



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



# Kolegij: PRIMJENA EKOTOKSIKOLOGIJE 2. predavanje

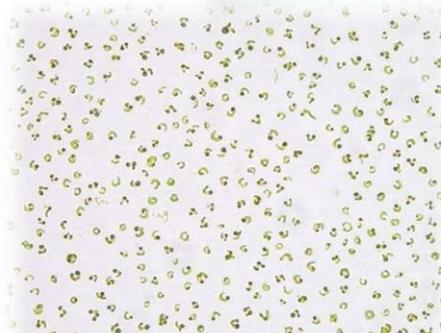
Doc. dr. sc. Dajana Kučić Grgić  
E-mail: [dkucic@fkit.hr](mailto:dkucic@fkit.hr)



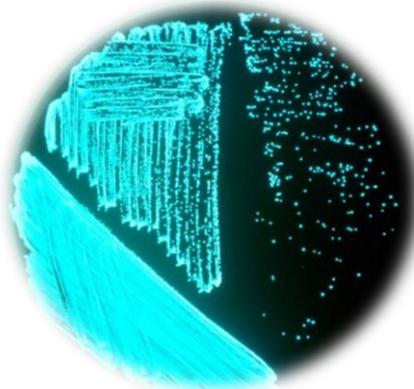
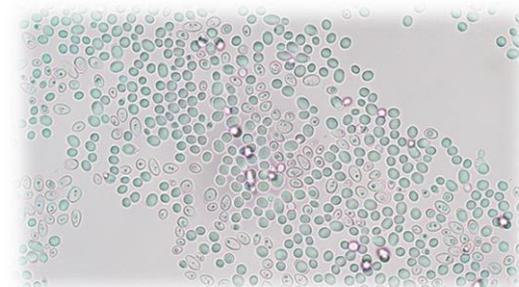
# SADRŽAJ

## Testovi ekotoksičnosti:

1. *Vibrio fischeri*
2. *Pseudomonas putida*
3. *Saccharomyces cerevisiae*
4. *Daphnia magna* (račić)
5. Zebrafish (*Danio rerio*)
6. Zelena alga (*Pseudokirchneriella subcapitata*)
7. Glodavci
8. Nematode



*Pseudokirchneriella subcapitata*  
Provided by Chemicals Evaluation and Research Institute, Japan



# TOKSIČNOST

- TOKSIČNOST - svojstvo ili svojstva tvari koja imaju štetan učinak na biološki sustav
- Odnos doze i biološkog učinka – doza-reakcija i temelj je za procjenu opasnosti i rizika koji predstavljaju tvari u okolišu
- **Pokazatelji toksičnosti:** BIOLOŠKI, FIZIOLOŠKI, REPRODUKTIVNI i UČINCI NA PONAŠANJE
- REACH regulativa (*eng. Registration, Evaluation, Authorisation and Restriction of Chemicals*) – sve kemikalije koje se na godišnjoj razini proizvode u količini većoj od jedne tone – nužno je provesti EKOTOKSIKOLOŠKA ispitivanja
- Utjecaj kemikalija na vodene sustave



# TOKSIČNOST

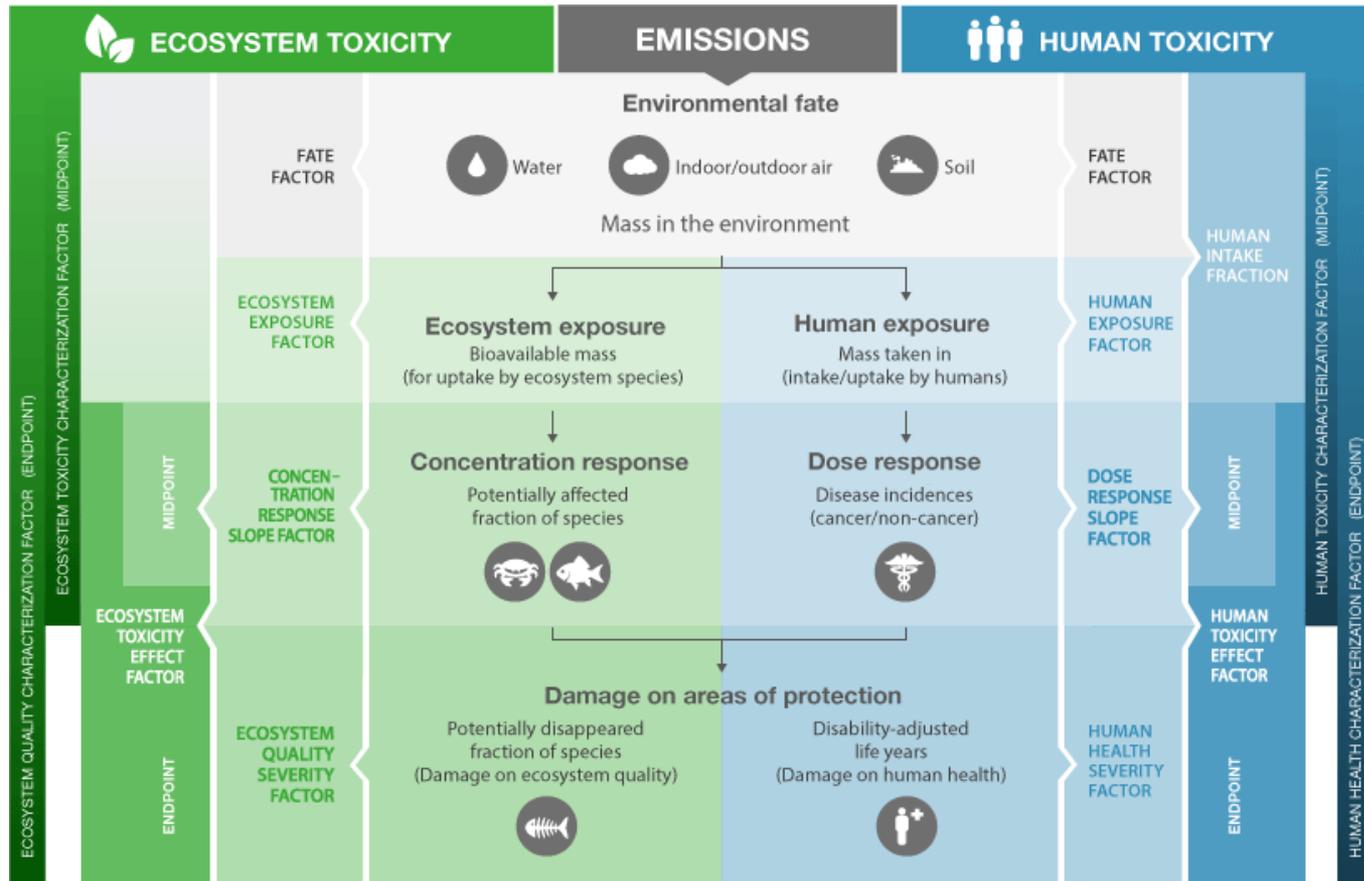


Figure 1: Framework for characterizing toxicity impacts with USEtox 2.0 with a set of factors (e.g. fate factor) linking different indicators (e.g. emissions and time-integrated mass in the environment) to characterize human toxicity and ecosystem toxicity of chemical emissions. The USEtox model is an environmental model for characterization of human toxicological and ecotoxicological life cycle impacts in LCA. It has been developed by a team of



# TOKSIČNOST

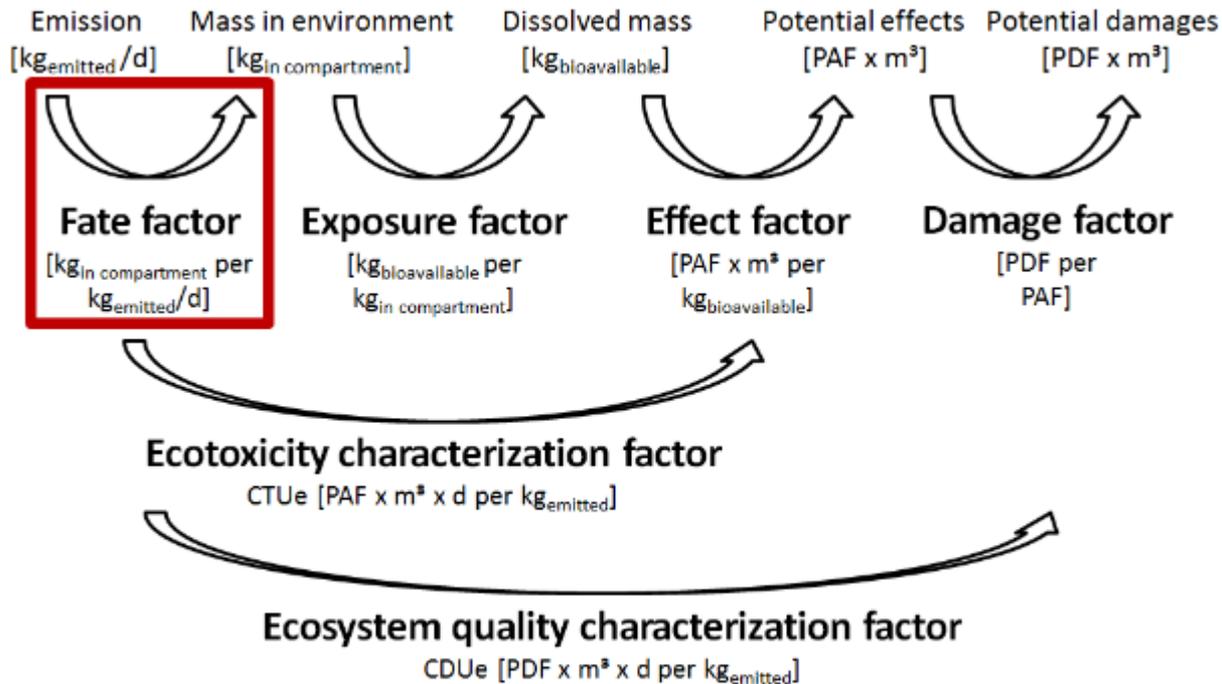


Figure 5: Symbolic description of the emission-to-damage framework for ecotoxicological impacts characterized with USEtox 2.0x.



# TOKSIČNOST

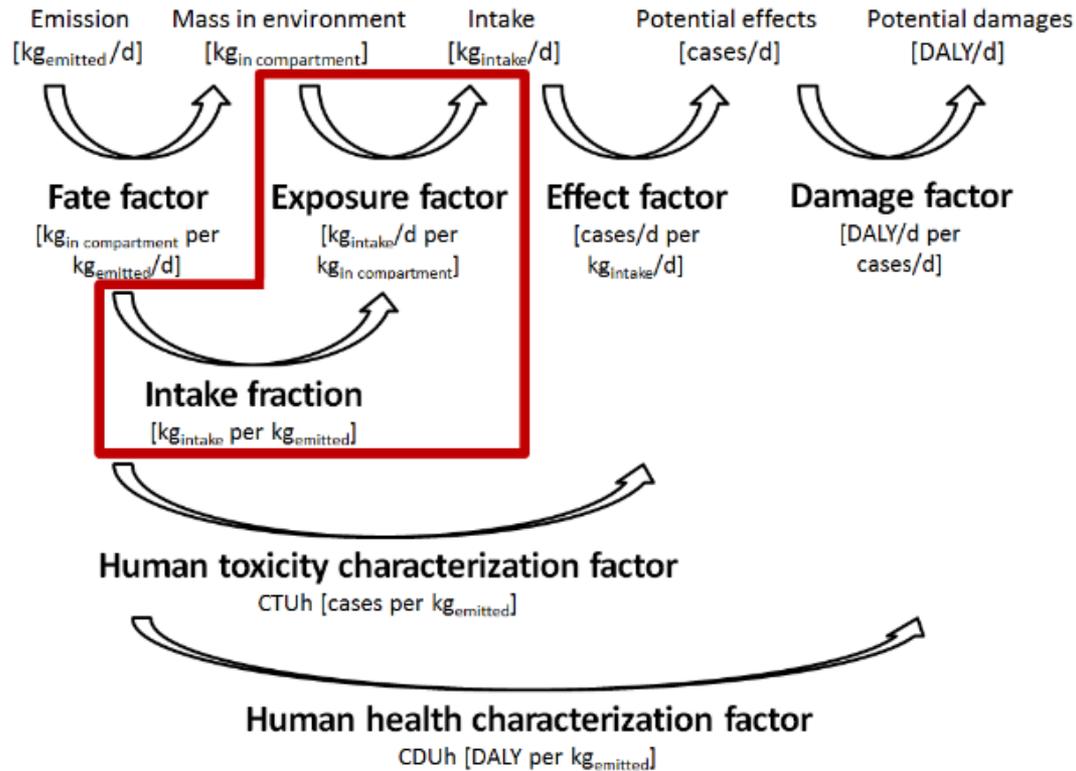


Figure 8: Symbolic description of the emission-to-damage framework for human toxicological impacts characterized with USEtox 2.0x.

- Inhalation of air,
- Ingestion of drinking water,
- Ingestion of meat and milk products,
- Ingestion of agricultural produce (distinguishing above-ground and below-ground),
- Ingestion of fish.



# TESTOVI TOKSIČNOSTI

- BIOTESTOVI ili laboratorijski testovi toksičnosti:
  - (1) Istraživanje djelovanja okolišnih uzoraka (vode, tla) ili kemikalije na organizme/stanice u kontroliranim laboratorijskim uvjetima
  - (2) Eksperimentalne metode za određivanje granične vrijednosti tolerancija pri izlaganju odabranih bioloških modela – test organizama – utjecaju različitih toksičnih tvari
- Biotestovi: DINAMIČNI (kontinuirani) i STATIČNI
- Dinamični test – testni organizam izlaže se testnom mediju – npr. riječnoj vodi , kontinuirano ili periodično u ciklusima od po nekoliko minuta
- Promatraju se promjene u METABOLIZMU, PONAŠANJU ili FIZIOLOŠKIM PARAMETRIMA testnih organizama koje su inducirane subletalnim učinkom onečišćujuće tvari

PRIMJER biotesta: Test s kanarincem – rudari nosili sa sobom u rudnike kako bi se osigurali ukoliko je u rudniku bio prisutan metan. Kanarinci su osjetljiviji na metan od ljudi, stoga su u slučaju da kanarinac ugine rudari znali da trebaju napustiti rudnik



# TESTOVI TOKSIČNOSTI

- STATIČNI (laboratorijski) testovi – izlaganje testnog organizma utjecaju testnog medija određeno vrijeme (npr. 24 ili 48 h) kako bi se utvrdili biološki učinci na organizam
- **ODABIR TESTA TOKSIČNOSTI** ovisi o:

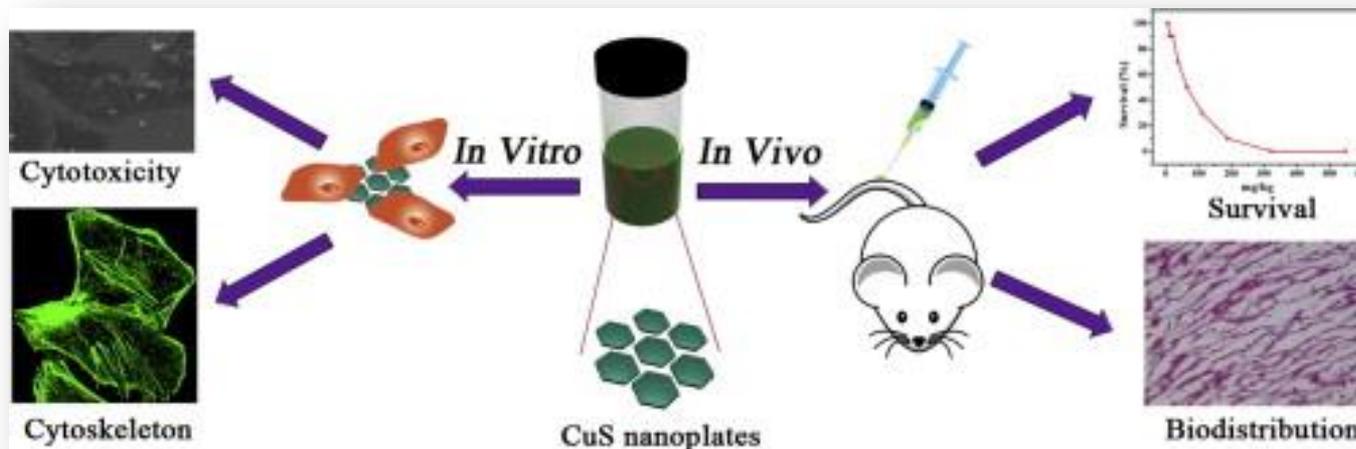
- vrsti ispitivanog medija
- svrsi testa
- osjetljivosti vrste prema toksičnoj tvari i čimbenicima koji je prate
- ekološkom značenju vrsta (karakteristične za ispitivani ekosustav)
- poznavanju životnog ciklusa vrste
- tehničkim i stručnim mogućnostima laboratorija



