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

Kolegij:
PRIMJENA EKOTOKSIKOLOGIJE
5. predavanje

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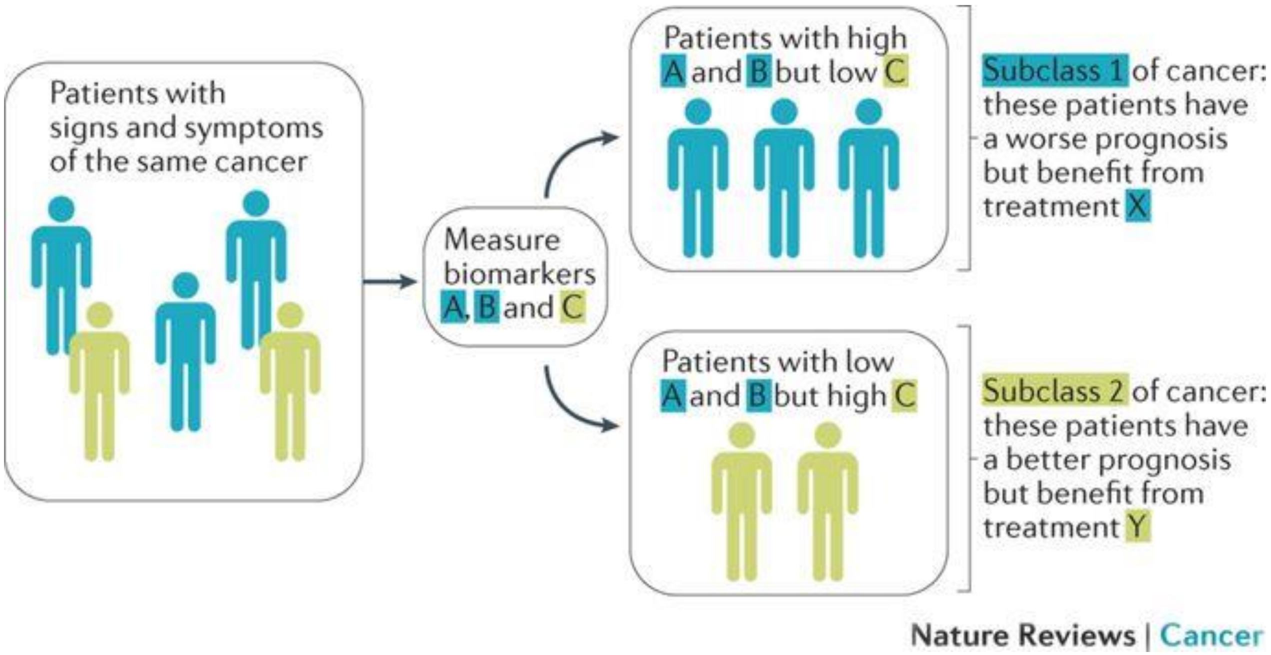
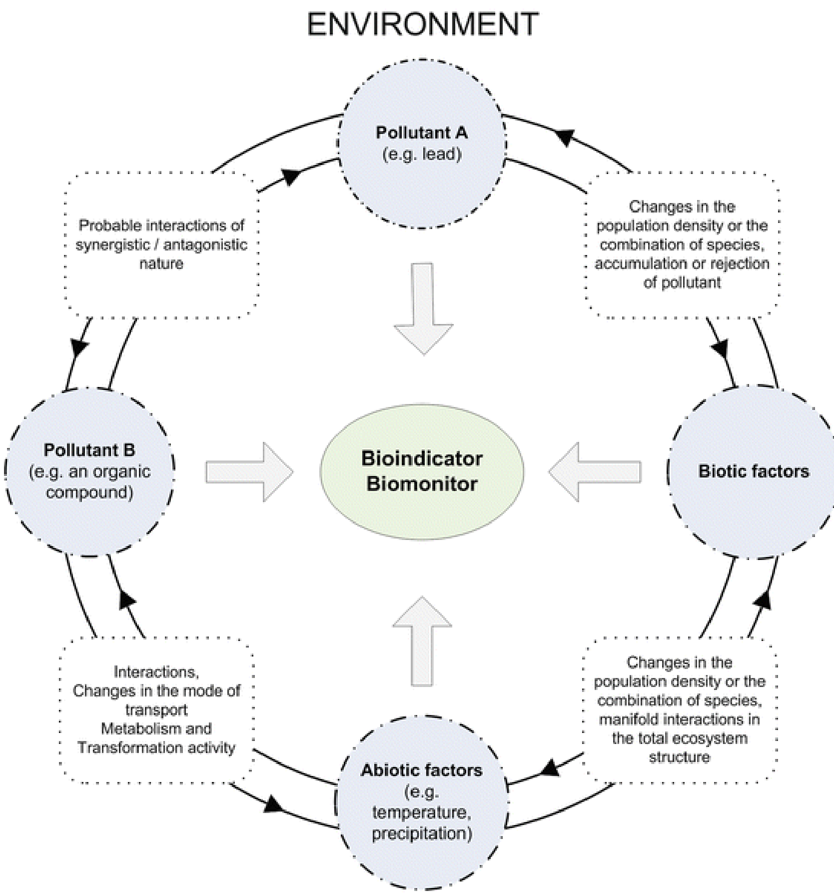
SADRŽAJ

BIOMONITORING I BIOMARKERI U EKOTOKSIKOLOGIJI



BIOMONITORING

- BIOLOŠKI MONITORING ili BIOMONITORING je primjena živih organizama kao bioindikatora promjena u okolišu tijekom nekog vremenskog razdoblja

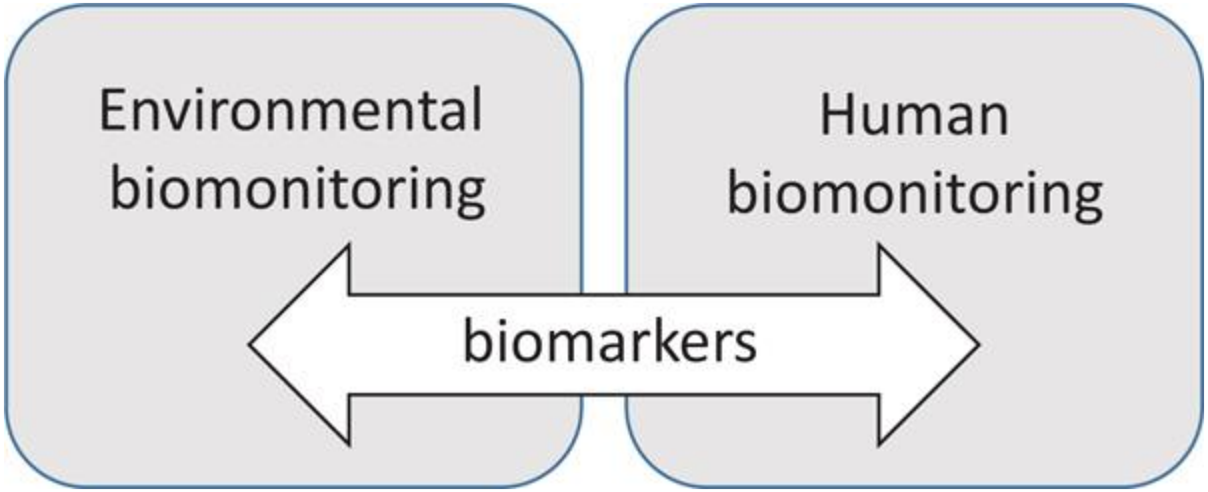


BIOMONITORING

- Može se podijeliti u dvije skupine:

