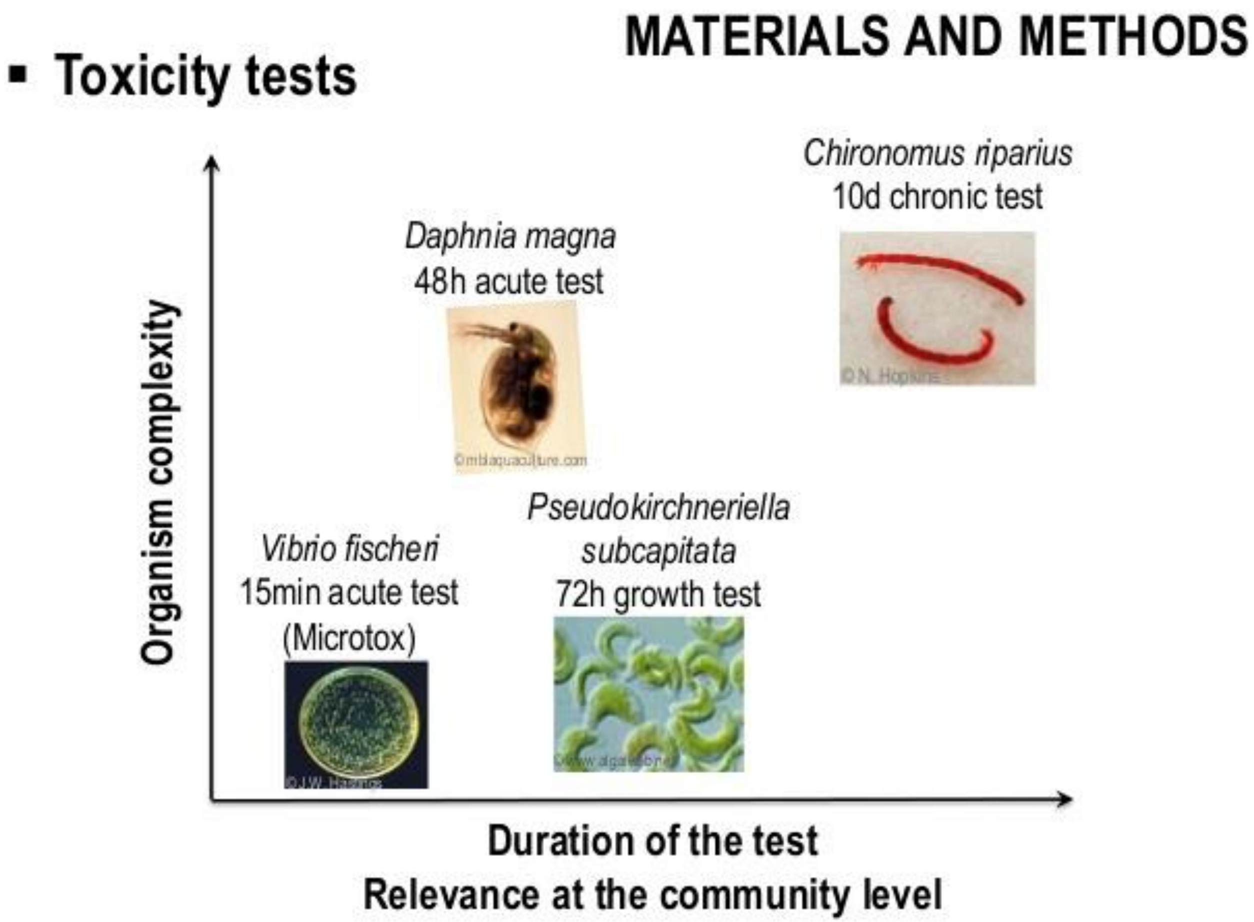
Table 1. OCDE standardized tests for the determination of the toxicity of chemical substances to soil and aquatic organisms

Taxonomic group	Title	Endpoint	Measurement variables	Assay time (days)	Test Number
Terrestrial invertebrates	Earthworm, acute toxicity test	Survival	Number of living worms	14	207
Terrestrial invertebrates	Enchytraeid, reproduction test	Reproduction	Number of juvenile worms	42	220
Terrestrial invertebrates	Earthworm reproduction test	Reproduction	Number of living offspring and cocoon numbers	56	222
Plants	Terrestrial plant test: seedling emergence and seedling growth test	Emergence of seedlings and Inhibition of growth	Emergence, dry shoot weight (fresh weight), shoot weight and assessment of visible detrimental effects	14-21	208
Plants	Terrestrial plants test: vegetative vigor test	Vegetative vigor and growth	Biomass (dry shoot weight) and visible detrimental effects	21-28	227
Microorganisms	Soil microorganisms: Nitrogen transformation tests	Nitrogen transformation	Rate of nitrate production	28	216
Microorganisms	Soil microorganisms: Carbon transformation tests	Carbon transformation	Glucose-induced respiration rates	28	217
Algae Cyanobacteria	Freshwater Alga and Cyanobacteria, Growth inhibition test	Inhibition of growth	Algal biomass: cell counts, cell volume, fluorescence, optical density, etc.	4	201
Aquatic Invertebrates	Daphnia s.p., Acute immobilization test	Survival	Immobilization	1	202
Aquatic Invertebrates	Daphnia magna, reproduction test	Reproduction	Number of living offspring	21	211
Fish	Fish, Acute toxicity test	Survival	Number of living fish	4	203
Fish	Fish, Prolonged toxicity test: 14-day study	Survival, appearance and behavior, and growth	Survival, abnormalities (appearance and behavior), length and weight	14	204