# TESTOVI TOKSIČNOSTI

- **In vivo** (bolje prilagođeno za promatranje ukupnih efekata odgovarajućih eksperimenata; provode na cijelim organizmima) i **in vitro** (izvode se na stanicama ili biološkim molekulama, izvan njihovog normalnog biološkog okruženja) testovi
- **In vitro** testovi provode se na bakterijama, bakteriofagima, jednostaničnim algama, kvascima, stanicama višestaničnih organizama

**PREDNOSTI:** jednostavnost i brzina, relativno niska cijena, izbjegavanje žrtvovanja pokusnih organizama, jasan mehanizam toksičnosti



# TESTOVI TOKSIČNOSTI

- Akutni testovi –  $LC_{50}$  i  $IC_{50}$   
Akutna toksična jedinica  $TU_a$  – predstavlja recipročnu vrijednost  $LC_{50}$  sa 100 ( $TU_a = 100/LC_{50}$ )
- Najčešća mjera toksičnosti je smrt organizama
- $LD_{50}$  – srednja letalna doza – doza koja uzrokuje mortalitet kod 50 % ispitivanih jedinki
- $EC_{50}$  – efektivna koncentracija koja uzrokuje učinak kod 50 % ispitivanih jedinki
- $IC_{50}$  – inhibicijska koncentracija koja smanjuje uobičajen odgovor organizama za 50 % (često se koristi za mjerenje rasta algi, bakterija)
- Preporučeni akutni testovi toksičnosti: *Daphnia magna*, *P. subcapitata* i *V. fischeri*



## KRONIČNI TESTOVI

- Duže se vrijeme proučava testni organizam
- CILJ: odrediti koncentraciju toksične tvari koja će u određenom periodu ostaviti posljedicu na ispitivani organizam
- **Promatra se: utjecaj toksične tvari na rast i razmnožavanje organizma, razvoj abnormalnih stanica, ponašanje jedinki**

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

Kronična toksična jedinica  $TU_c$  – predstavlja recipročnu vrijednost NOEC sa 100 ( $TU_c = 100/NOEC$ )

- Preporuka – procjena toksičnosti na 2 do 3 standardizirana testa



# TESTOVI TOKSIČNOSTI

- 2 vrste kroničnih testova:

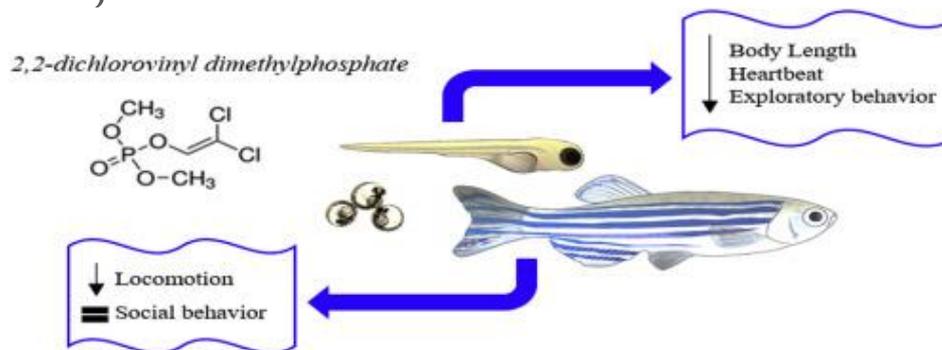
## 1. Full life cycle test

– utjecaj različitih koncentracija onečišćujuće tvari na rast i razmnožavanje, preživljavanje i na ostale pokazatelje (ISO 8692:1989; ISO 10712:1995; ISO 10253:1995; ISO 20079:2005)

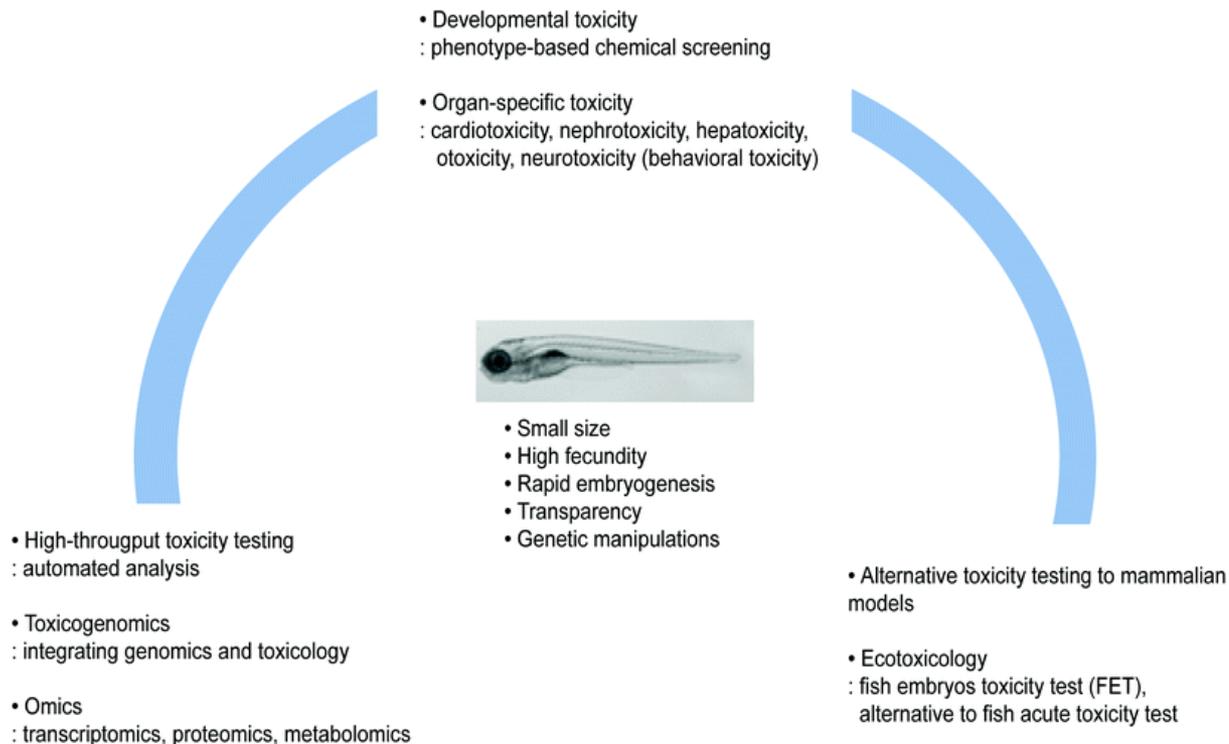
- primjenjuje se na algama i beskičmenjacima
- na ribama se ne provodi zbog dugotrajnosti (1-2 godine)

## 2. Partial life cycle test (sensitive life stage tests)

- testiranja se provode na **EMBRIJIMA RIBA** – u tom stadiju su **NAJOSJETLJIVIJI**
- trajanje od 7 do 60 dana
- promatra se preživljavanje i razvoj abnormalnosti



# TESTOVI TOKSIČNOSTI



# TESTOVI ZA ODREĐIVANJE AKUTNE TOKSIČNOSTI

Organizam	Test vrsta	EN ISO	Slatka / Slana voda
Bakterija	<i>Vibrio fischeri</i>	ISO 11348-2	Slatka / Slana voda
Alga	<i>P. subcapitata</i>	ISO 8692:2012	Slatka
	<i>Scenedesmus subspicatus</i>	DIN 3841233	Slatka
	<i>Skeletonema costatum</i>	ISO 10253:2006	Slana
Račić	<i>Daphnia magna</i>	ISO 10706/2000	Slatka
	<i>Tisbe battagliai</i>	ISO 14669	Slana
	<i>Acartia tonsa</i>	ISO 14669	Slana
Riba	<i>Oncorhynchus mykiss</i>	OECD 203	Slatka
	Zebrafish ( <i>Danio rerio</i> (test s ribljim jajima)	ISO 15088:2007	Slatka



# TESTOVI ZA ODREĐIVANJE KRONIČNE TOKSIČNOSTI

Organizam	Test vrsta	EN ISO	Slatka / Slana voda
Bakterija	<i>Pseudomonas putida</i>	ISO 10712:1995	Slatka
Mikroorganizam	Mikrobiološka zajednica aktivnog mulja (“Zahn-Wellens” test)	ISO 15522:1999	Slatka
Alga	<i>Desmodesmus subcapitata</i>	DIN 38412- 33:1991	Slatka
	<i>Skeletonema costatum</i>	ISO 10253:2006	Slana
Račić	<i>Daphnia magna</i>	ISO 10706/2000	Slatka
	<i>Daphnia magna</i>	OECD 211	Slatka



# TESTOVI TOKSIČNOSTI

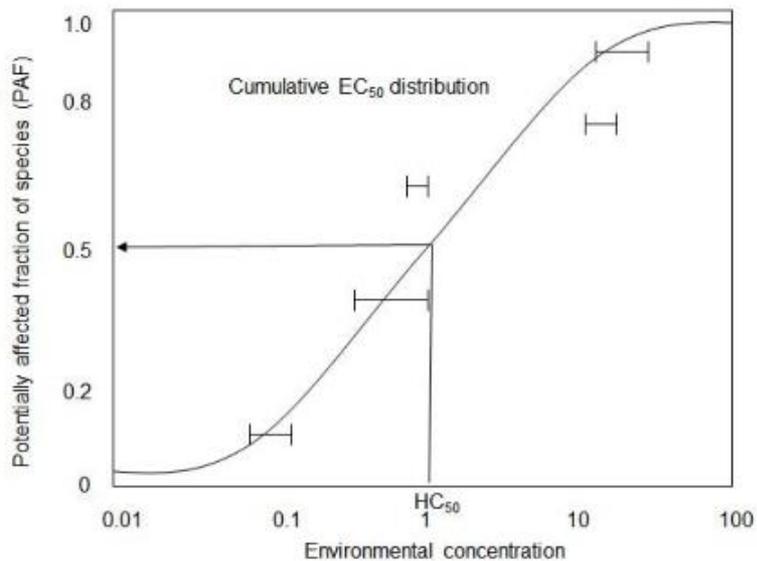


Figure 16: Species Sensitivity Distribution (SSD) curve showing the cumulative distribution of  $EC_{50}$  values across tested species for a chemical (ranges of test- $EC_{50}$ s represent variation between available data for a given species).

Table 21: Duration requirement for classification of a test as acute, sub-chronic or chronic.

	Acute	Sub-chronic	Chronic
Vertebrates	< 7 days	$\geq 7$ days; < 32 days	$\geq 32$ days
Invertebrates	< 7 days	$\geq 7$ days ; < 21 days	$\geq 21$ days
Plants	< 7 days	-	$\geq 7$ days
Algae	< 3 days	-	$\geq 3$ days



# STANDARDI



International  
Organization for  
Standardization



ISO – *International Organization for standardization* (Internacionalna organizacija za standardizaciju)

DIN – *Deutches Institut für Normung* (Kratika njemačkog standarda i istoimenog Instituta (DIN EN – njemačka verzija europskih standarda; DIN ISO – njemačka verzija ISO standarda)

HRN – hrvatske norme

OECD – *Organization for Economic Cooperation and Development* (organizacija za gospodarsku suradnju i razvoj)

USEPA – U.S. Environmental Protection Agency

ASTM – American Society for Testing and Materials



HOW STANDARDS PROLIFERATE:  
(SEE: A/C CHARGERS, CHARACTER ENCODINGS, INSTANT MESSAGING, ETC.)



# Određivanje akutne toksičnosti s *Vibrio fischeri*

- **BIOLUMINISCENCIJA** – pojava svjetlucanja živih organizama
- Razvijena kod velikog broja organizama
- Najveći broj bioluminiscentnih organizama – more
- Organizmi bioluminiscenciju koriste za: **komunikaciju, osvjetljavanje, mimikriju, zbunjivanje predatora** i dr.
- Bioluminiscentne bakterije:
  - Vibrio fischeri*,
  - Photobacterium phosphoreum* (*Vibrio phosphoreum*),
  - Vibrio harveyi*,
  - Pseudomonas fluorescens*
- Mnoge luminiscentne bakterije – žive u **simbiozi** s drugim morskim organizmima koji tu svjetlost koriste u razne svrhe (zooplanktonski račići ih konzumiraju – u probavilu se razmnožavaju – svjetlucanjem u utrobi primanjuju ribe)



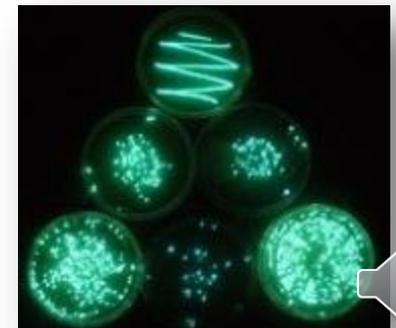
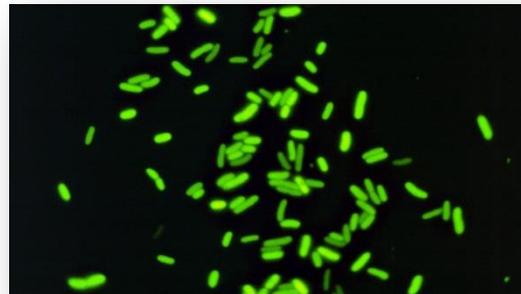
# Određivanje akutne toksičnosti s *Vibrio fischeri*

- Određivanje akutne toksičnosti na temelju BIOLUMINISCENCIJE
- Bioluminiscencija – nastaje oksidacijom molekule **LUCIFERIN** uz pomoć enzima **LUCIFERAZE**
- Luciferin + Luciferaza = FOTOPROTEIN
- Luciferaza – složeni protein i specifična samo za jednu reakciju
- Aktivnost luciferaze u stanicama – prirodno regulirana – vidljivo prema odsutnosti ili prisutnosti luminiscencije i njezinom intenzitetu
- Osim prirodne regulacije, na aktivnost luciferaze (intenzitet bioluminiscencije) utječu i mnoge druge tvari koje možemo naći u vodi



# Određivanje akutne toksičnosti s *Vibrio fischeri*

- 1979. - Bulich razvio standardizirani test za određivanje toksičnosti vode - **Microtox® Acute Toxicity Test**
- ISO 11348-3:2007 Water Quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) – Part 3: Method using freeze-dried bacteria
- Test kratkog trajanja (30 min), jednostavan test
- Test mikroorganizam – *Vibrio fischeri* (*Aliivibrio fischeri*) – G (-) bakterija, heterotrofna, pokreću se pomoću flagela, živi kao simbiot u bioluminiscentnim ribama i lignjama
- Osjetljivost na veliki raspon koncentracija onečišćujućih tvari





# Određivanje akutne toksičnosti s *Vibrio fischeri*

## ■ NEDOSTACI *V. fischeri*:

- Kratko trajanje – manje učinkovito za određivanje toksičnosti nekih onečišćujućih tvari poput antibiotika i mikrotoksina
- Ima slabu osjetljivost na oksitetraciklin i teške metale