  (1) OKOLIŠNI BIOMONITORING (OBM) – prati se utjecaj štetnih tvari dospjelih u okoliš na promjene odnosno štetne učinke na zrak, vodu i tlo

  (2) HUMANI BIOMONITORING (HBM) – bavi se istraživanjem utjecaja štetnih tvari na ljudski organizam, te omogućuje procjenu tjelesnog opterećenja štetnim čimbenicima mjerenjem pokazatelja - biomarkera



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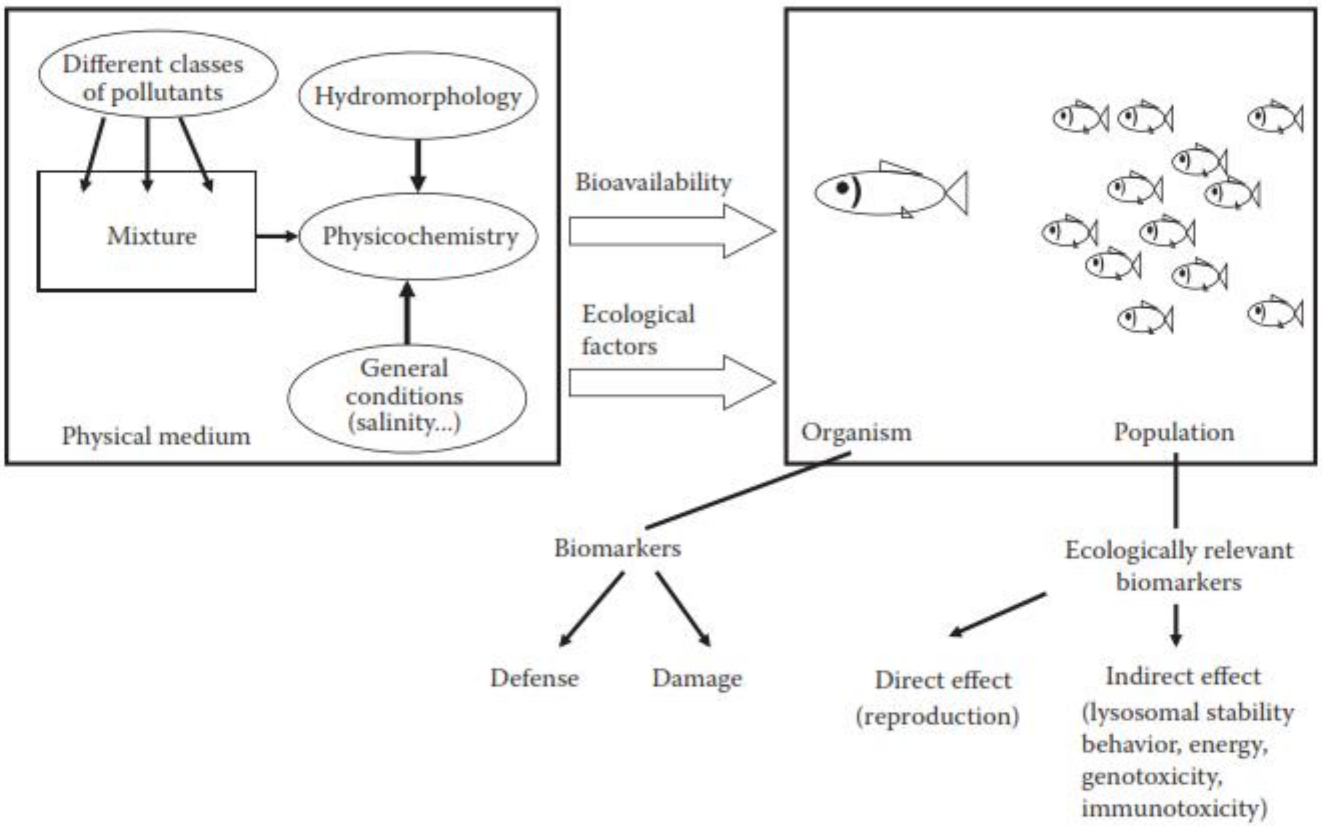


FIGURE 16.1
Methods for quality assessment of aquatic environments.