Table 1. (Continued)

Taxonomic group	Title	Endpoint	Measurement variables	Assay time (days)	Test Number
Fish	Fish, early-life stage toxicity test	Survival, appearance and behavior, and growth	Hatching and survival (at different stages), abnormalities (appearance and behavior), length and weight	30-60	210
Fish	Fish, short-term toxicity test on embryo and sac-fry stages	Survival, appearance and behavior, and growth	Hatching and survival (at different stages), abnormalities (appearance and behavior), length and weight	8-55	212
Fish	Fish, juvenile growth test	Inhibition of growth	Weight	≥ 28	215
Aquatic plants	Lemna, sp. Growth inhibition test	Inhibition of growth	Frond number, total frond area, dry weight or fresh weight	7-10	221

EKOTOKSIČNOST



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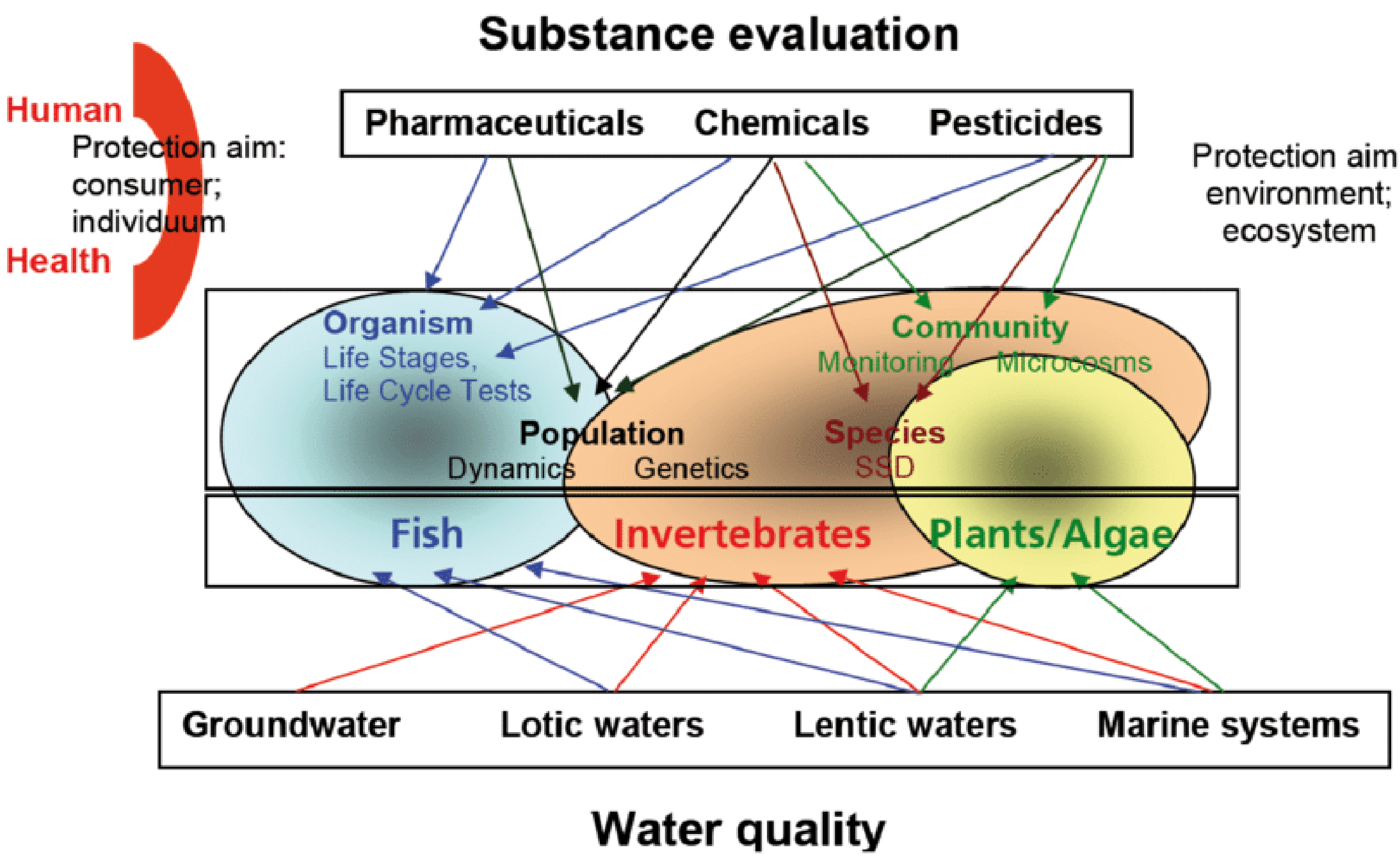