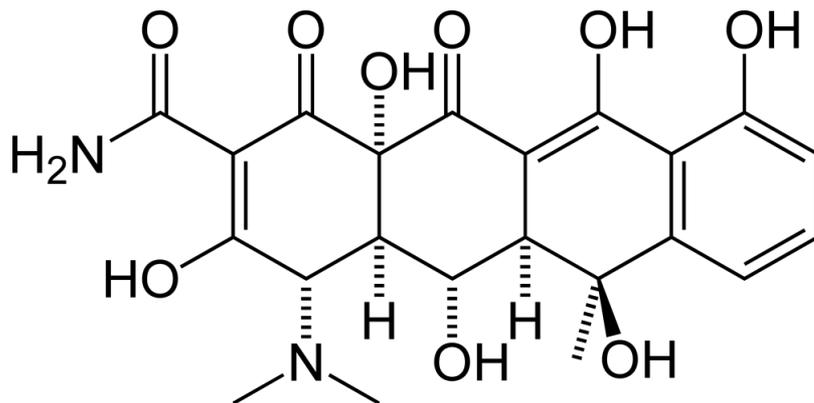


Table 2

Studies on comparative sensitivities of VFBLA and other reference toxicity bioassays

Samples	Comparative sensitivities of applied bioassays	References
Industrial waste effluents	<i>A. fischeri</i> assay provided highest sensitivity than respirometry, nitrification inhibition, adenosine triphosphate luminescence and enzyme inhibition assays	Dalzell, 2002
Electroplating industry effluents	<i>A. fischeri</i> and <i>Daphnia similis</i> based assays exhibited comparable sensitivities	Liu et al., 2002
Metal ions	<i>A. fischeri</i> and <i>Pseudomonas fluorescens</i> were equally sensitive	Abbondanzi et al., 2003
Copper and chromium	<i>A. fischeri</i> was more sensitive than bioassays utilizing fish, daphnids, shrimps and <i>Pseudomonas fluorescens</i>	Hsieh et al., 2004
Food and dying industries effluents	<i>A. fischeri</i> and <i>D. magna</i> tests demonstrated equivalent sensitivities	Kungolos, 2004
Benzimidazole anthelmintics	<i>A. fischeri</i> was less sensitive than <i>D. magna</i>	Oh et al., 2006
Pharmaceutical industry effluents	<i>A. fischeri</i> test was more effective than <i>D. magna</i> assay	Larsson et al., 2007
Ammonia and metals	Bioluminescent dinoflagellate ( <i>QwikLite</i> ) assay was relatively more sensitive than <i>A. fischeri</i> test	Rosen et al., 2008
Fosthiazate, metalaxyl-M, imidacloprid and copper	<i>A. fischeri</i> test was highly sensitive than assays involving <i>Pseudokirchneriella subcapitata</i> and <i>Daphnia magna</i>	Kungolos et al., 2009
Wastewater samples	<i>A. fischeri</i> was more sensitive than <i>Pseudokirchneriella subcapitata</i> , <i>Daphnia magna</i> , <i>Lemna minor</i> and <i>Thamnocephalus platyurus</i>	Mendonça et al., 2009
Cadmium, zinc, manganese and lead	<i>Daphnia magna</i> acute toxicity test was highly sensitive than <i>Pseudomonas putida</i> and <i>A. fischeri</i> based assays	Teodorovic et al., 2009
Pharmaceutical wastewater	<i>A. fischeri</i> and <i>D. magna</i> based assays exhibited comparable sensitivities	Sponza and Demirden, 2010
Pyrene-contaminated soil samples	<i>A. fischeri</i> was the most sensitive marker of pyrene toxicity as compared to <i>Brassica rapa</i> and <i>Eisenia fetida</i>	Khan et al., 2012
Wastewater produced in the photocatalytic degradation process	<i>A. fischeri</i> displayed more sensitivity than fern ( <i>Polystichum setiferum</i> ) spore	Marugan et al., 2012
Pharmaceuticals, personal care products and resultant metabolites	<i>A. fischeri</i> test exhibited maximum sensitivity than assays involving algae, fish, crustaceans and respirometry	de García et al., 2014
Pharmaceutical waste water	<i>A. fischeri</i> was more effective than <i>Scenedesmus obliquus</i>	Yu et al., 2014
Pesticides and heavy metals	<i>A. fischeri</i> and <i>Daphnia magna</i> assays exhibited comparable sensitivity	Mansour et al., 2015



# Određivanje akutne toksičnosti s *Vibrio fischeri*

## PRIMJER 1: Teški metali

Species	ranking
<i>Daphnia magna</i>	Cd>Zn>Mn>Pb
<i>Vibrio fischeri</i>	Zn>Pb>Cd>Mn
<i>Pseudomonas putida</i>	Pb>Cd>Zn>Mn

**Table 3.** Comparative toxicity ranking of 4 tested metals and 3 test species: ranking from the highest to the lowest toxicity (from left to right).

metal	Sensitivity ranking (based on EC/LC <sub>50</sub> values)
Cd	<i>D. magna</i> > <i>P. putida</i> > <i>V. fischeri</i>
Zn	<i>D. magna</i> > <i>V. fischeri</i> > <i>P. putida</i>
Pb	<i>P. putida</i> > <i>V. fischeri</i> > <i>D. magna</i>
Mn	<i>D. magna</i> > <i>P. putida</i> > <i>V. fischeri</i>

**Table 4.** Sensitivity ranking of *D. magna*, *V. fischeri* and *P. putida* to Cd<sup>2+</sup>, Zn<sup>2+</sup>, Pb<sup>2+</sup> and Mn<sup>2+</sup>: ranking from the most sensitive to the least sensitive (from left to right)

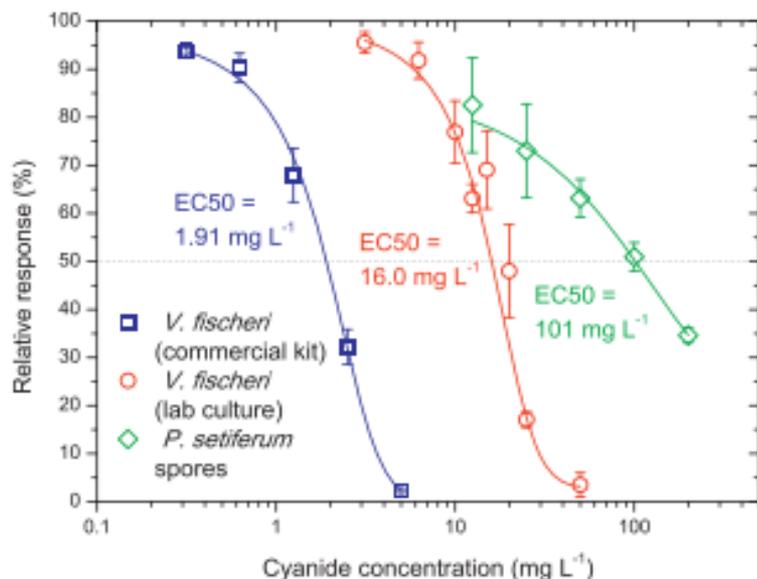
*Daphnia magna* – osjetljivija na teške metale od *V. fischeri* i *P. putide*

U 15 min EC<sub>50</sub> – *V. fischeri* – 36,08 mg/L

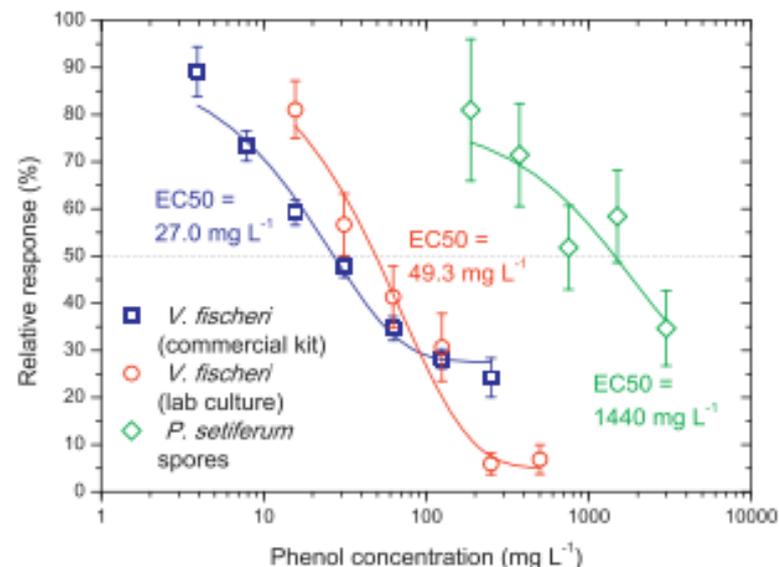
*D. magna* – 0,29 mg/L



## PRIMJER 2: Cijanidi i fenoli



**Fig. 1.** Effect of cyanide concentration on the inhibition of *V. fischeri* bioluminescence and *P. setiferum* mitochondrial activity. EC<sub>50</sub> values have been calculated by fitting the experimental data to dose-response curves with variable Hill slope.



**Fig. 2.** Effect of phenol concentration on the inhibition of *V. fischeri* bioluminescence and *P. setiferum* mitochondrial activity. EC<sub>50</sub> values have been calculated by fitting the experimental data to dose-response curves with variable Hill slope.

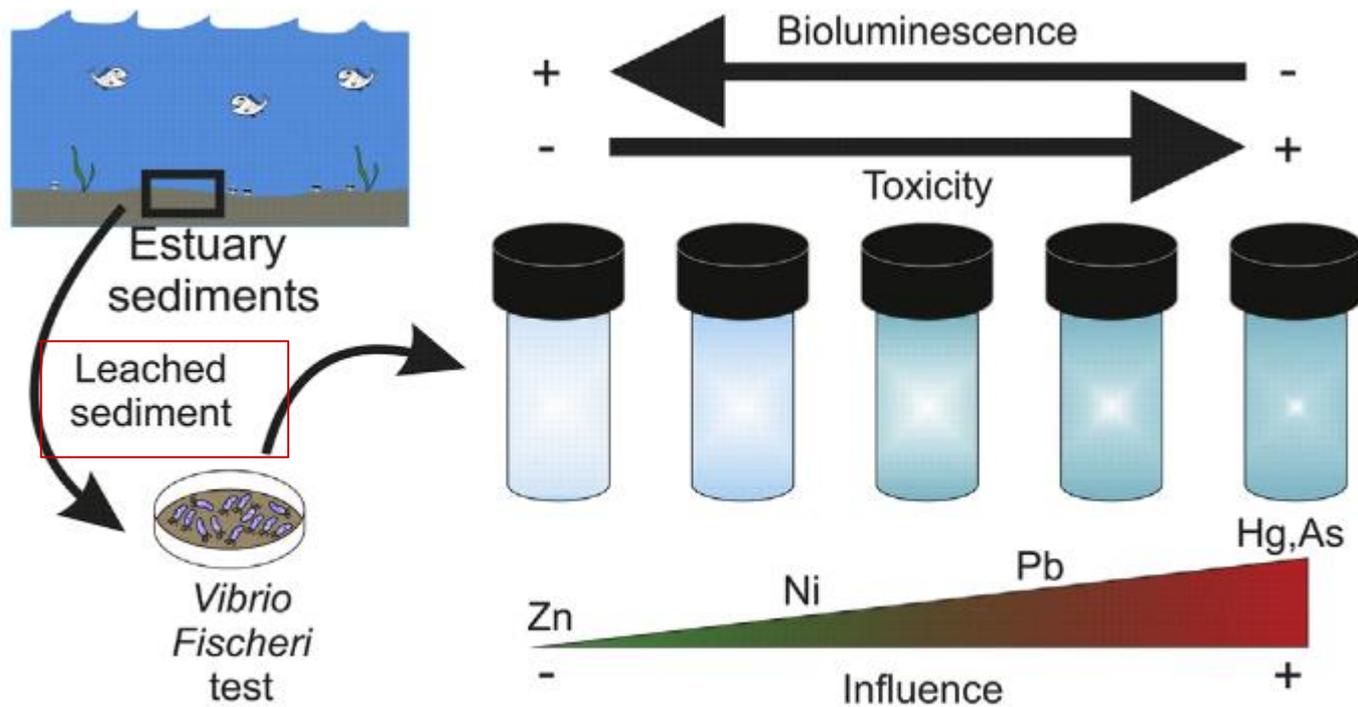
Lab. kultura *V. fischeri* u boljem je fiziološkom stanju od liofilizirane kulture – lakše podnosi toksičan stres

Liofilizacija kultura – može uzrokovati oštećenje stanične membrane – toksične tvari ulaze u stanicu



# Određivanje akutne toksičnosti s *Vibrio fischeri*

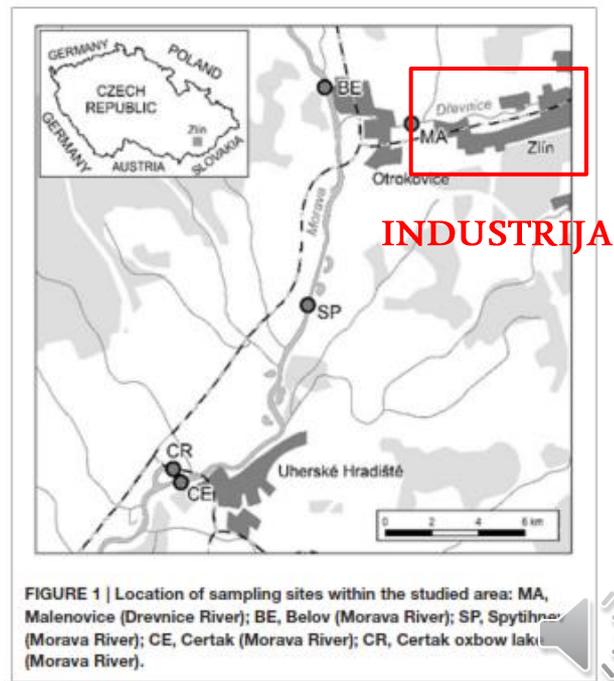
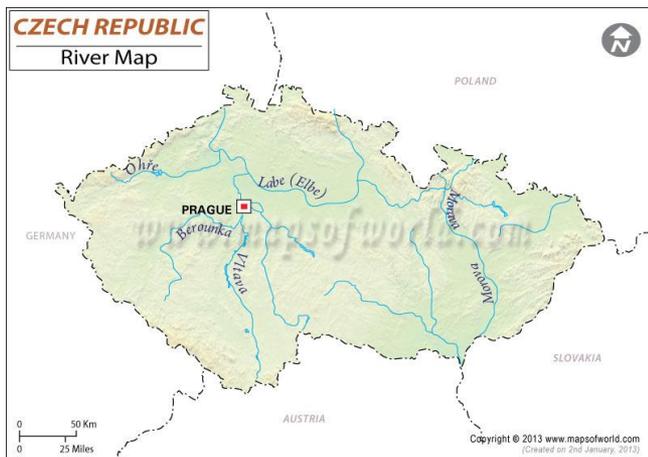
## TOKSIČNOST SEDIMENTA



# Određivanje akutne toksičnosti s *Vibrio fischeri*

## PRIMJER 3: Ispitivanje toksičnosti sedimenta u rijeci Moravi i pritoka Drevnice u Češkoj Republici

- Sedimenti – važan dio vodenog sustava
- Sedimenti – “klopka” za akumulaciju onečišćujućih tvari
- Gustoća populacije, antropogene aktivnosti (industrija), kemijski sastav sedimenta i geokemijski procesi – utječu na koncentraciju onečišćujućih tvari – promjena u sastavu tijekom godišnjih doba



# Određivanje akutne toksičnosti s *Vibrio fischeri*

TABLE 1 | Annual and seasonal toxicity (IC<sub>50</sub>) levels obtained with the 30-s kinetic *Vibrio fischeri* assay in bottom and recent sediments tested in suspensions.