BIOMONITORING

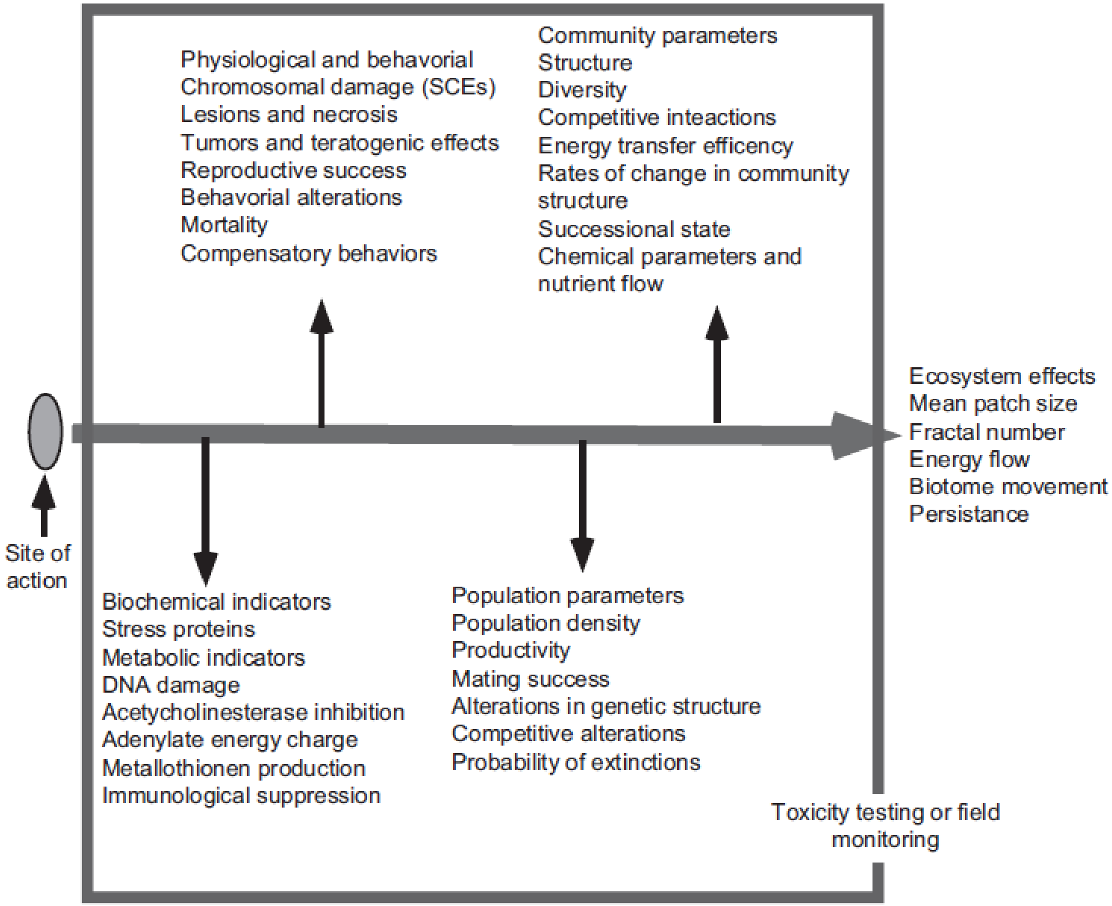


Figure 13.6 Methods and measurements used in biomonitoring for ecological effects. A number of methods are used both in a laboratory situation and in the field to attempt to classify the effects of xenobiotics upon ecological systems. Toxicity tests can be used to examine effects at several levels of biological organization and can be performed with species introduced as monitors for a particular environment.

BIOINDIKATORI

PREDNOSTI BIOINDIKATORA:

- Biološki utjecaji se mogu utvrditi
- Praćenje sinergističkih i antagonističkih utjecaja onečišćujućih tvari na organizme
- U ranoj fazi se mogu odrediti utjecaji štetnih tvari na organizme
- Ekonomski prihvatljiv način praćenja promjena u okolišu

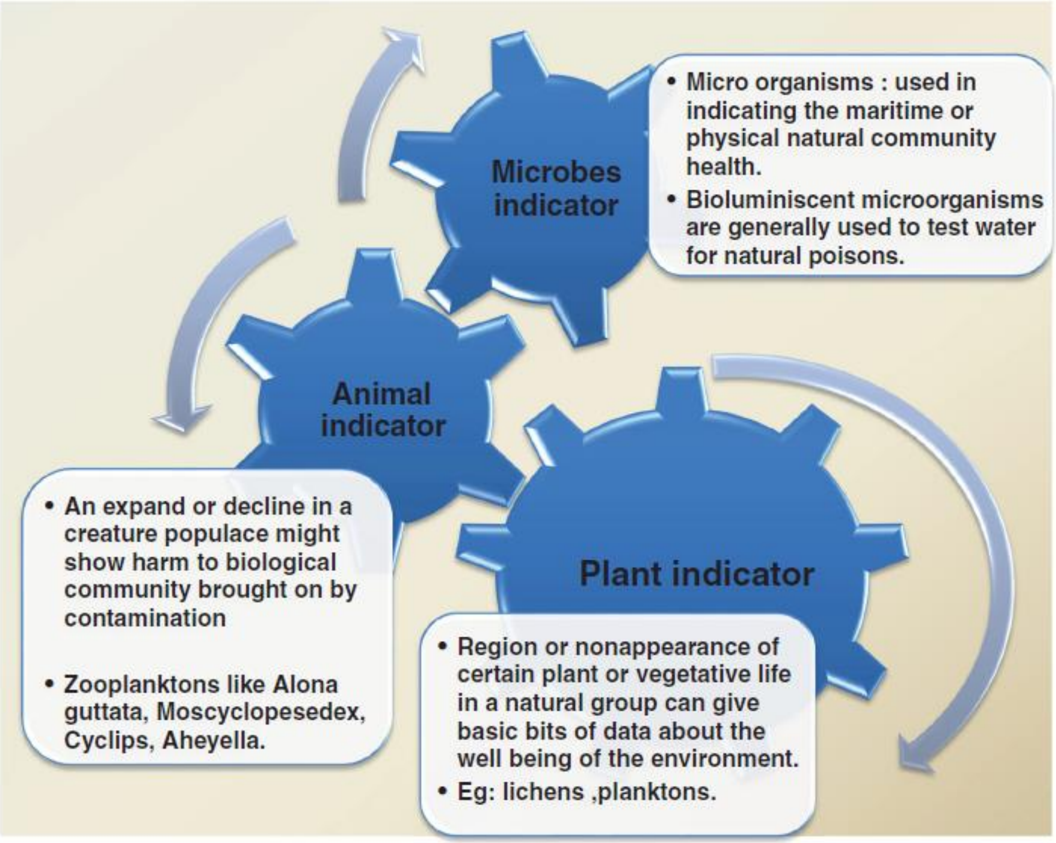


Figure 1. Types of Bioindicator.

BIOINDIKATORI

Biljke se koriste kao vrlo osjetljivi alati za predviđanje i prepoznavanje promjena u okolišu (lišaji)
Wolffia globosa – zelena alga – onečišćenje kadmijem