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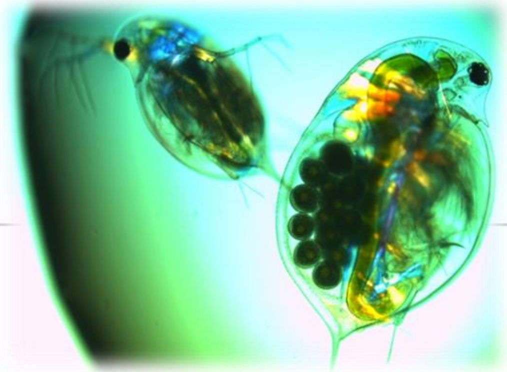

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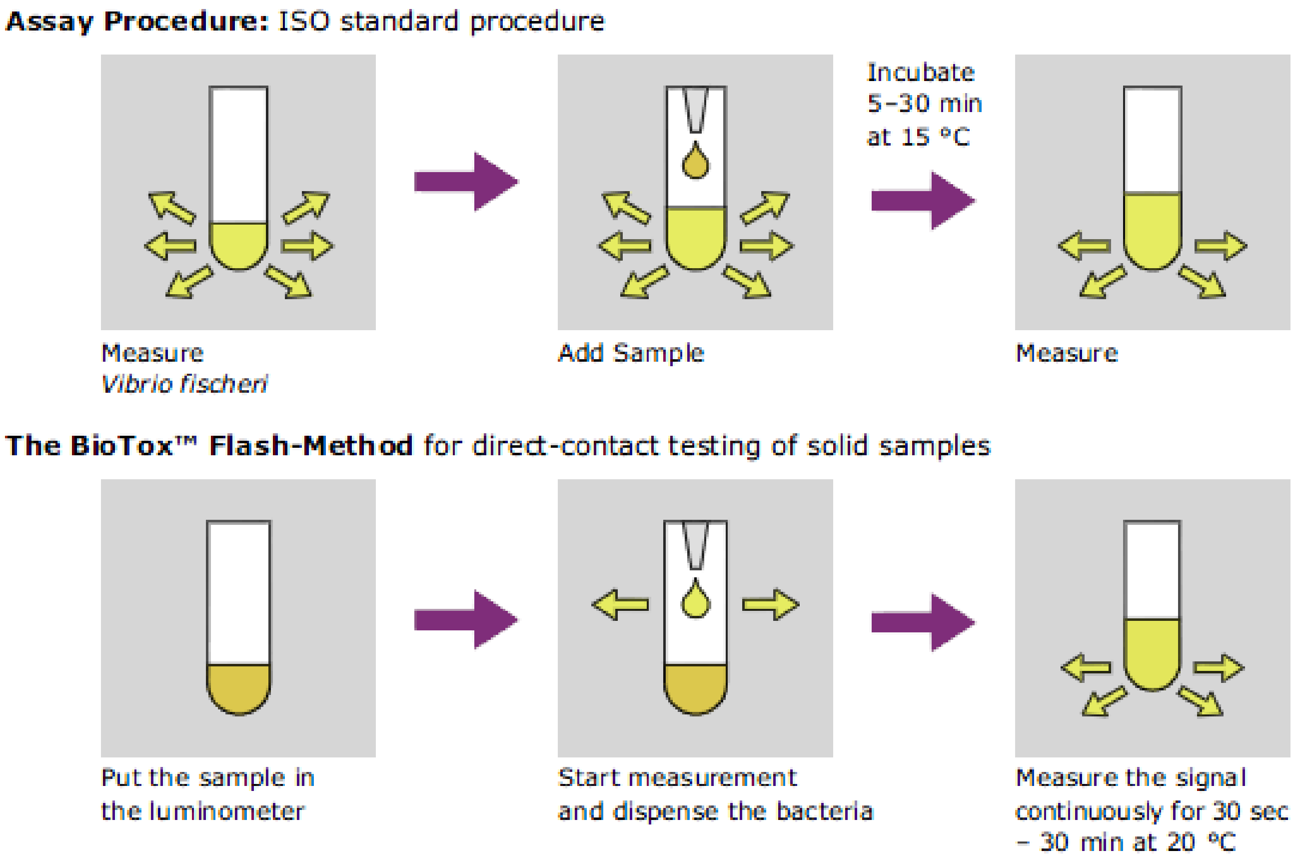