Locality <sup>b</sup>	N <sup>c</sup>	Toxicity (IC <sub>50</sub> , mg/ml) <sup>a</sup>				
		Spring	Summer	Autumn	Winter	All seasons
<b>Bottom sediment</b>	<b>73</b> (15 + 24 + 15 + 19)	<b>53.6</b> (12.4 – >75.0)	<b>&gt;75.0</b> (30.6 – >75.0)	<b>63.2</b> (36.5 – >75.0)	<b>55.8</b> (13.5 – >75.0)	<b>63.8</b> (12.4 – >75.0)
MA	15 (3 + 5 + 3 + 4)	39.4 (12.4 – 74.1)	71.0 (30.6 – >75.0)	60.6 (36.5 – 65.0)	23.6 (13.5 – 43.8)	43.8 (12.4 – >75.0)
BE	14 (3 + 4 + 3 + 4)	27.9 (20.1 – 34.9)	72.5 (63.3 – >75.0)	41.9 (37.6 – 54.3)	34.4 (19.2 – 58.3)	46.1 (19.2 – >75.0)
SP	14 (3 + 5 + 3 + 3)	53.6 (38.5 – 58.3)	60.5 (49.1 – 63.8)	62.1 (61.2 – 63.2)	73.9 (46.4 – >75.0)	60.9 (38.5 – >75.0)
CE	15 (3 + 5 + 3 + 4)	>75.0 (>75.0 – >75.0)	>75.0 (>75.0 – >75.0)	>75.0 (70.3 – >75.0)	>75.0 (>75.0 – >75.0)	>75.0 (70.3 – >75.0)
CR	15 (3 + 5 + 3 + 4)	54.4 (25.4 – >75.0)	>75.0 (>75.0 – >75.0)	>75.0 (>75.0 – >75.0)	67.9 (48.0 – >75.0)	>75.0 (25.4 – >75.0)
<b>Recent (trap) sediment</b>	<b>49</b> (10 + 16 + 10 + 13)	<b>26.0</b> (4.6 – 53.7)	<b>43.5</b> (10.4 – 63.7)	<b>29.6</b> (18.0 – 75.8)	<b>21.2</b> (4.9 – >75.0)	<b>28.4</b> (4.6 – >75.0)
MA	12 (2 + 4 + 3 + 3)	7.8 (4.6 – 11.0)	38.2 (10.4 – 56.0)	22.3 (20.3 – 37.7)	8.1 (4.9 – 9.4)	15.7 (4.6 – 56.0)
BE	9 (1 + 3 + 2 + 3)	30.8 <sup>d</sup>	42.3 (41.9 – 42.5)	39.1 (23.1 – 55.2)	27.5 (21.2 – 27.6)	30.8 (21.2 – 55.2)
SP	11 (3 + 4 + 3 + 1)	27.6 (24.5 – 29.8)	50.0 (33.5 – 63.0)	36.3 (30.5 – 75.8)	20.5 <sup>d</sup>	33.5 (20.5 – 75.8)
CE	7 (1 + 2 + 1 + 3)	53.7 <sup>d</sup>	54.5 (45.4 – 63.7)	28.8 <sup>d</sup>	45.7 (21.7 – >75.0)	45.7 (21.7 – >75.0)
CR	10 (3 + 3 + 1 + 3)	19.4 (17.3 – 28.4)	33.1 (16.9 – 48.8)	18.0 <sup>d</sup>	14.7 (14.2 – 22.9)	18.7 (14.2 – 48.8)

Svježi sediment

<sup>a</sup> Values indicate concentrations causing 50% inhibition of bioluminescence after 30-s exposures (mg sediment dry weight/ml). Expressed as median (min–max).

<sup>b</sup> MA, Malenovice; BE, Balov; SP, Spytihnev; CE, Certak; CR, Certak oxbow lake.

<sup>c</sup> Number of samples – total number and numbers from different seasons (Spring + Summer + Autumn + Winter).

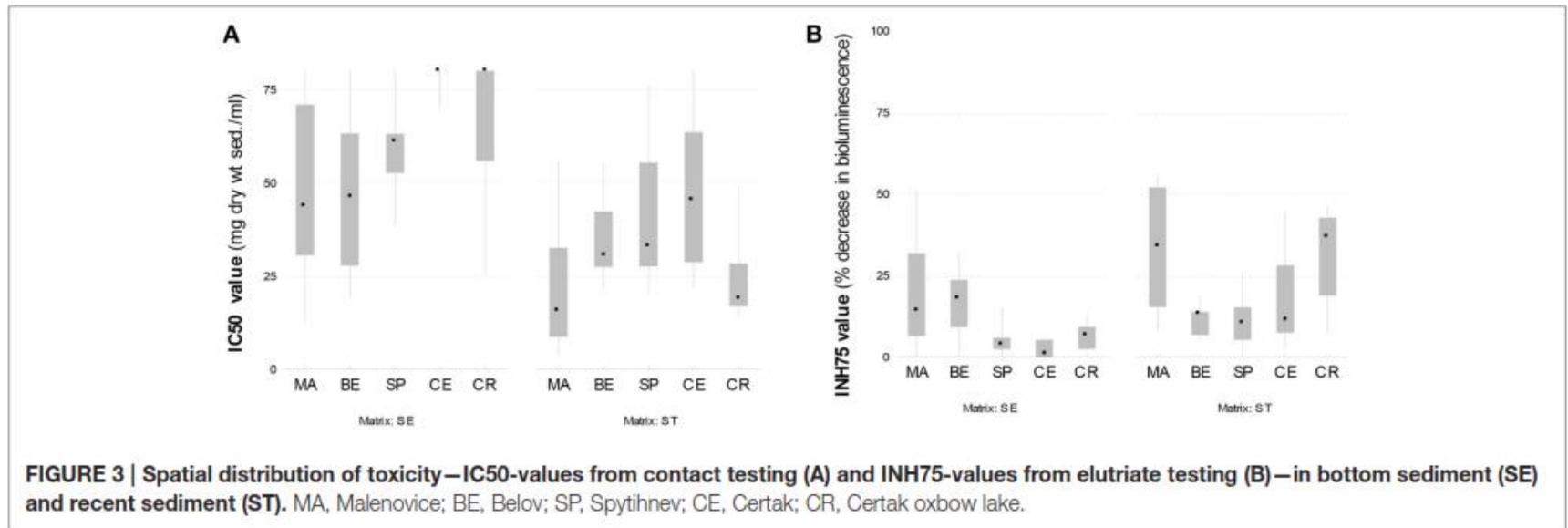
<sup>d</sup> The only detected value.



# Određivanje akutne toksičnosti s *Vibrio fischeri*

## Ispitivanje toksičnosti sedimenta i pripremljenog eluata

- Eluat – pokazao manju toksičnost – PCB, PAH – doprinose jako toksičnosti – nepolarne molekule – slabo topljive u vodi



# Određivanje akutne toksičnosti s *Vibrio fischeri*

## PRIMJER 4: Određivanje toksičnosti farmaceutika

**Table 1**

Toxic effects ( $EC_{50}$ ) of typed PPCPs in freshwater organisms.

Pharmaceutical	Toxicity index	Test	Reference
<b>DIKLOFENAK</b>	$EC_{50}$ 11.45 mg/L	<i>V. fischeri</i>	Zhang et al., 2008
	$EC_{50}/96$ h 0.09 mg/L	<i>Zebra fish embryos</i>	Zhang et al., 2008
	$EC_{50}$ 71.9 mg/L	Algal test	Cleuvers, 2004
	$EC_{50}$ 68.0 mg/L	<i>D. magna</i>	Cleuvers, 2004
	$EC_{50}$ 22.7 mg/L	<i>Ceriodaphnia dubia</i>	Ferrari et al., 2003
	$EC_{50}$ 66.81 mg/L	<i>D. magna</i>	Cleuvers, 2004
	$EC_{50}$ 39.9–69.8 mg/L	<i>D. magna</i>	Haap et al., 2008
<b>KLORAMFENIKOL</b>	LOEC 2.00 mg/L	<i>Ceriodaphnia dubia</i>	Ferrari et al., 2003
	NOEC 1.00 mg/L	<i>D. magna</i>	Ferrari et al., 2003
	NOEC 0.246 mg/L	<i>B. calyciflorus</i>	
<b>CPL</b>	$EC_{50}$ 1086 mg/L	<i>D. magna</i>	Calleja et al., 1994
	$EC_{50}$ 227–600 mg/L	<i>D. magna</i>	Muller, 1982
	$EC_{50}$ 542.86 mg/L	<i>D. magna</i>	Lilius et al., 1994
<b>METOPROLOL</b>	$EC_{50}$ 20.68 mg/L	<i>V. fischeri</i>	Choi et al., 2008
	LOEC 0.0012 mg/L	<i>D. magna</i>	Dietrich et al., 2010
<b>MT</b>	LOEC 0.0012 mg/L	<i>D. magna</i>	Dietrich et al., 2010
	$EC_{50}$ 100 mg/L	<i>D. magna</i>	Cleuvers, 2004

$EC_{50}$ —concentration that causes 50% of effect.

NOEC—no observed effect concentration.

LOEC—lowest observed effect concentration.



# Određivanje akutne toksičnosti s *Vibrio fischeri*

*D. magna*  
osjetljivija od *V. fischeri*

**Table 2**

The changes of the toxicity of wastewater in the contexts of toxicity categories established according with the Directive 79/831/EEC: very toxic ( $EC_{50} \leq 1$  mg/L); toxic ( $1$  mg/L  $< EC_{50} \leq 10$  mg/L); harmful ( $10$  mg/L  $< EC_{50} \leq 100$  mg/L).

Compound	Raw wastewater		After treatment $EC_{50}$ (mg/L)		After 60 days post treatment $EC_{50}$ (mg/L)				
		$EC_{50}$ (mg/L)	TU	$EC_{50}$ (mg/L)	TU	$EC_{50}$ (mg/L)	TU		
<i>D. magna</i> 24 h									
DCF	toxic	3.18	0.31	toxic	5	0.2	harmful	50	0.02
CPL	toxic	5	0.2	harmful	40	0.025	non-toxic	> 50	0.02
MT	toxic	2.59	0.39	harmful	40	0.025	harmful	30	0.033
<i>D. magna</i> 48 h									
DCF	toxic	2.69	0.37	toxic	5	0.2	harmful	50	0.02
CPL	toxic	5	0.2	harmful	25	0.04	non-toxic	≈ 100	0.01
MT	toxic	2.59	0.39	harmful	25	0.04	harmful	25	0.04
<i>V. fischeri</i>									
DCF	harmful	14.04	0.07	toxic	10	0.1	harmful	85	0.012
CPL	harmful	27.06	0.04	harmful	80	0.0125	harmful ≈ non toxic	100	0.01
MT	harmful	14.48	0.07	non-toxic	100	0.01	non-toxic	130	0.008

**JAKO TOKSIČNO  $EC_{50} \leq 1$  mg/L**

**TOKSIČNO  $1$  mg/L  $< EC_{50} \leq 10$  mg/L**

**OPASNO  $10$  mg/L  $< EC_{50} \leq 100$  mg/L**



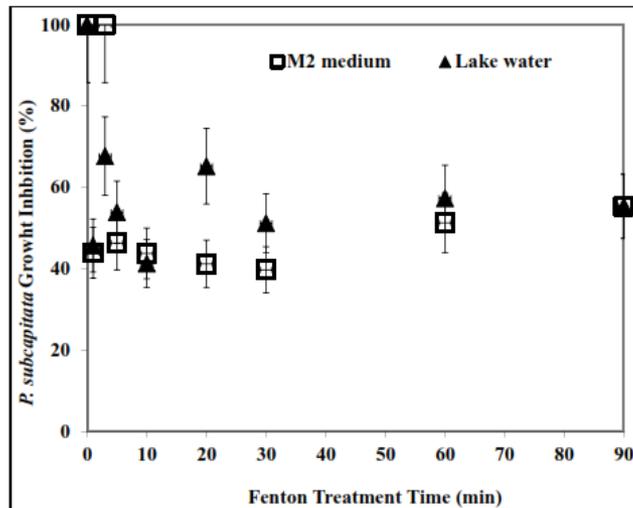
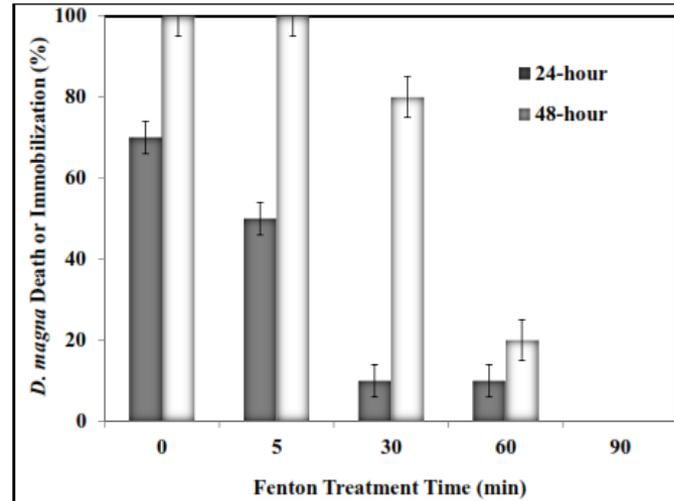
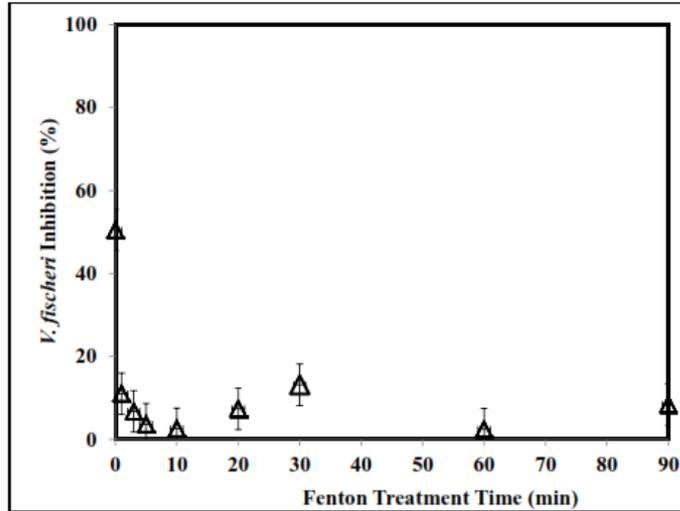
# Određivanje akutne toksičnosti s *Vibrio fischeri*

## PRIMJER 4: Utjecaj Fentonovog procesa na toksičnost vode onečišćene bisfenolom A (BPA)

- BPA - osnovna građevna jedinica u proizvodnji polikarbonata, epoksi smola, stomatoloških potrepština te drugih materijala
- Polikarbonati se koriste u proizvodnji plastičnih ambalaža za hranu i piće, automobila, bočica za djecu, pakiranju lijekova, elektroničke opreme, kuhinjskih pomagala, optičkih medija i raznih drugih materijala
- BPA utječe na ravnotežu hormona kod ljudi i životinja, odnosno uzrokuje poremećaje endokrinog sustava
- Ispitivala se toksičnost na *V. fischeri*, *D. magna* i *P. subcapitata*
- *D. magna* – test se provodio na kulturi mlađoj od 24 h – ispitale različite koncentracije, nakon 24 i 48 h broj umrlih i imobiliziranih stanica se odredio