Životinje – promjena u gustoći populacije
ŽABE – važni bioindikatori bara

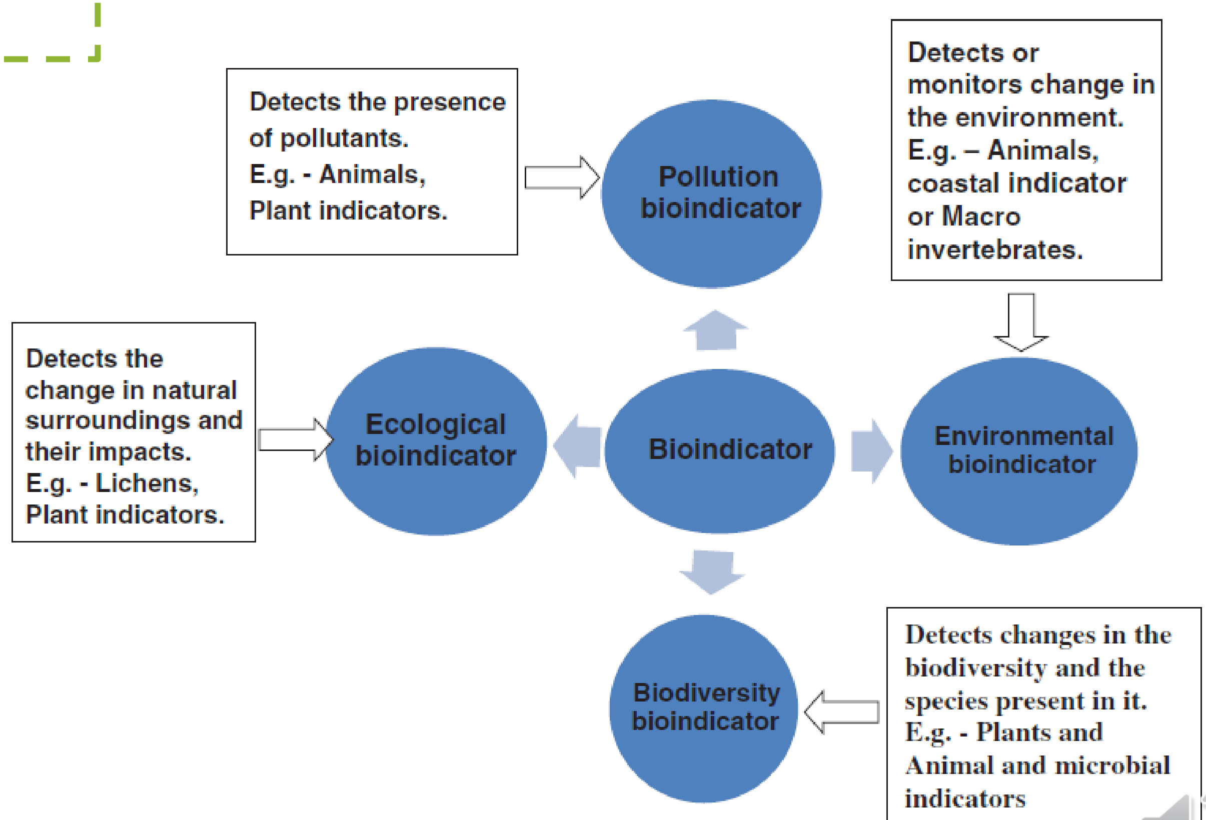
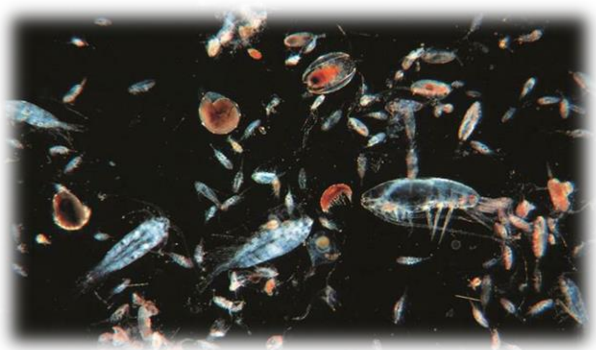


Figure 2. Sub-types of Bioindicators.

BIOINDIKATORI

PLANKTONI – ukazuju na onečišćenje vode



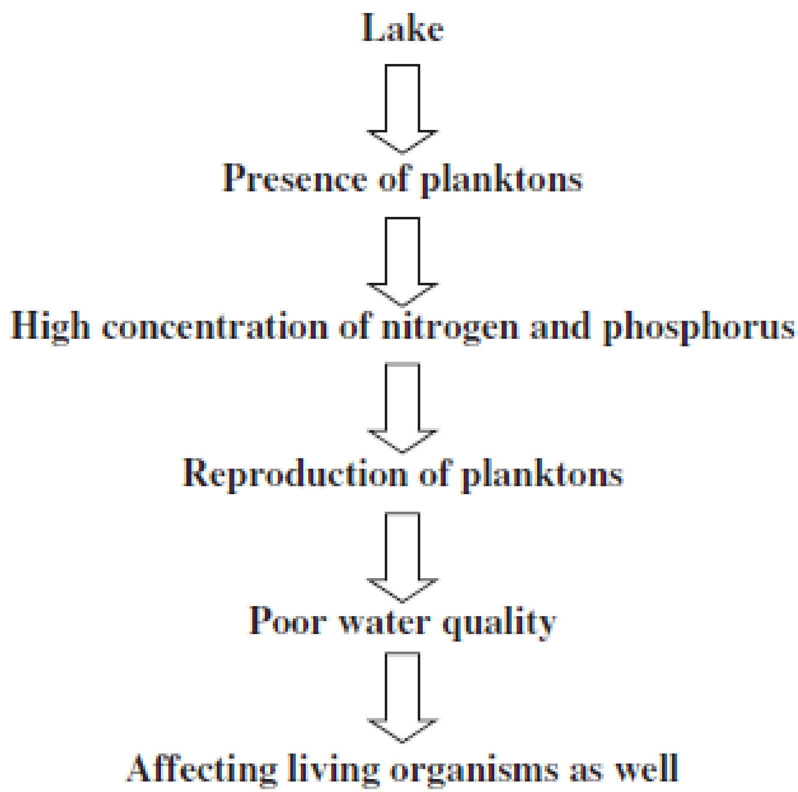


Figure 4. Flow chart of planktons indicating pollution (lake condition).

BIOINDIKATORI

Table 1. Types of phytoplankton and its indications.

Names of phytoplankton	Indications	References
Reen algae	Facilitates the growth of fishes	Khatri and Tyagi (2015)
Mosses, liverworts	Pollution by accu-mulation of metals	Uttah et al. (2008)
Charophytes	Quality of water	Uttah et al. (2008)
Selanastrum	Water pollution	Uttah et al. (2008)
Wolffiaglobose	Contamination of cadmium	Uttah et al. (2008)
Euglena gracilis	Organic pollution in lakes	Hosmani (2014)
Chlorella vulgaris	Helps in removal of heavy metal contamination from water and soil	Lilian (2009)
Chlorococcales like *C. vulgaris* and *A. falcatus* et.al Paramasivam and Sreenivasan (1981)	Indicators of the paper industry and sewage waste	Lilian (2009)

Table 2. Types of zooplanktons and its indications.