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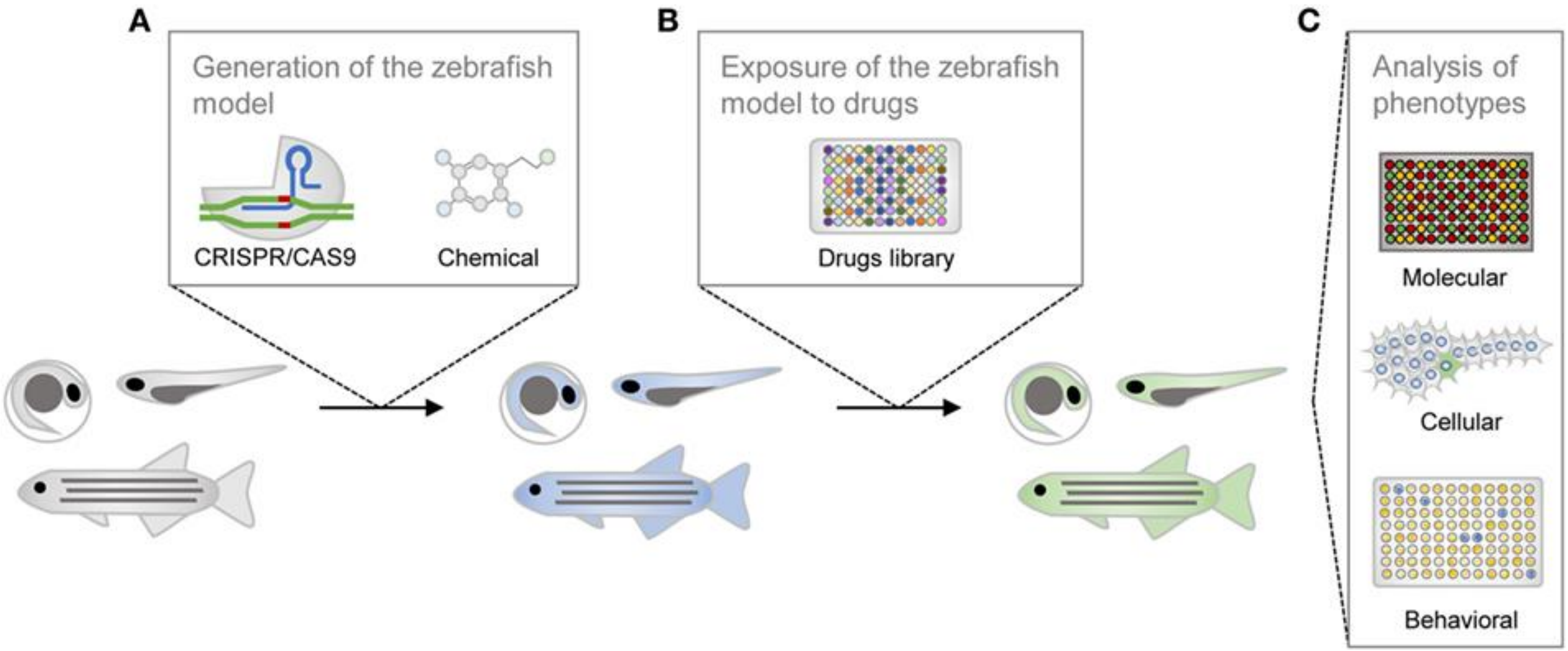

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Fig. 2: **Scheme of the zebrafish embryo acute toxicity test procedure (from left to right)**: production of eggs, collection of the eggs, pre-exposure immediately after fertilisation in glass vessels, selection of fertilised eggs with an inverted microscope or binocular and distribution of fertilised eggs into 24-well plates prepared with the respective test concentrations/controls, n = number of eggs required per test concentration/control (here 20), hpf = hours post-fertilisation.