# Određivanje akutne toksičnosti s *Vibrio fischeri*



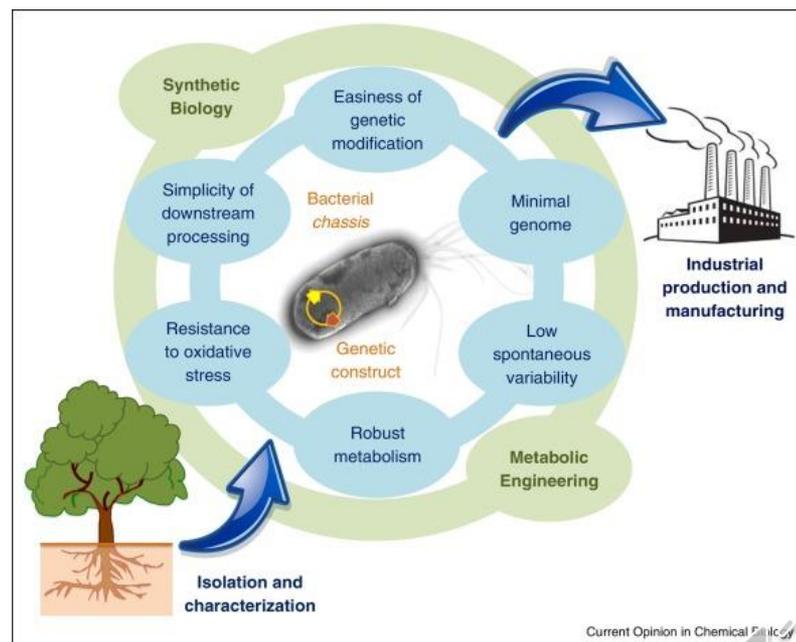
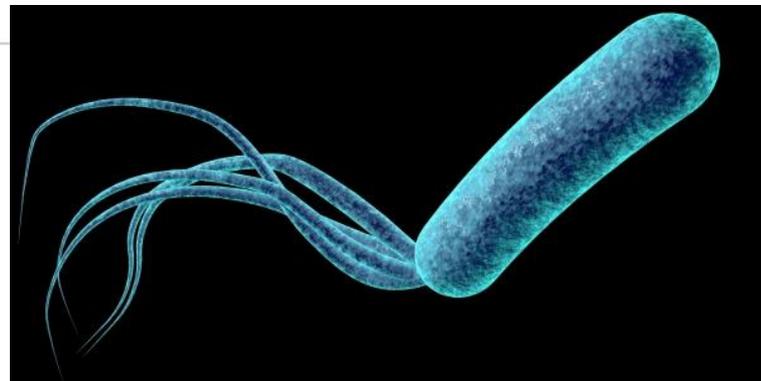
*P. subcapitata* > *D. magna* > *V. fischeri*



# Određivanje toksičnosti sa *Pseudomonas putida*

## *Pseudomonas putida*

- G (-), štapičasta, aerobna bakterija
- Uloga u procesu bioremedijacije
- Water quality *Pseudomonas putida* growth inhibition test (*Pseudomonas* cell multiplication inhibition test) (ISO 10712:1995)
- Ispituje se utjecaj toksične tvari na rast *P. putida* (optička gustoća ili CFU)
- Test traje 16 h
- Primjenjuje se za ispitivanje toksičnosti površinskih, podzemnih i otpadnih voda
- Nedostatak – nije primjenjiv za obojane uzorke vode i vode koje sadrže suspendirane i hlapive tvari
- $EC_{10}$  i  $EC_{50}$



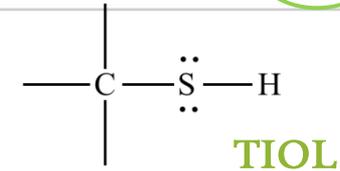
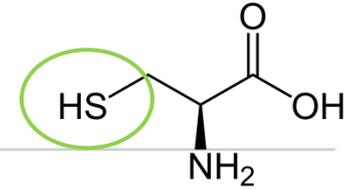
# Određivanje toksičnosti sa *Pseudomonas putidom*

**PRIMJER:** Ispitivanje toksičnosti nanočestica (AgNPs) i srebra ( $\text{AgNO}_3$ ) na *Pseudomonas putidu*

- AgNPs – **dominantni materijal u proizvodnji medicinske opreme, u ambalaži za prehrambene proizvode, kozmetici i dr.**
- Antimikrobna, katalitička i plazmonska svojstva
- Godišnje se u svijetu proizvede 500 t nanosrebra
- Ag – negativan utjecaj na okoliš (otpadne vode, površinske vode, tlo)
- Ag – negativno utječe na bakterije koje sudjeluju u kruženju N, S i P
- Toksičnost AgNPs uspoređuje se s toksičnosti ionskog oblika srebra
- Antibakterijsko djelovanje AgNPs - temelji se na kontinuiranom otpuštanju iona srebra s površine AgNPs
- AgNPs izaziva – **GENOTOKSIČNOST**, potiče apoptoze (kontrolirane stanične smrti), izaziva oksidativni stres



# Određivanje toksičnosti sa *Pseudomonas putidom*



- Srebro – **ANTIMIKROBNA** svojstva:

Interakcija Ag sa tiolom (dio proteina CISTEIN – aminokiselina koja sadrži tiolni bočni lanac koji sudjeluje u enzimskim reakcijama – tiolna skupina doprinosi velikoj reaktivnosti cisteina) – dovodi do inaktivacije proteina

**Posljedice:** poticanje oksidativnog stresa, štetno djelovanje na staničnu membranu i nukleinske kiseline, utječu na proizvodnju ATP, smanjena sposobnost replikacije DNA

Tiol – podložan oksidaciji nakon čega nastaje cistin koji ima važnu ulogu u proteinima

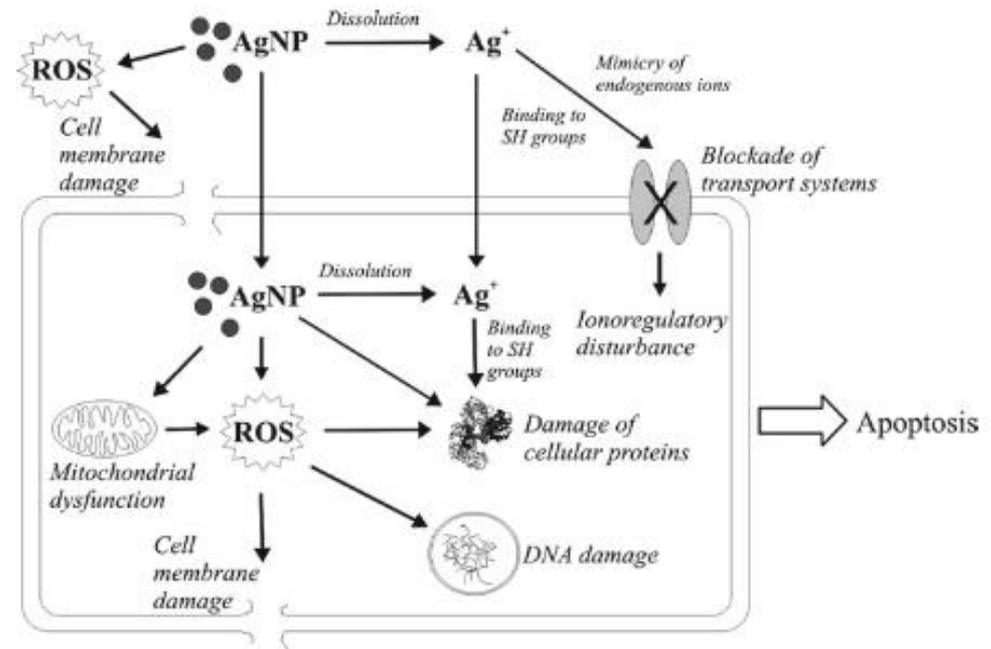


Fig. 1 Possible modes of action of nanosilver toxicity

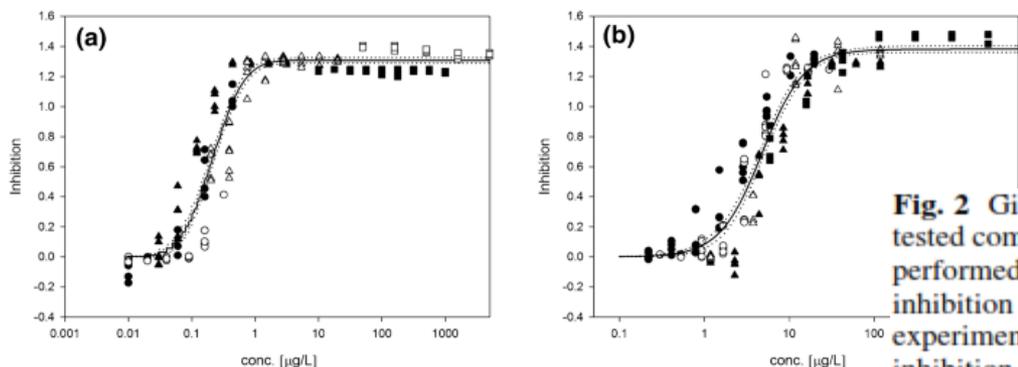


# Određivanje toksičnosti sa *Pseudomonas putidom*

**Table 4** Overview of  $EC_{05}$ ,  $EC_{10}$  and  $EC_{50}$  values in  $\mu\text{g L}^{-1}$  total silver. Details on parameter estimates and concentration–response models are given in the supporting information, Table 1 and an overview on the curve fits to the raw data is given in Fig. 2

Acronym	Particle size (TEM based)	Particle coating	$EC_{05}$	$EC_{10}$	$EC_{50}$
AgNO <sub>3</sub>	None	None	0.043 [0.053–0.036]	0.058 [0.071–0.05]	0.16 [0.18–1.69]
nAg1	8 nm	None	0.73 [0.94–0.59]	1.11 [1.36–0.92]	3.46 [3.84–3.10]
nAg2	14 nm	None	1.96 [–]	3.24 [3.75–2.77]	11.6 [12.5–11]
nAg3	20 nm	None	0.13 [0.15–0.11]	0.15 [0.17–0.13]	0.25 [0.28–0.26]
nAg4	20 nm	Citrate	3.41 [4.82–2.59]	4.93 [6.52–3.88]	13.4 [15.3–11.6]
nAg5	20 nm	Tannic acid	0.22 [0.29–0.18]	0.34 [0.41–0.28]	1.03 [1.16–0.93]
nAg6	40 nm	Citrate	0.42 [0.57–0.33]	0.69 [0.86–0.55]	2.40 [2.68–2.13]
nAg7	60 nm	None	0.98 [1.43–0.71]	1.66 [2.22–1.25]	6.9 [7.95–5.90]

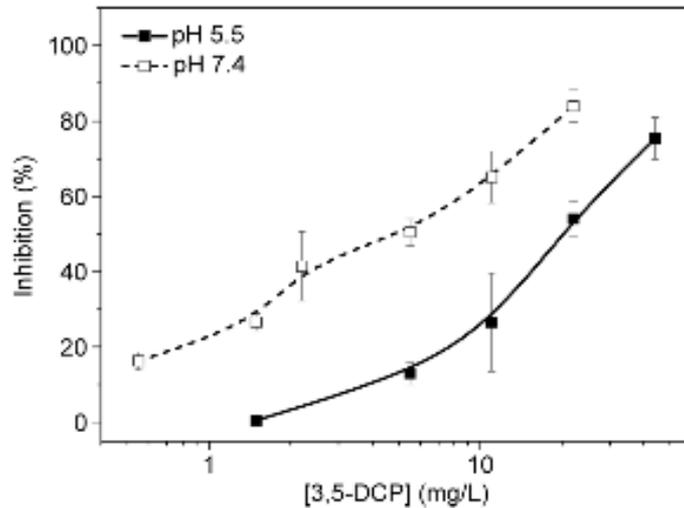
ANTAGONISTIČKI  
UČINAK



**Fig. 2** Gives an overview on the raw data and the curve fits for all tested compounds, stating the respective models well as the number of performed independent experiments. **a** Curve fit (Probit) to the growth inhibition data for silver nitrate; results pooled from 6 independent experiments. **b** Curve fit (Morgan-Mercer-Flodin) to the growth inhibition data for nAg1; results pooled from 5 independent experiments. **c** Curve fit (Weibull) to the growth inhibition data for



# Određivanje toksičnosti sa *Pseudomonas putidom*



**Fig. 4** Inhibition of the respiration rate of *P. putida* exposed to 3,5-DCP. The concentration-response data was obtained by performing the CO<sub>2</sub>-TOX bioassay at neutral and acidic conditions (pH 7.4 and 5.5). Vertical bars represent  $\pm$  standard deviation of the media ( $n=3$ )

**Table 2** EC<sub>50</sub> values for 3,5-DCP reported in the literature for different toxicity bioassays, and in this paper

Bioassay (exposure time)	EC <sub>50</sub> (mg/L)	CV%	Reference
CO <sub>2</sub> -TOX- <i>P. putida</i> pH 7.4 (50 min)	4.93	22.11	This paper
CO <sub>2</sub> -TOX- <i>P. putida</i> pH 5.5 (50 min)	19.14	8.15	This paper
mPCMIT- <i>P. putida</i> (16 h)	10.06	12.73	This paper
FM-TOX- <i>P. putida</i> (60 min)	9.2	28.26	Yong et al. (2011)
FM-TOX- <i>E. coli</i> (60 min)	4.88	n.a.	Catterall et al. (2010)
Baroxymeter- <i>P. putida</i> (5 min)	25 <sup>a</sup>	10	Tzoris et al. (2005)
Micredox- <i>P. putida</i> (60 min)	8.5	n.a.	Tizzard et al. (2004)
Microtox- <i>V. fischeri</i> (30 min)	3.39 <sup>b</sup>	11.25 <sup>b</sup>	Dalzell et al. (2002)
ASRIT (3 h)	12.97	30.06	Gutiérrez et al. (2002)
PCMIT- <i>P. putida</i> (16 h)	21.4	23	ISO 10712 (1995)

<sup>a</sup> 48 % of inhibition

<sup>b</sup> Calculated from presented results

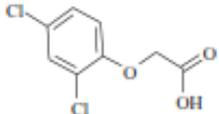
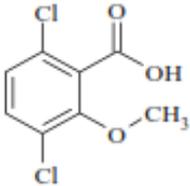
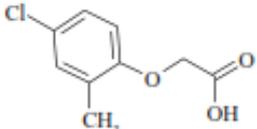
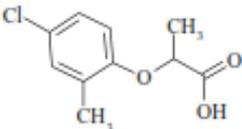
n.a. not available



# Određivanje toksičnosti sa *Pseudomonas putidom*

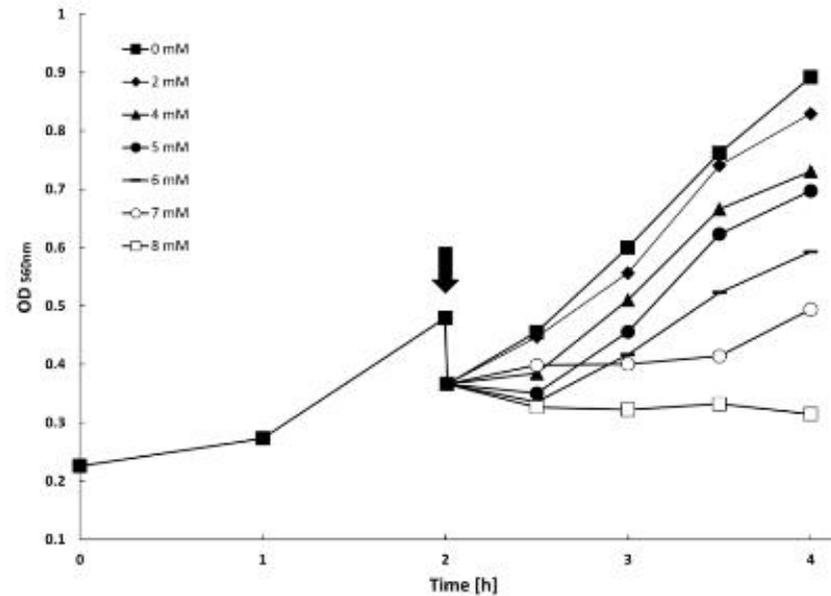
PRIMJER: Određivanje toksičnosti herbicida

**Table 2.** Chemical structures of herbicides

Herbicide	Chemical structure	Herbicide	Chemical structure
2,4-D		dicamba	
MCPA		MCPP	



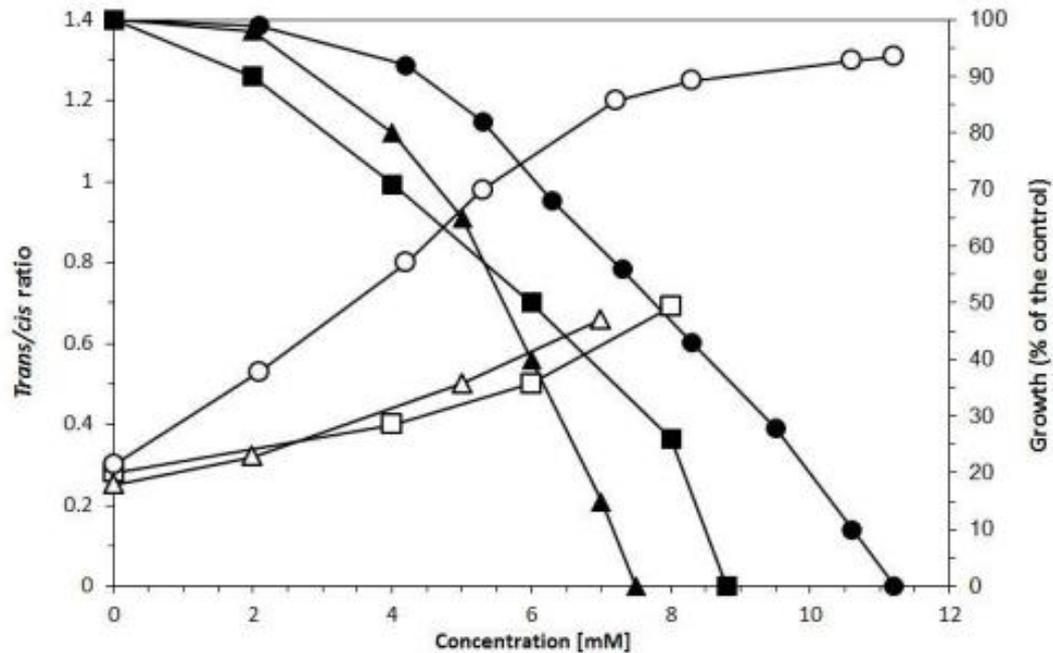
# Određivanje toksičnosti sa *Pseudomonas putidom*



**Fig 11.** Growth curves of *Pseudomonas putida* in experiment with 2,4-D at different concentrations (0 mM, 2 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM). Moment of *Pseudomonas putida* pellet suspension into media with different concentration of toxicant is indicated by the arrow.