Names of zooplanktons	Indications	References
Rotifers	Trophic status	Walsh (1978)
Keratellatropica, Hexarthramira	High turbidity due to suspended sediments	Thakur et al. (2013)
Brachionuscalyciflorus	Eutrophic conditions and organic pollution of lakes	Jain et al. (2010)
Cladocerans group (unspecified)	Low concentration of contaminants	Hosmani (2014)
Trichotriatetratis	Pollution caused by accumulation of phosphorous and heavy metal ions	Aslam et al. (2012)
Thermocyclops, argyrodiaptomus	Eutrophic conditions	(Markert et al. 2003)
B.angularis, *Rotatoria*	Eutrophic conditions	(Markert et al. 2003)
Leeches	Indicates contamination because of presence of PCB (polychlorinated biphenyl) in a river	Uttah et al. (2008)
Leeches	Sensor-bioindicator of river contamination of PCB's	Uttah et al. (2008)
Oyster (Crassostreagi-gas), crabs (Geoticadepressa)	Presence of lead	Uttah et al. (2008)
B. dolabrotus	High turbidity due to suspended sediments	Grizzle (1984)
Copepods (Cyclops & phyllodiaptomus)	Health of the marine body	Aslam et al. (2012)
Cladocerans (moina, daphnia, bosmina)	Health of the marine body	Aslam et al. (2012)

BIOINDIKATORI

- Mediteranska dagnja *Mytilus galloprovincialis* – široko rasprostranjena vrsta u Jadranu, te je od visoke ekonomske važnosti
- Školjkaši su FILTRATORI i mogu u sebi nakupiti razne onečišćujuće tvari i zbog toga se smatraju bioindikatorima kvalitete mora, te se koriste u raznim “mussel watch” programima
- *M. galloprovincialis* ima ljušturu crne ili tamno-plave boje koja često može biti prekrivena raznim obraštajem
- Hrani se tako što FILTRIRA MORSKU VODU i uklanja iz nje organsku tvar
- HRANJIVE ČESTICE SE HVATAJU NA ŠKRGAMA I ZATIM INGESTIRAJU
- Svaki školjkaš filtrira i do 6 litara vode tokom perioda od jednog sata

BIOINDIKATORI

- zbog široke rasprostranjenosti i dostupnosti često se koristi u raznim fiziološko-kemijskim, biokemijskim i genetičkim istraživanjima
- BIOMARKERI koji su se pratili u ovom istraživanju:

o INDEKS KONDICIJE (IK):

✓ daje uvid u kvalitetu mesa dagnji za komercijalne svrhe;

✓ stanje uzorkovanih organizama koje proizlazi iz njihovih fizioloških aktivnosti pod određenim okolišnim uvjetima;

✓ u dagnji indeks kondicije varira ovisno o veličini jedinke, godišnjem dobu i lokalnim okolišnim uvjetima, a najviše o količini dostupne hrane i reproduktivnom ciklusu),

o INDEKS PROBAVNE ŽLIJEZDE (IPŽ)

✓ pokazatelj ishranjenosti odnosno izgladnjelosti školjkaša;

✓ ima ulogu skladištenja rezervi metaboličke energije dagnji;

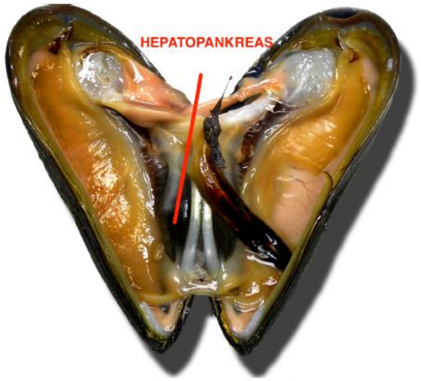
✓ njezin indeks se (IPŽ) mijenja ovisno o promjeni godišnjeg doba ili ovisno o promjenama okolišnih uvjeta i parametara),

BIOINDIKATORI

- VRIJEME PREŽIVLJAVANJA NA ZRAKU:
- ✓ pokazuje promjene u fiziologiji organizma, uslijed prirodnih ili antropogenih čimbenika;
- ✓ DOBAR POKAZATELJ ONEČIŠĆENJA OKOLIŠA;
- ✓ test je široko primijenjen u laboratorijskim istraživanjima za praćenje kvalitete mora, iako je vjerojatnost da organizmi ostanu izloženi na zraku duži vremenski period vrlo mala
- TOKSIČNOST TKIVA DAGNJE
- ✓ Bioluminiscentna bakterija *V. fischeri*

$$IP\check{Z} = \frac{m(probavne\ \check{z}lijezde)}{m(ukupna)}$$

$$IK = \frac{m(tkivo)}{m(lju\check{s}tura)}$$



Slika 5. Probavna žlijezda (hepatopankreas) mediteranske dagnje *Mytilus galloprovincialis*.

BIOINDIKATORI

Svakodnevno su se brojale uginule jedinke, te se vagala ukupna masa dagnje i masa prazne ljušture

Slika 6. Određivanje preživljavanja na zraku mediteranske dagnje *M. galloprovincialis* (SOS test).

BIOINDIKATORI

- *Herring Gulls* (galeb) – idealni bioindikator ekosustava i zdravlja okoliša zbog:
  - dnevne aktivnosti,
  - široke rasprostranjenosti,
  - dugog životnog ciklusa, i
  - veličine
- ONEČIŠĆUJUĆE TVARI se prate u:
  - JAJIMA
  - PERJU
  - UNUTARNJIM ORGANIMA
- Perje i jaja – ne utječu na dinamiku populacije
- Galebovi izlegu 3 jaja – odgoje samo 1 ili 2
- Uklanjanje jednog jaja iz gnijezda ne utječe na reproduktivni uspjeh

Figure 1 Picture of Herring Gull with young. Photo by Joanna Burger.

BIOINDIKATORI

- Analiza tkiva daje informacije o lokalnom onečišćenju
- PRIMJER: onečišćujuća tvar u jaju UKAZUJE NA izloženost ženke onečišćujućoj tvari prije izlijeganja jaja
- Jedno od ključnih pitanja u ekotoksikologiji – DA LI UČINCI UTVRĐENI U LABORATORIJU POSTOJE U DIVLJINI I DA LI IZRAVNO UTJEČU NA PREŽIVLJAVANJE?
- Istraživanja na terenu ukazuju da su neki učinci (broj padova, šetanja, hranjenje) bili manje izraženiji u laboratorijskim ispitivanjima