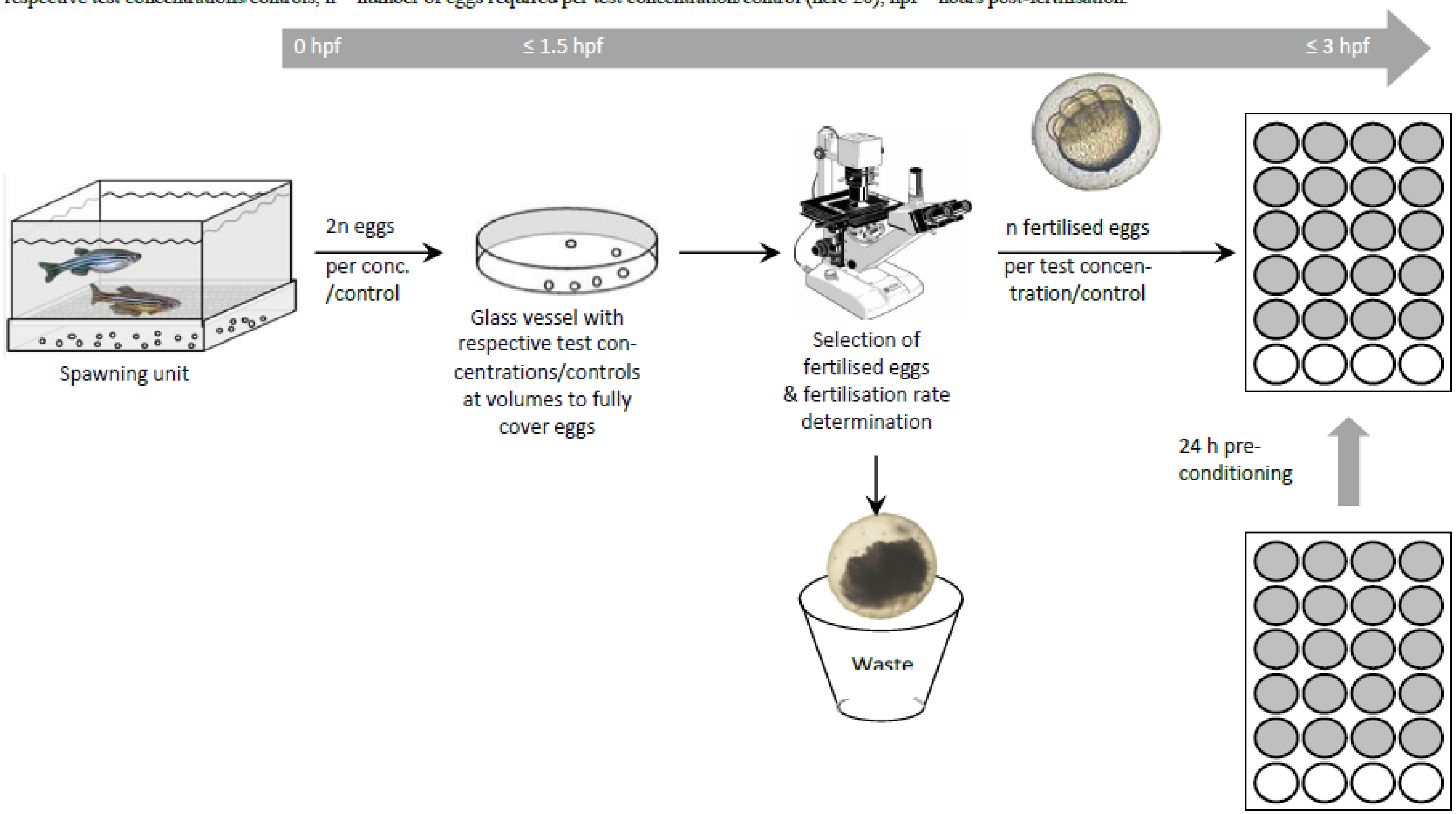

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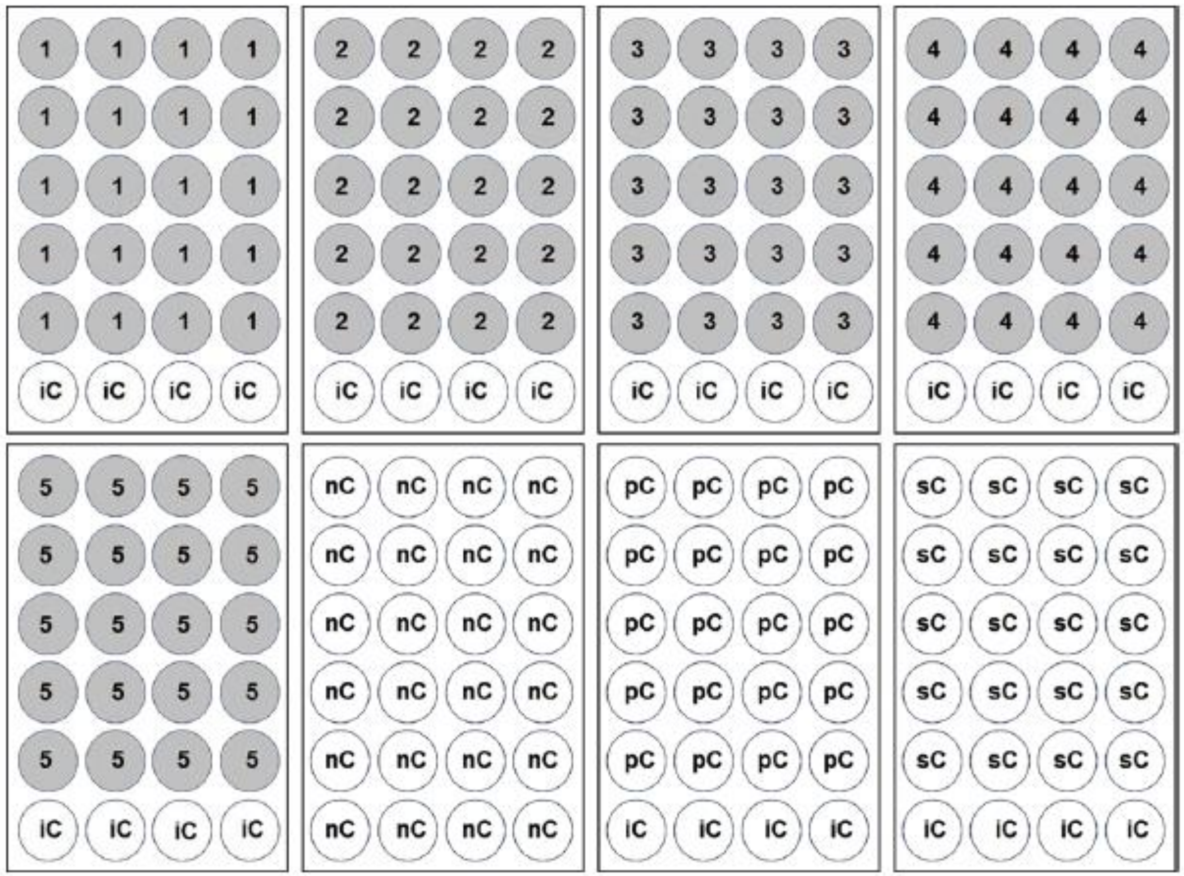
Fig. 1: **Layout of 24-well plates**



1-5 = five test concentrations / chemical; nC = negative control (dilution water); iC = internal plate control (dilution water); pC = positive control (3,4-DCA 4mg/L); sC = solvent control