# Određivanje toksičnosti sa *Pseudomonas putidom*



**Fig 12.** Effect of 2,4-D (▲,△), [2,4-DDAEC<sub>6</sub>][Br] (■,□), and [2,4-DDAEC<sub>10</sub>][Br] (●,○) on growth (closed symbols) and the *trans/cis* ratio of unsaturated fatty acids (open symbols) of *Pseudomonas putida*; adapted from (Piotrowska et al. 2016).



# Određivanje toksičnosti sa *Pseudomonas putidom*

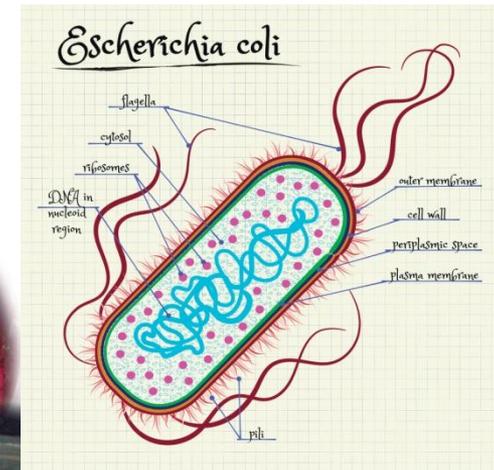
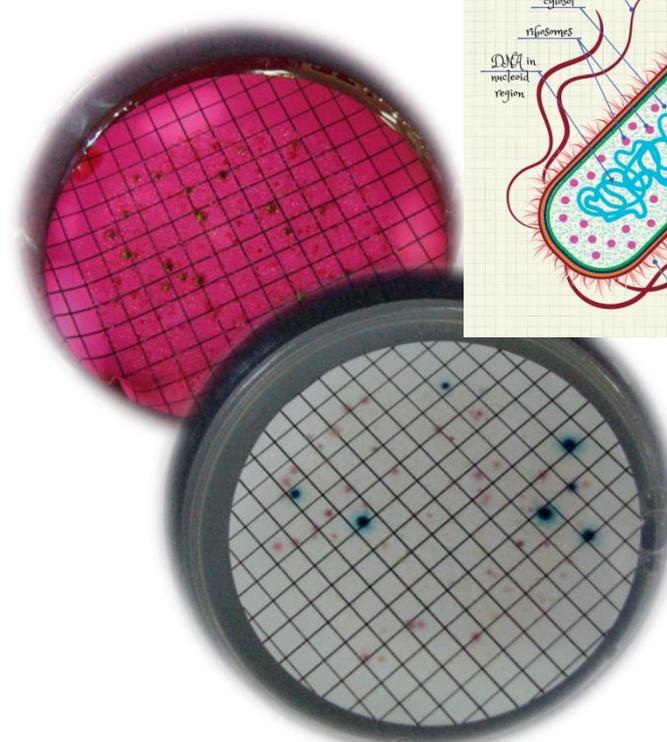
**Table 6.** Toxicity and its effect on *cis-trans* isomerisation of the investigated compounds; <sup>(a)</sup> EC<sub>50</sub> - concentrations (50% growth inhibition) measured with *Pseudomonas putida* cells; <sup>(b)</sup> the concentration which caused an increase in the *trans/cis* ratio of unsaturated fatty acids to 50% of the maximum *trans/cis* level; the standard deviation is calculated based on triplicate data analysis

Compound	EC <sub>50</sub> <sup>a</sup> [mM]	<i>trans/cis</i> 50% <sup>b</sup> [mM]
2,4-D	5.6 ± 0.28	4.5 ± 0.24
[2,4-DDAEC <sub>6</sub> ][Br]	6.0 ± 0.31	6.2 ± 0.33
[2,4-DDAEC <sub>10</sub> ][Br]	7.8 ± 0.40	4.2 ± 0.21
MCPA	6.7 ± 0.34	6.2 ± 0.31
[MCPADAEC <sub>6</sub> ][Br]	8.3 ± 0.39	3.7 ± 0.18
[MCPADAEC <sub>10</sub> ][Br]	13.4 ± 0.71	4.4 ± 0.20

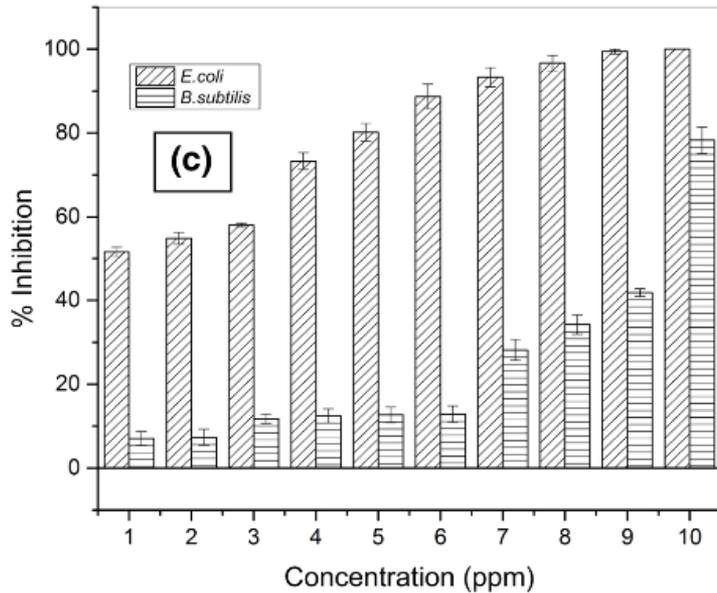


# Određivanje toksičnosti s *Escherichia coli*

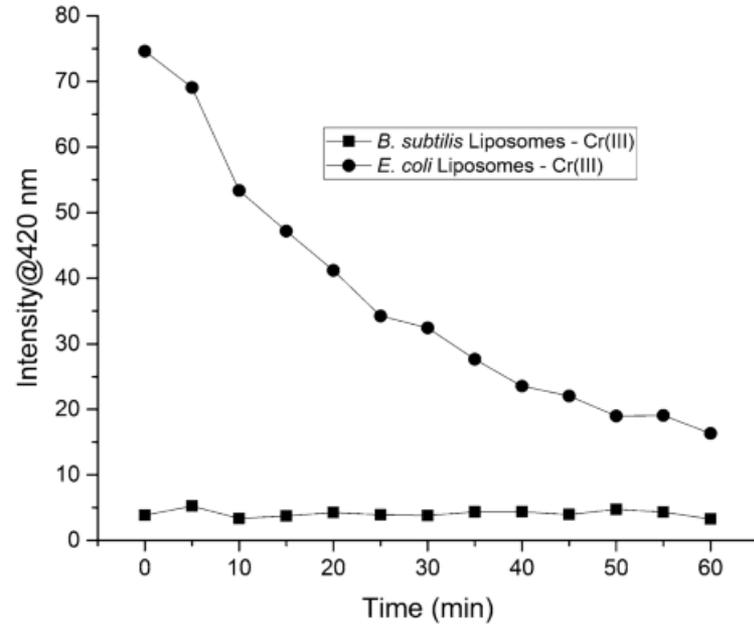
- *E. coli*- G (-) bakterija, štapićastog oblika, fakultativni anaerob
- Živi u donjem dijelu probavnog trakta sisavca
- Prisutnost u vodama – fekalno onečišćenje
- Uzrokuje infekcije mokraćnog mjehura



## PRIMJER: Određivanje toksičnosti Cr(III)



**Fig. 1** Growth profile pattern of *Bacillus subtilis* and *E. coli* in the presence and absence of Cr(III) (a, b) and growth inhibition of bacteria by Cr(III) (c)



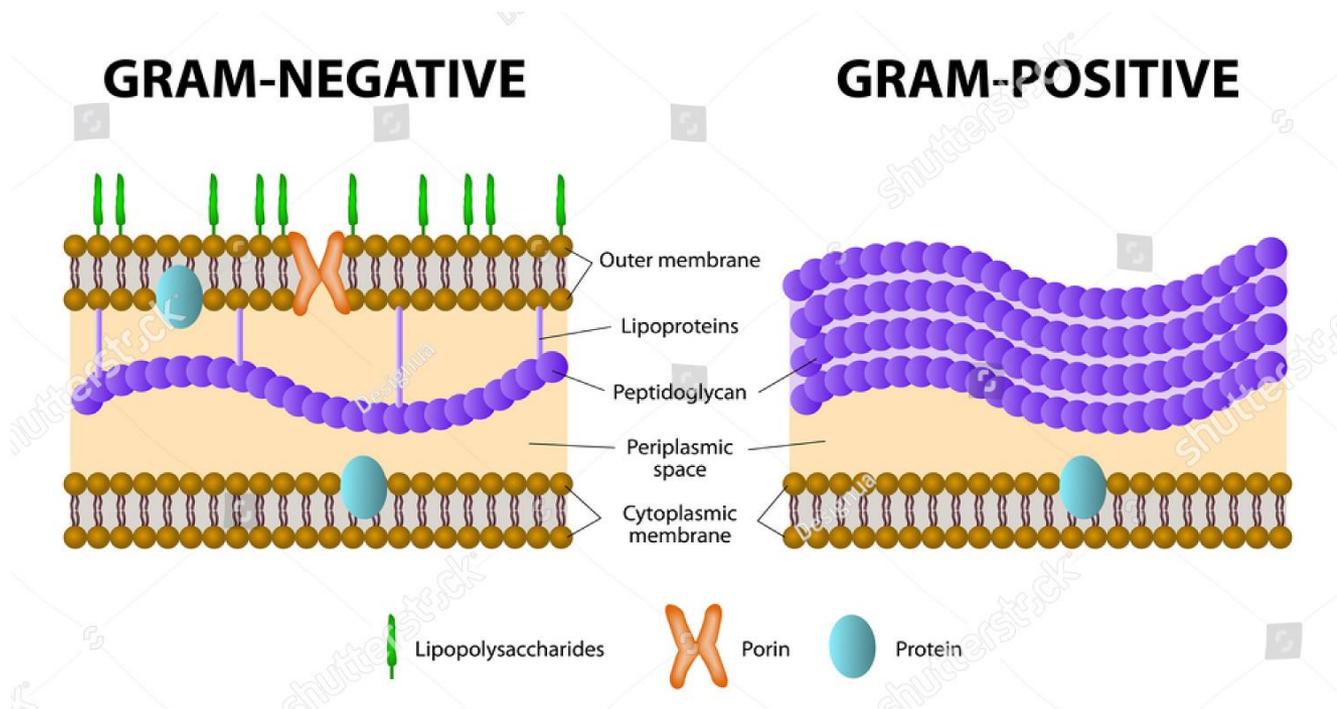
**Fig. 4** Membrane permeabilization studies with 1-*N*-phenyl-naphthylamine (NPN) by *Bacillus subtilis* and *E. coli* after treatment with CrCl<sub>3</sub>



# Određivanje toksičnosti s *E. coli*

Zbog čega je *E. coli* osjetljivija na Cr(III) od *B. subtilis*?

- Cr(III) toksičnost *E. coli* > Cr(III) toksičnost *B. subtilis*
- *B. subtilis* – G (+) bakterija, deblja stanična stjenka od peptidoglikana
- G (-) bakterije – negativno nabijen lipopolisaharidni sloj – jaki afinitet Cr (+3) iona



# Određivanje akutne toksičnosti sa *Saccharomyces cerevisiae*



- *Saccharomyces cerevisiae* – pivski ili pekarski kvasac
- Jednostanična gljiva, stanice su jajolikog oblika (5-8  $\mu\text{m}$  u promjeru), glatke površine
- Stvara blastospore – **razmnožava** se nespolnim načinom – **pupanjem**
- **Fakultativni anaerob**
- Raste u širokom rasponu pH-vrijednosti
- *S. cerevisiae* – najproučavaniji eukariotski modalni mikroorganizam, u molekularnoj i staničnoj biologiji
- Veliki značaj u istraživanjima na području **genetike**, **fiziologije** i **medicine**
- **Karakteristike** *S. cerevisiae*: nepatogen, lako se kultivira, kratko generacijsko vrijeme, poznat mu je životni ciklus i dr.



# Određivanje akutne toksičnosti sa *Saccharomyces cerevisiae*

- *Saccharomyces cerevisiae* test toksičnosti:

(1) Yeast Toxicity Test (YTT)

(2) Bojanje stanica kvasca *S. cerevisiae* metilenskim modrilom



# Određivanje akutne toksičnosti s *Saccharomyces cerevisiae*

## Yeast Toxicity Test (YTT)

- zasniva se na činjenici da kvasac može fermentirati saharozu do ugljikova(IV) dioksida
- **PRINCIP rada:**
- Fermentacija – odvija u hermetički zatvorenim bočicama koje sadrže tekući medij (inkubacija 16 h, 28 °C)
- Tijekom fermentacije nastaje plin koji u otvoreni injekcijski cilindar istiskuje ekvivalentni volumen tekućine

1. UZORAK = SAHAROZA (Slijepa proba)

2. UZORAK = SAHAROZA + ONEČIŠĆUJUĆA TVAR

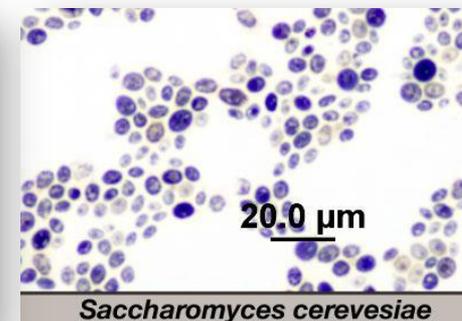
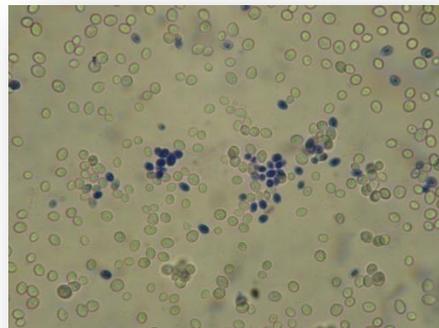
$$V(\text{CO}_2 \text{ uzorak 1}) > V(\text{CO}_2 \text{ uzorak 2})$$
$$\% I = (V_{\text{kontrola}} - V_{\text{uzorak}}) / V_{\text{kontrola}} * 100$$