BIOINDIKATORI

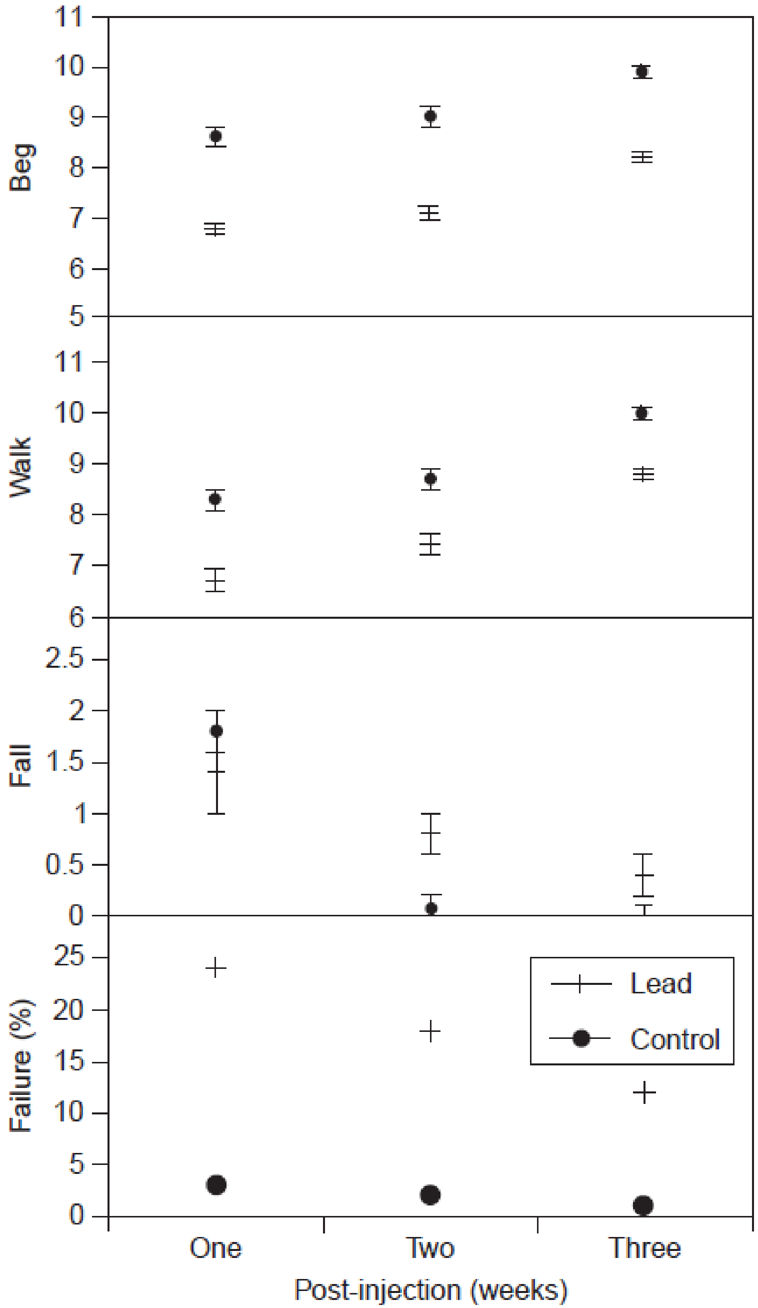


Figure 3 Behavioral responses of herring gulls in the wild. Shown are mean scores (± standard error) of lead-injected and control chick (all significantly different, dose = 100 mg kg^{-1}. Reproduced from Burger, J and Gochfeld, M (1994). Behavioral impairments of lead-injected young Herring Gulls in nature. *Fundamental and Applied Toxicology* 23: 553–561; Burger, J and Gochfeld, M (1997). Lead and neurobehavioral development in gulls: A model for understanding effects in the laboratory and the field. *NeuroToxicology* 18: 279–287. Walking and begging ability were scored on a scale of 10 (the highest score). Fall refers to the number of times a chick fell when walking a 1-m distance; failure refers to the percent of times a chick missed its parent's bill when pecking for food.

BIOINDIKATORI

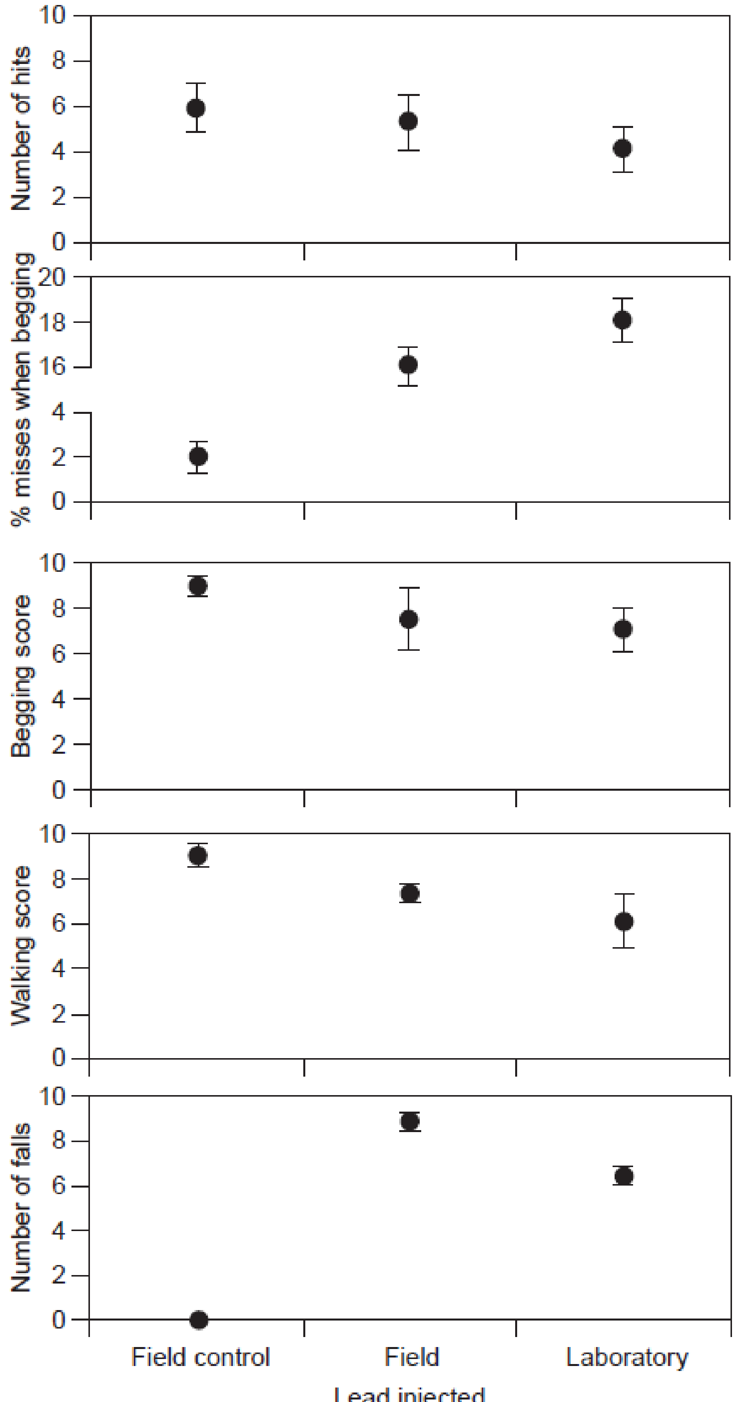


Figure 4 Comparison of behavior herring gulls raised in the field (lead-injected and controls) with lead-injected herring gulls raised in the laboratory (all injections were at 2 days of age, dose = 100 mg kg^{-1}). In all cases the lead-injected chicks differed from the field controls. Reproduced from Burger, J and Gochfeld, M (1994). Behavioral impairments of lead-injected young Herring Gulls in nature. *Fundamental and Applied Toxicology* 23: 553–561; Burger, J and Gochfeld, M (1997). Lead and neurobehavioral development in gulls: A model for understanding effects in the laboratory and the field. NeuroToxicology 18: 279–287. Hits are successful pecks at parent's bill.