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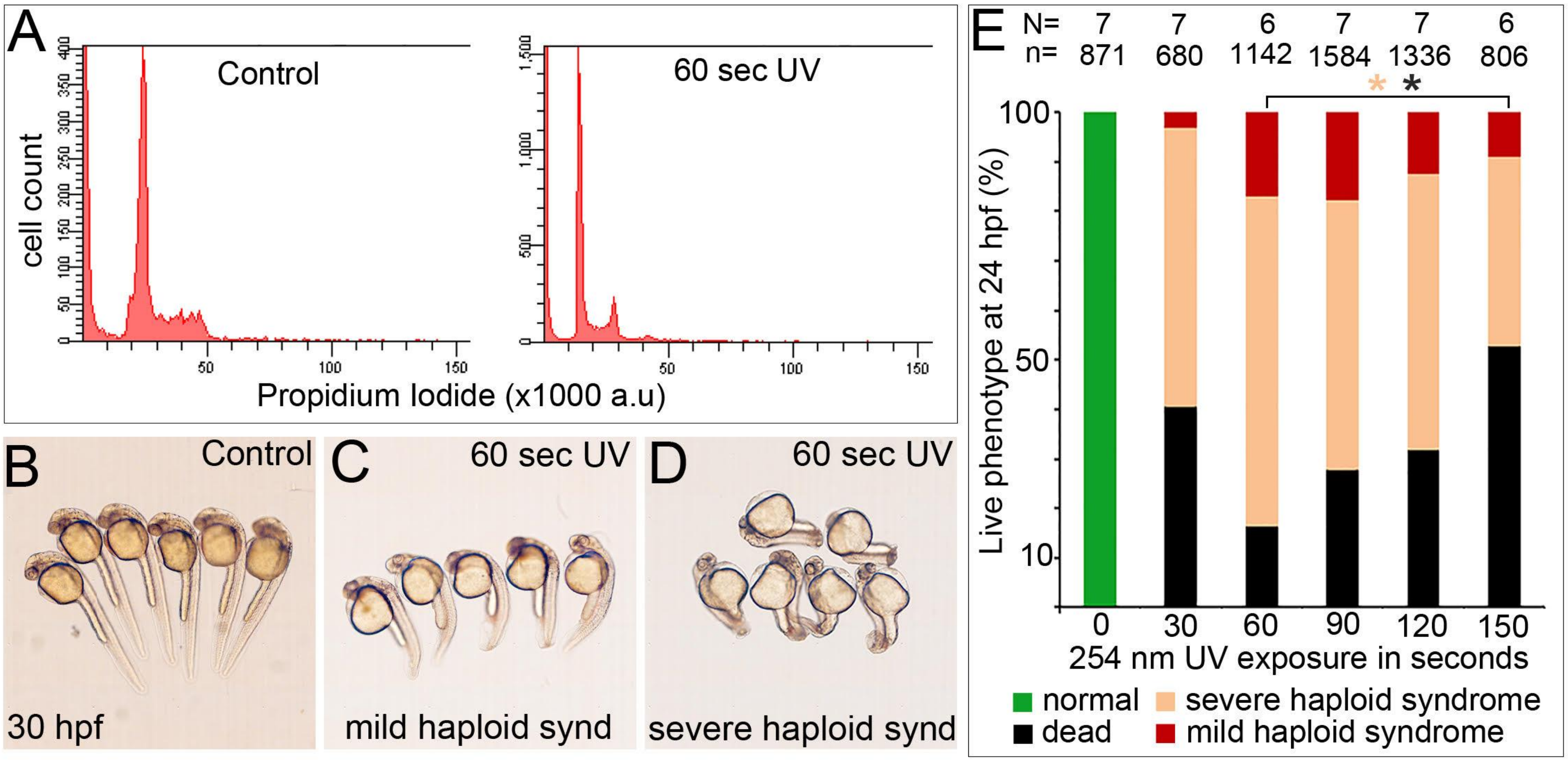
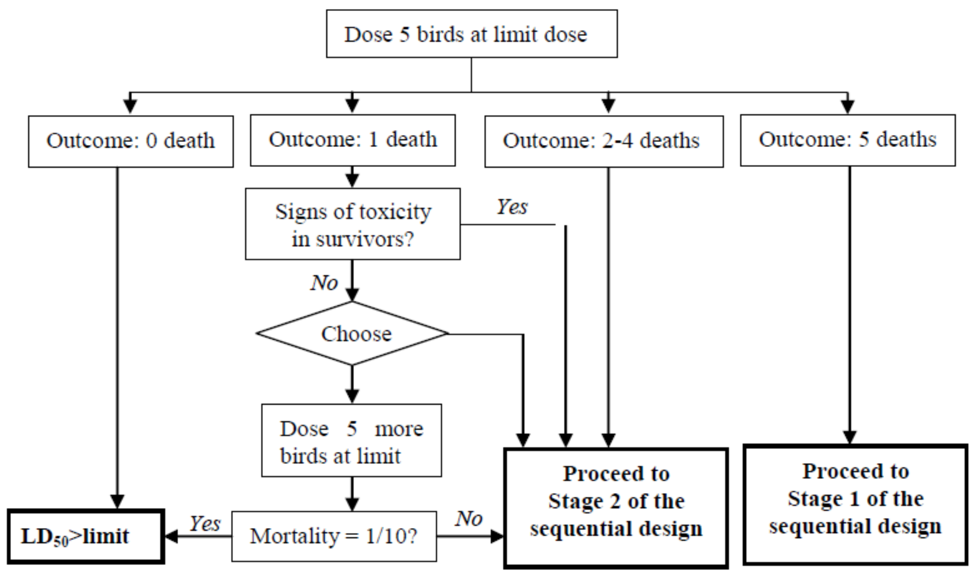