# Određivanje akutne toksičnosti sa *Saccharomyces cerevisiae*

## Bojanje stanica kvasca *S. cerevisiae* metilenskim modrilom

- Metilensko modrilo – indikator da li su stanice kvasaca žive ili mrtve
- Metilensko modrilo – **inhibira respiraciju** jer služi kao akceptor vodikovih iona nastalih pri respiraciji – kvasci ih dalje ne mogu koristiti u metabolizmu za proizvodnju energije
- Dolazi do redukcije boje vodikovim protonima – metilensko modrilo se obezboji
- Ako je stanica **ŽIVA** (enzimi aktivni) – stanica se **OBEZBOJI** (stanice kvasaca pod mikroskopom se vide kao bijele/prozirne)
- Plava boja ne mora nužno značiti da je stanica mrtva – stanica oslabljena (enzimi inaktivirani)

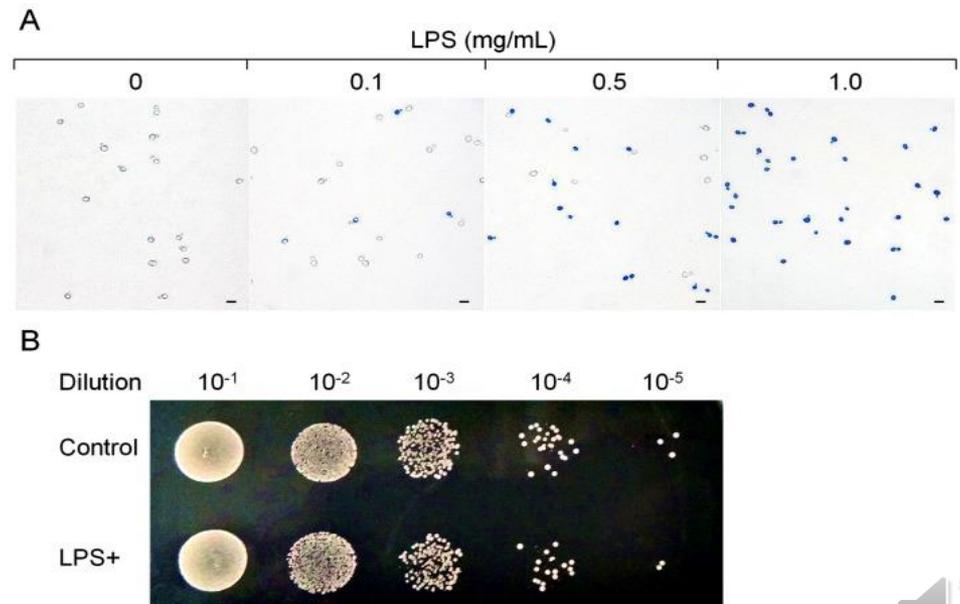


# Određivanje akutne toksičnosti s *Saccharomyces cerevisiae*

## PRINCIP RADA:

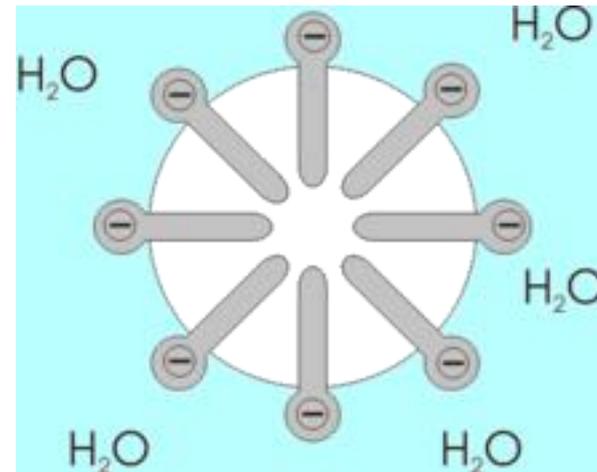
- Direktno brojanje stanica pod mikroskopom
- Računa se brojčani odnos živih i mrtvih stanica na 100 izbrojenih
- Izračuna postotak inhibicije pri određenoj koncentraciji toksične tvari
- Iz grafičkog prikaza izračunava se efektivna koncentracija ( $EC_{50}$ ) za pojedinu toksičnu supstancu
- Inkubacija: 15 min, 28 °C

$$\% I = (\text{Broj mrtvih stanica} - \text{Broj mrtvih stanica u kontroli}) / \text{Broj mrtvih stanica} * 100$$



# Određivanje akutne toksičnosti sa *Saccharomyces cerevisiae* - PRIMJER

- SURFAKTANTI - (površinske aktivne tvari) – skupina molekula najpoznatijih po upotrebi u sastavu detergenata i ostalih sredstva za pranje i čišćenje
- Nakon upotrebe ispuštaju se u kanalizacijski sustav, te većina surfaktanata završi raspršena u vodi, tlu ili sedimentu
- Većina surfaktanata – biološki razgradiva



# Određivanje akutne toksičnosti sa *Saccharomyces cerevisiae* - PRIMJER

**Tablica 3.** Srednja EC<sub>50</sub> vrijednost za surfaktante u testu fermentacije. SD = standardna devijacija, CV = koeficijent varijacije ( % ).

Surfaktant	EC <sub>50</sub> (mg/L)	SD	CV
Kutriacid 95A	80,0	10,0	12,50
Ethomeen T/15	3,0	0,2	6,90
Oxidet DM-4	68,0	8,0	11,76
Arlypon VPC	3,7	0,6	16,22
Servamine KOO 330	71,0	0,0	2,82
Empilan KI 8	-	-	-
Lutensit TC-EHS	-	-	-
Genapol PF 20	-	-	-
Na-Cumolsulfonat	-	-	-
Texapon LS 35	400,0	20,0	4,88
Hospatur SAS 60	1520,0	60,0	3,95
Solfodac AC-3-I	10,0	0,0	14,29
SDS	135,0	5,0	3,70
N-hexadecylpyridinium	52,0	2,0	3,85
N-dodecylpyridinium	26,0	3,0	11,54

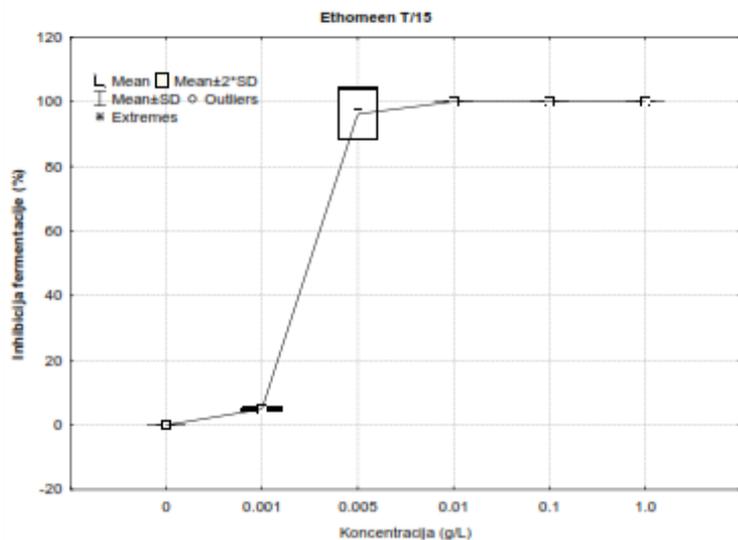
**Tablica 4.** Srednja EC<sub>50</sub> vrijednost za različite surfaktante u testu metilenskim modrilom. SD = standardna devijacija CV = koeficijent varijacije ( % ).

Surfaktant	EC <sub>50</sub> (mg/L)	SD	CV
Kutriacid 95A	50,0	10,0	20,00
Ethomeen T/15	3,0	1,0	33,33
Oxidet DM-4	7,0	1,0	14,29
Arlypon VPC	4,3	0,3	6,98
Servamine KOO 330	7,7	1,2	15,58
Empilan KI 8	7800,0	1,2	15,38
Lutensit TC-EHS	2000,0	500,0	25,00
Genapol PF 20	8500,0	400,0	4,71
Na-Cumolsulfonat	-	-	-
Texapon LS 35	70,0	20,0	28,57
Hospatur SAS 60	1900,0	400,0	21,05
Solfodac AC-3-I	6,0	2,0	33,33
SDS	110,0	30,0	27,27
N-hexadecylpyridinium	19,0	5,0	26,32
N-dodecylpyridinium	70,0	10,0	14,29

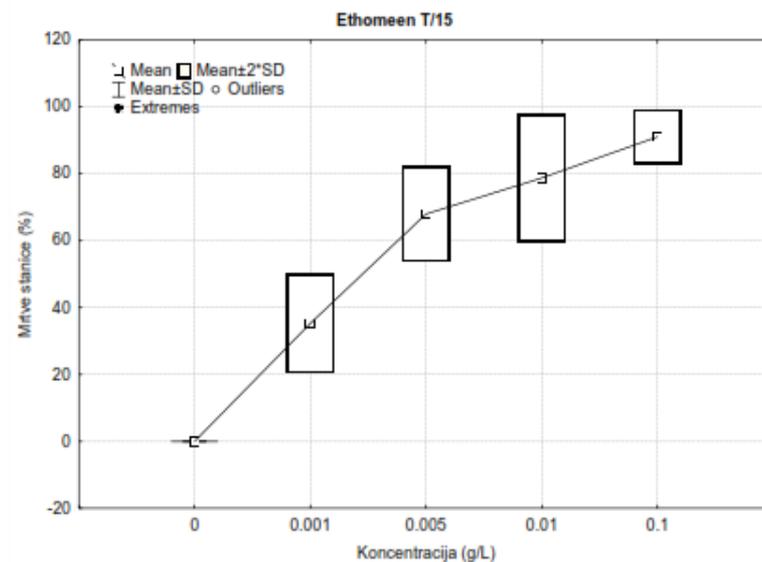
STUPANJ ŠTETNOSTI	EC <sub>50</sub> (mg/L)
VRLO TOKSIČAN	0,1 – 1
TOKSIČAN	1 – 10
ŠTETAN	10 – 100
NIJE ŠTETAN	> 100



# Određivanje akutne toksičnosti sa *Saccharomyces cerevisiae* - PRIMJER



**Slika 18.** Grafički prikaz odnosa inhibicije za Ethomeen T/15 u testu fermentacije.  $EC_{50} = 3,0$  mg/L.

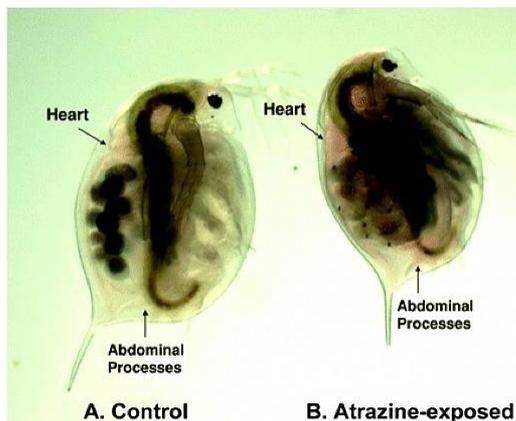
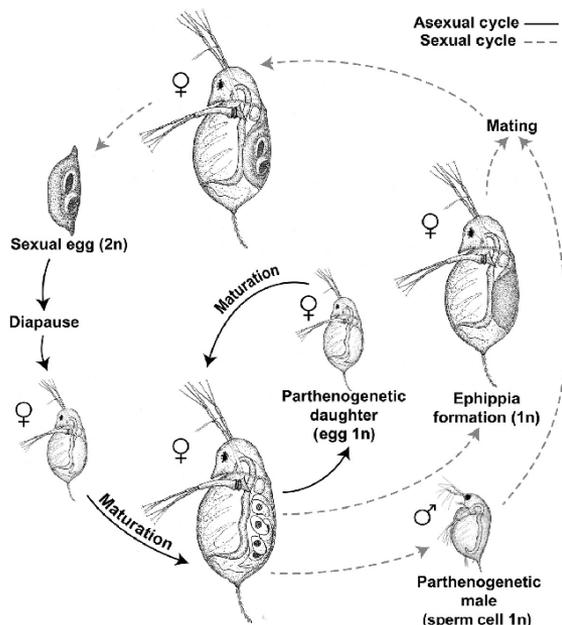


**Slika 19.** Grafički prikaz odnosa inhibicije za Ethomeen T/15 u testu metilenskog modrila.  $EC_{50} = 3,0$  mg/L.

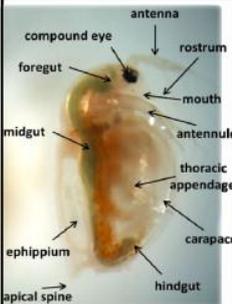


# Određivanje akutne toksičnosti s *Daphnia magna*

- *Daphnia magna* – vodenbuha – pripada potkoljenu rakova (Crustacea)
- Malih veličina – od 0,2 do 6 mm
- Hrane se algama, bakterijama, organskim i anorganskim česticama
- Spolno i nespolno razmnožavanje



AKUMULACIJA  
HEMOGLOBINA

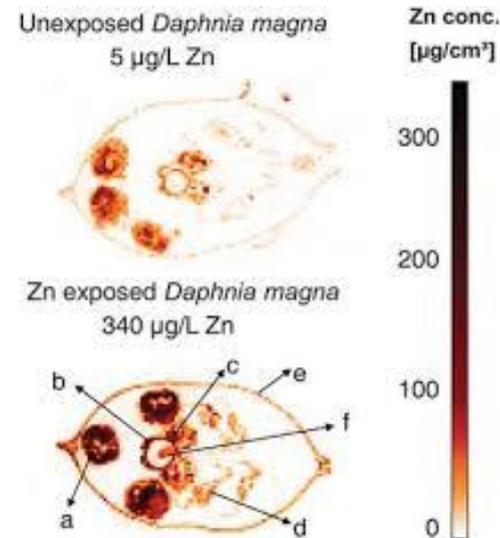
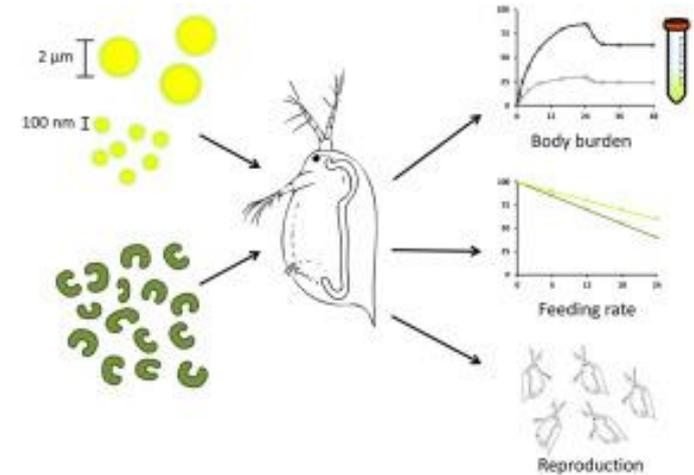
	Adults	Neonate
Control		
AgNP 24 h		

# Određivanje akutne toksičnosti s *Daphnia magna*

- Razlozi odabira vodenbuhe kao modalnog organizma:

- brojnost;
- uloga u prehrambenom lancu (prehrana fitoplanktonom, učinak na kruženje hranjivih tvari, uloga plijena za sekundarne konzumente
- geografska distribucija
- kratko generacijsko vrijeme – visok rast populacije

- Indikatorska vrsta slatkovodnih rijeka i ribnjaka – pad brojnosti ukazuje na okolišne probleme
- Vodenbuha – plijen beskralješnjacima i ribama koje se prehranjuju planktonom
- Imaju veliki **EKONOMSKI ZNAČAJ** – koriste se u akvakulturi za prehranu riba ili kontroliranje brojnosti fitoplanktona
- Mogu prenositi parazite – prijetnja čovjekovom zdravlju