BIOMARKERI

https://www.youtube.com/watch?v=cT-iUJwEy0E

BIOMARKERI

- mjera koja reflektira interakciju između biološkog sustava i potencijalnog kemijskog, biološkog i fizikalnog štetnog čimbenika
- indikator normalnih bioloških ili patoloških procesa ili farmakoloških odgovora na terapijsku intervenciju
- Svjetska zdarvstvena organizacija – bilo koja tvar, struktura ili proces koja se može mjeriti u tijelu i može utjecati na ili predvidjeti tijek bolesti
- Izmjereni odgovor organizma može biti FUNKCIONALNI ili FIZIOLOŠKI, BIOKEMIJSKI na staničnom nivou ili molekulska interakcija

BIOMARKERI

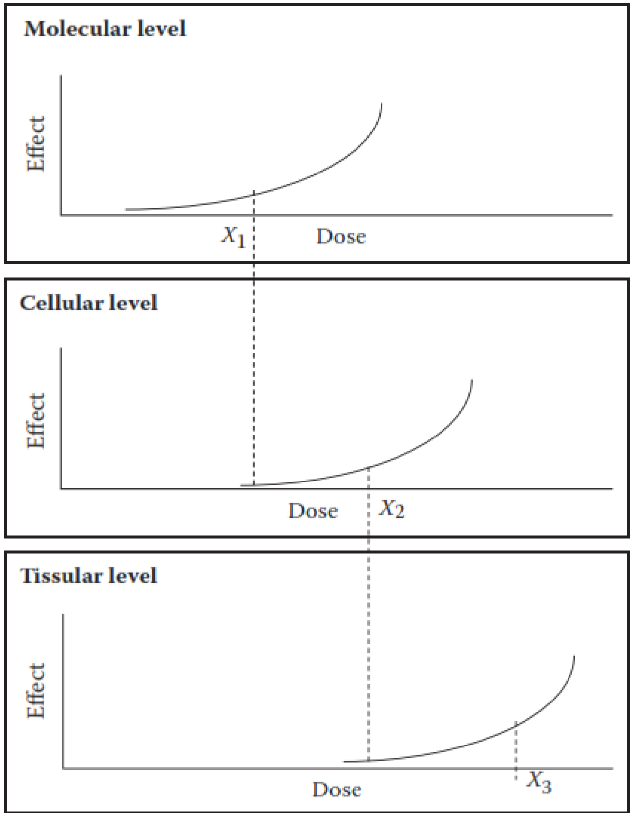


FIGURE 1.2
Biomarkers of damage: progression of the dose–effect relationship according to the level of biological organization.

Niža razina biološke organizacije – osjetljivija od više razine

Krivi rezultat - biomarkeri

ODNOS BIOMARKERA i BIOINDIKATORA

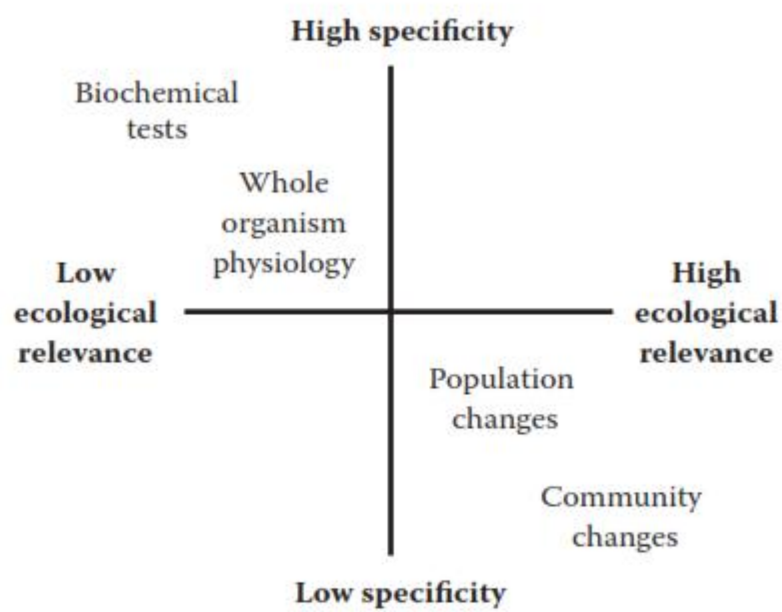


FIGURE 10.1
Specificity and ecological relevance of biochemical effects measurements. (*Source:* Addison, F. (1996). *Environmental Reviews* 4, 225–237. With permission.)

- Odnos biomarkera i bioindikatora na temelju specifičnosti i ekološke značajnosti
- Teško je povezati biokemijske promjene s ekološkim promjenama
- Iako u nekim slučajevima je moguće – primjer: kod štenca TBT je uzrokovao – imposex – fiziološka promjena koja može dovesti do masivne promjene u populaciji

BIOMARKERI

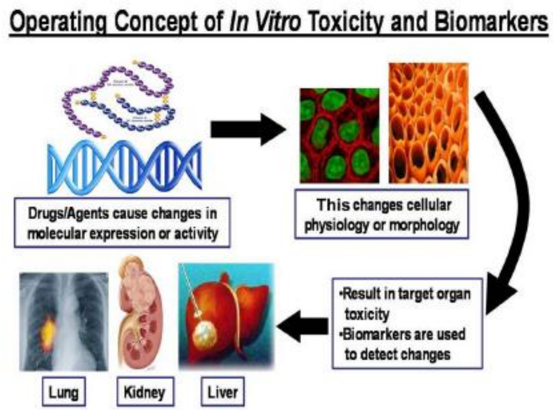


FIGURE 7.2 Toxic test substances cause changes at the molecular level (DNA or protein), which are expressed at the cellular level, which in turn result in target organ toxicity (e.g. of liver, kidney), which can be studied and extrapolated to whole animal or humans based on *in vitro* studies.

FIGURE 7.3 Drugs or agents interact with various cells, organelles, and organ systems to produce their pharmacological and/or toxicological effects. These effects can now be studied using various *in silico* or *in vitro* tools, minimizing the use of animal testing.