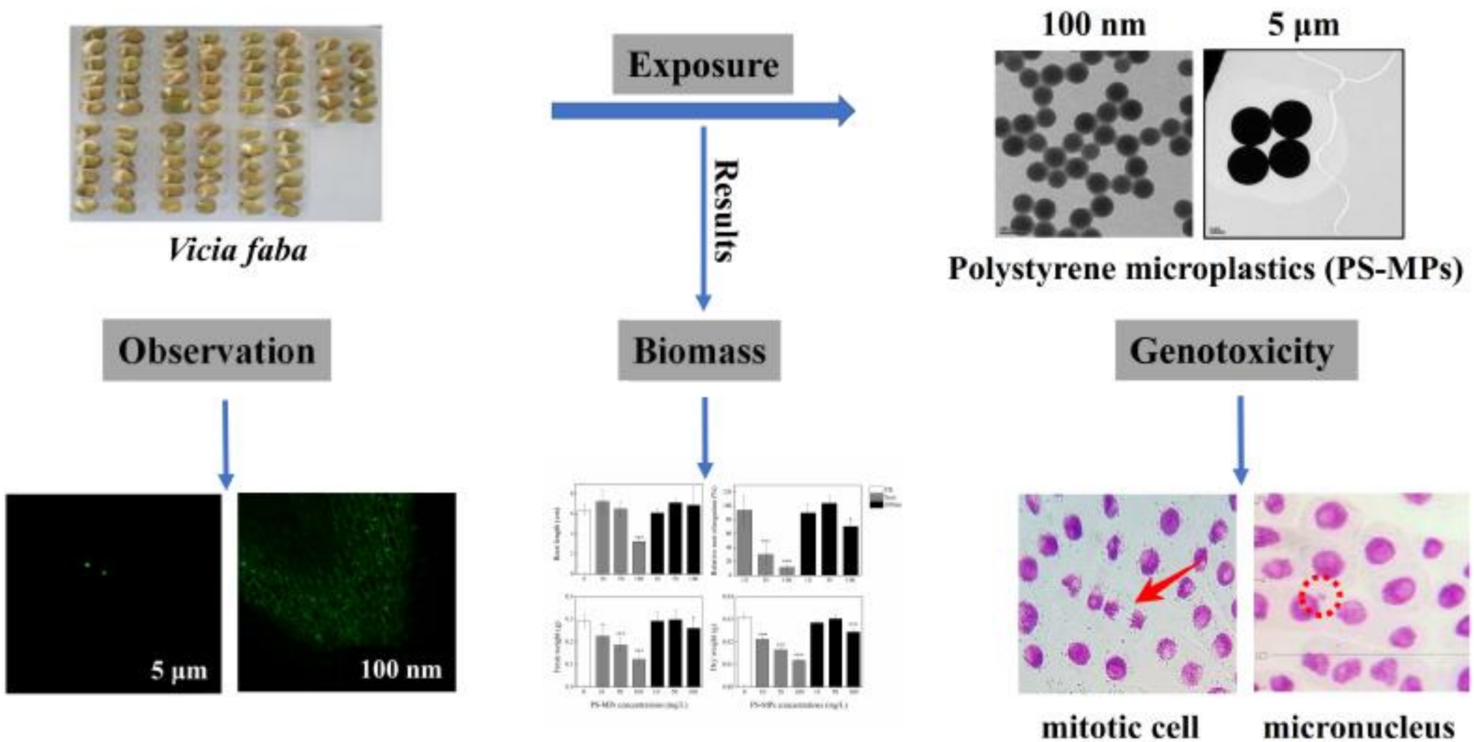
OECD/OCDE 223

Figure 1: Limit dose test procedure; figure does not include control birds



- **Limit dose test** – This is the preferred test when toxicity is expected to be low and lethality is unlikely at the limit dose. The limit dose must be adequate for assessment purposes, and it is usually 2000 mg/kg-bwt. The needs of some regulatory authorities may necessitate using 2,000 mg/Kg-bwt or the highest environmentally relevant concentration, whichever is higher. Five or ten birds are tested at the limit dose in addition to a control group (Figure 1).