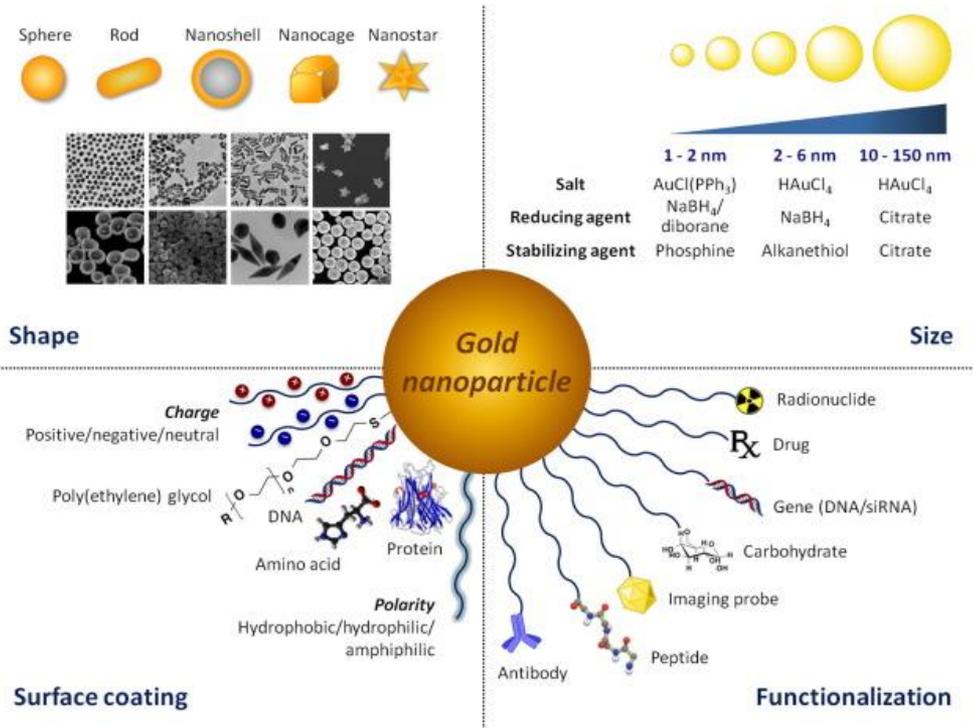


# Određivanje akutne toksičnosti s *Daphnia magna* - PRIMJER

**PRIMJER:** Ispitivanje ekotoksičnosti nanočestica zlata i srebra i njihovih ionskih oblika ( $\text{Ag}^+$  i  $\text{Au}^{3+}$ )

- Karakteristike elementarnog zlata: inertnost, mala toksičnost i biokompatibilnost
- Kontradiktorna istraživanja – nanočestice zlata – toksičan/netoksičan
- Prednost - nanočestica zlata - vrlo stabilne (nisu podložne otapanju ili značajnim promjenama veličine ili oblika u određenim okolišnim uvjetima)
- Primjenjuju se u različitim područjima znanosti, tehnologije te u medicini (terapijskim i dijagnostičkim sustavima)

<https://www.krenizdravo.rtl.hr/zdravlje/rak-gusterace-bi-se-mogao-lijeciti-sitnim-cesticama-zlata>



# Određivanje akutne toksičnosti s *Daphnia magna* - PRIMJER

- CILJ: odrediti EC<sub>50</sub>, LOAEL i NOAEL nakon 24 i 48 h za svaki pojedini ispitivani nano i ionski oblik srebra i zlata na testni organizam vodenbuhe

**Tablica 1.** Toksičnost nanočestica zlata i srebra stabiliziranih cisteinom i glutationom te ionskih oblika zlata i srebra na vodenbuhe. Vrijednosti EC<sub>50</sub> su izražene kao srednje vrijednosti ± SD (s 95% CI u zagradama) i to u µg Ag ili Au/L. / označava kako EC<sub>50</sub> vrijednosti nisu dobivene.

Ispitivana vrsta (raspon korištenih koncentracija)	Vrijeme izloženosti (h)	EC <sub>50</sub> (95% CI)
CYSnanoAg (5 - 1000 µg/L)	24	347.6 ± 5.39 (344.19 - 364.39)
	48	193.1 ± 5.20 (192.73 - 206.39)
GSHnanoAg (5 - 750 µg/L)	24	152.5 ± 6.20 (150.76 - 166.14)
	48	119.0 ± 5.79 (116.92 - 131.31)
CYSnanoAu (5 - 5000 µg/L)	24	/
	48	/
GSHnanoAu (5 - 5000 µg/L)	24	/
	48	/
Ag <sup>+</sup> (0,1 - 10 µg/L)	24	1.06 ± 5.69 (0 - 12.75)
	48	0.97 ± 5.13 (0 - 11.74)
Au <sup>3+</sup> (0,5 - 50 µg/L)	24	12.7 ± 6.23 (12.15 - 27.61)
	48	10.4 ± 6.19 (8.63 - 24.01)

Ionski oblik Ag<sup>+</sup> i Au<sup>3+</sup> toksičniji od nanočestica

Nanočestice AU nisu toksične za vodenbuhu

Ag toksičniji od Au

CY – stabilizacija cisteinom  
GS – stabilizacija glutationom



# Određivanje akutne toksičnosti s *Daphnia magna* - PRIMJER

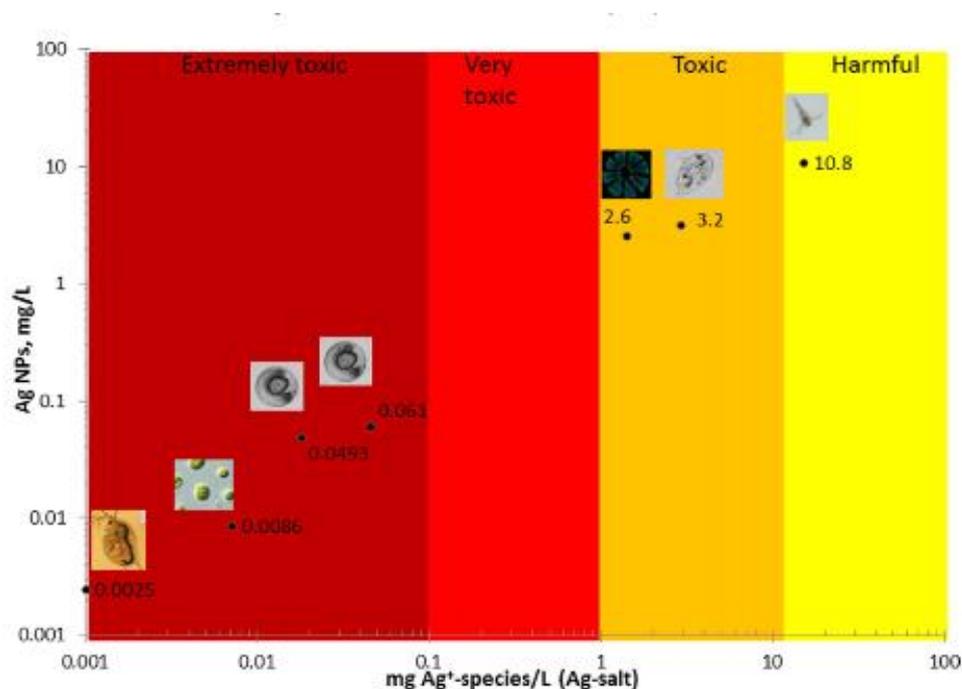
**Table 1**

Toxicity of AgNPs and AgNO<sub>3</sub> towards the different test organisms. EC50 values are arranged from the least sensitive test organism to the most sensitive based on the exposure protocols used. Toxicity of AgNPs and AgNO<sub>3</sub> were ranked to different hazard categories according to EC50 values. For the ranking, we applied the criteria used in Kahru and Dubourguier (2010) and color coding applied by Bondarenko et al. (2013): <0.1 mg/L = extremely toxic (dark red); 0.1–1 mg/L = very toxic (red); 1–10 mg/L = toxic (orange); 10–100 mg/L = harmful (yellow). *D. rerio* test was done at two different times (May 2013, and August 2014) and different developmental stages (hpf–hours post-fertilisation). Cartoons depicting the organisms are symbolic (*V. fischeri* and *P. subcapitata* are from [www.wikipedia.com](http://www.wikipedia.com)).

Test organism	Biological organization	Toxicity Endpoint (biomarker)	International standardization	Effect value (mg AgNPs/L)	Ranking (Ag NPs)	Effect value mg Ag <sup>+</sup> /L (AgNO <sub>3</sub> )	Ranking (Ag <sup>+</sup> )salt
Artemia franciscana	Marine crustacean, arthropoda	Immobility	No	24h EC50 = 1.3 48h EC50 = 10.8	Yellow	48h EC50 = 15.0	Yellow
		Hatching success (% of hatched artemia)	No	24h EC50 = 1.3	Orange		
Tetrahymena thermophila	Freshwater protozoa	Viability (ATP content)	No	2h EC50 = 3.2 24h EC50 = 3.9	Orange	2h EC50 = 2.9 24h EC50 = 2.9	Orange
Vibrio fischeri	Bacteria, prokaryotic microorganisms	Viability (luminescence)	ISO 21338:2010	30min EC50 = 2.6	Orange	30 min EC50 = 1.4	Orange
RTgutGC cells	Rainbow trout's (fish) gut cells in vitro	Viability (metabolic activity)	No	24h EC50 = 0.551	Red		
		Viability (membrane integrity)	No	24h EC50 = 0.732	Red		
		Viability (Lysosomal integrity)	No	24h EC50 = 0.576	Red		
Danio rerio, 2 hpf embryo (May 2013)	Freshwater fish	Lethality (lethal malformations)	OECD 236: 2013	24h LC50 = 0.084 48h LC50 = 0.066	Red		Red
Danio rerio, 2 hpf embryo (August 2014)	Freshwater fish	Lethality	OECD 236: 2013	24h LC50 = 0.061 48h LC50 = 0.061	Red	24h LC50 = 0.045 48h LC50 = 0.032	Red
Danio rerio, 28 hpf, dechorionated (May 2013)	Freshwater fish	Lethality	No	24h LC50 = 0.0324 48h LC50 = 0.0334	Red		Red
Danio rerio, 72 hpf, hatched embryos (May 2013)	Freshwater fish	Lethality	No	24h LC50 = 0.0493	Red	24h LC50 = 0.018 48h LC50 = 0.014	Red
Pseudokirchneriella subcapitata	Freshwater green microalgae	Growth (cell density)	OECD 201:2011	72h EC50 = 0.0086	Red	72h EC50 = 0.0071	Red
Daphnia magna, ADaM medium	Freshwater crustacean, arthropoda	Lethality (Immobility)	OECD 202:2004	48h EC50 = 0.0034	Red	48h EC50 = 0.0014	Red
Daphnia magna, OECD medium	Freshwater crustacean, arthropoda	Lethality (Immobility)	OECD 202:2004	48h EC50 = 0.0025	Red	48h EC50 = 0.001	Red



# Određivanje akutne toksičnosti s *Daphnia magna* - PRIMJER



**Fig. 7.** The correlation between the toxicity of AgNPs and Ag<sup>+</sup>-species to different test organisms. Those values from Table 1 were included, which were available for both AgNPs and AgNO<sub>3</sub> (eight of them) (for *D. magna* two values in two different test media are reported). The numerical values for AgNP toxicity are indicated for each test near the pictogram. The hazard ranking criteria are as described in Kahru and Dubourguier (2010) and color coding as in Bondarenko et al. (2013): <math>< 0.1</math> mg/L = extremely toxic (dark red); <math>0.1 - 1</math> mg/L = very toxic (red); <math>1 - 10</math> mg/L = toxic (orange); <math>10 - 100</math> mg/L = harmful (yellow). Cartoons depicting the organisms are symbolic (*V. fischeri* and *P. subcapitata* are from www.wikipedia.com).



# Određivanje akutne toksičnosti s *Daphnia magna* - PRIMJER

**PRIMJER:** Ispitivanje **ekotoksičnosti mikroplastike** na *Daphniu magnu*

- Mikroplastika – veličina plastike < 5 mm
- Nanoplastika – veličina plastike od 1 μm do 100 nm
- Mnogi organizmi – ingestija mikro i nanoplastike
- Ispitana toksičnost mikroplastike na:



*Daphnia magna*,

*Notodromas monacha*,

*Gammarus pulex* (rakušci),

*Lumbriculus variegatus*,



*Potamopyrgus antipodarum* i na različite riblje vrste



# Određivanje akutne toksičnosti s *Daphnia magna* - PRIMJER

- Mikroplastika uzrokuje: OKSIDACIJSKI STRES, OŠTEĆENJE TKIVA, PROMJENE U METABOLIZMU, SMANJENU MOGUĆNOST REPRODUKCIJE, UTJEČE NA PONAŠANJE
- 29 % smanjena prehrana *D. magne* ukoliko je izložena mikroplastici

**Table 2**

Feeding rates of *Daphnia magna* (1 week old) during 24 h exposure to algae only (control group) or algae in the presence of 1 mg/l of 100 nm or 2  $\mu$ m polystyrene (PS) particles. Results of linear regression analysis are shown (SE=Standard Error).

Group	Feeding rate (ingested algal cells/h)	SE (cells/h)	R <sup>2</sup>	P-value (diff. to control)
Control (algae)	$2.05 \cdot 10^4$	$2.0 \cdot 10^3$	0.97	–
Algae + 100 nm PS	$1.63 \cdot 10^4$	$1.6 \cdot 10^3$	0.82	0.013
Algae + 2 $\mu$ m PS	$1.91 \cdot 10^4$	$1.9 \cdot 10^3$	0.85	0.093

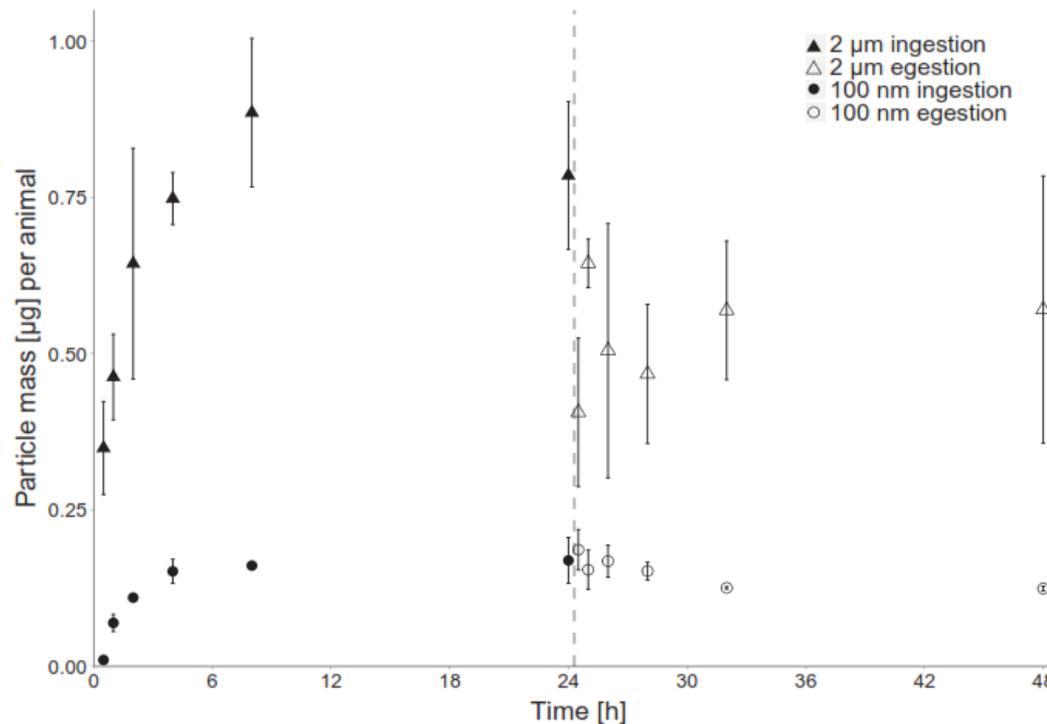


# Određivanje akutne toksičnosti s *Daphnia magna* - PRIMJER

402

S. Rist et al. / Environmental Pollution 228 (2017) 398–407

Bolja ingestija i egestija mikroplastike (2  $\mu\text{m}$ ) od nanoplastike 100 nm



**Fig. 1.** Body burdens of particles (mass/animal) for *Daphnia magna* (1 week old) exposed to 100 nm (round symbols) and 2  $\mu\text{m}$  (triangular symbols) polystyrene particles during a 24 h ingestion phase (closed symbols) in a particle suspension of 1 mg/l, followed by a 24 h egestion phase (open symbols) in clean medium. The mean and standard deviation for the 3 replicates per sampling point are shown.