EKOTOKSIČNOST



ECOLOGICAL RISK ASSESSMENT (Procjena rizika po okoliš)

- Procjena vjerojatnosti pojave štetnih učinka na okoliš
- Proces kojim se identificira opasnost i kvantificira rizik za ljudsko zdravlje i oštećenje ekosustava zbog utjecaja kemikalija u okolišu
- EPA (Environmental Protection Agency) – Framework for Ecological Risk Assessment – 3 faze:

1. FORMULACIJA PROBLEMA - faza planiranja, procjena ekološkog rizika sustavno se planira na temelju raspoloživih podataka i informacija, postojeći podaci se prikupljaju, istražuju se propisi
2. ANALIZA - sastoji se od dvije pod-aktivnosti, *karakterizacije izloženosti* (predviđa ili mjeri prostornu i vremensku raspodjelu stresora (kemijski ili biološki agens koji izaziva stres u organizmu) i identificira njegovu zajedničku pojavu ili kontakt s ekološkim komponentama koje izazivaju zabrinutosti) i *karakterizacije ekoloških učinaka* (identificira i kvantificira štetne učinke koji nastaju zbog stresora i, gdje je to moguće, uspostavlja odnos uzroka i posljedica) – REZULTAT – PROFILI IZLOŽENOSTI I UČINKA
3. KARAKTERIZACIJA RIZIKA - integracijska je faza u kojoj su profili izloženosti i učinaka razvijeni u drugoj fazi integrirani kako bi se *procijenio potencijalni rizik* ili *vjerojatnost štetnih ekoloških učinaka* povezanih s izlaganjem stresoru

ECOLOGICAL RISK ASSESSMENT
(Procjena rizika po okoliš)

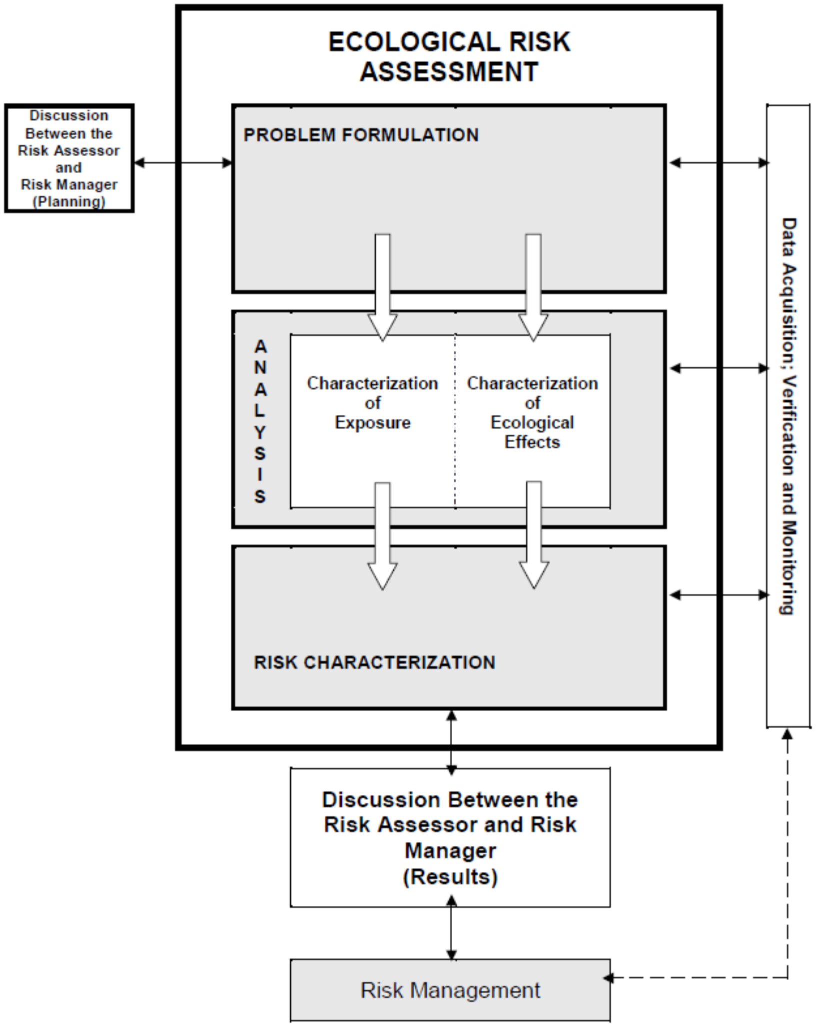


Figure I-2. Framework for Ecological Risk Assessment (Taken from EPA 1992a)

ECOLOGICAL RISK ASSESSMENT
(Procjena rizika po okoliš)

Table I-1. Critical Phases of the Ecological Risk Assessment Process

Phase I	Problem Formulation	• Determine stressor characteristics (e.g. type, intensity, duration, frequency, timing, scale) • Determine the ecosystem potentially at risk • Evaluate existing data of ecological effects • Select appropriate endpoints, considering ecological relevance, policy goals and societal values, susceptibility to the stressor • Develop a conceptual model, working hypothesis regarding how the stressor might affect the ecological components of the ecosystem
Phase II	Analysis	<u>Characterization of exposure:</u> • Characterize the stressor, in terms of distribution or pattern of change • Characterize the ecosystem • Analyze the potential exposure • Develop an exposure profile <u>Characterization of ecological effects:</u> • Evaluate the relevant effects data • Analyze the ecological response in terms of stressor –response determinations or extrapolations and causal evidence evaluation • Develop a stressor-response profile
Phase III	Risk Characterization	• Estimate the risk • Integrate the stressor-response and exposure profiles • Identify uncertainty in the analyses • Describe the risk • Summarize the risk assessment • Interpret the ecological significance