BIOMARKERI

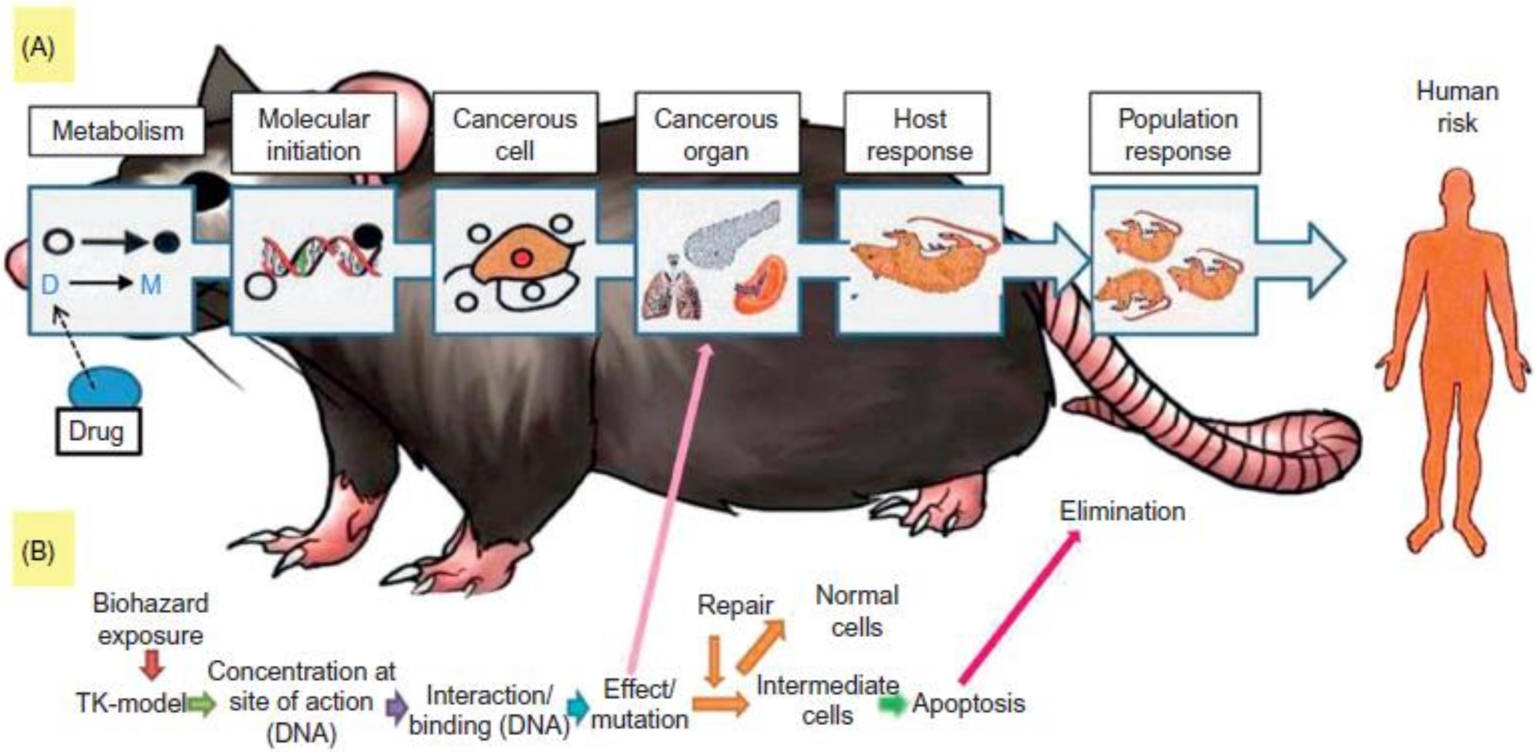


FIGURE 7.11 Toxicodynamics: (A) An illustration of how the animal handles the drug and its relationship to human risk. (B) Molecular toxicodynamics interactions.

BIOMARKERI

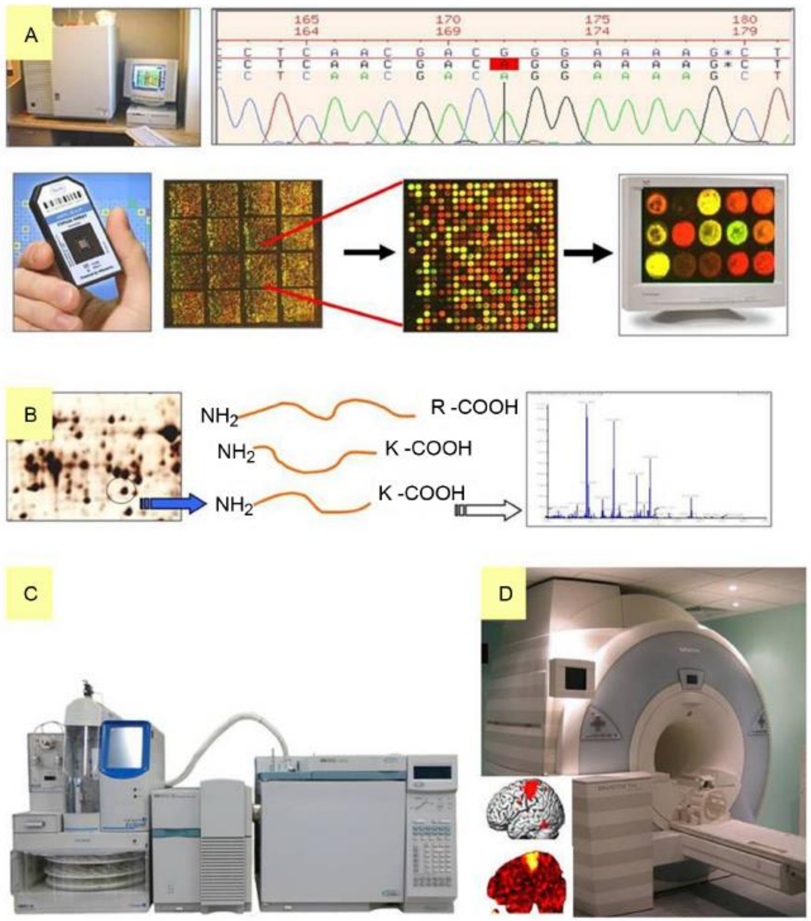


FIGURE 7.12 Select tools of modern molecular toxicology: (A) Genomics, (B) Proteomics, (C) Metabonomics and (D) functional Magnetic Resonance Imaging (fMRI).

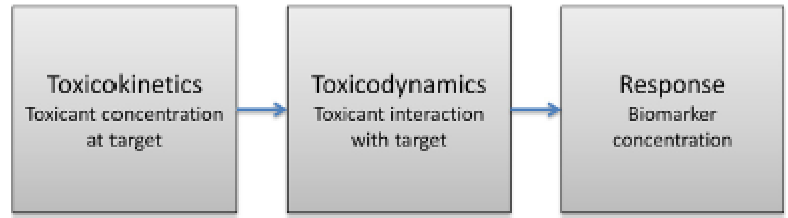
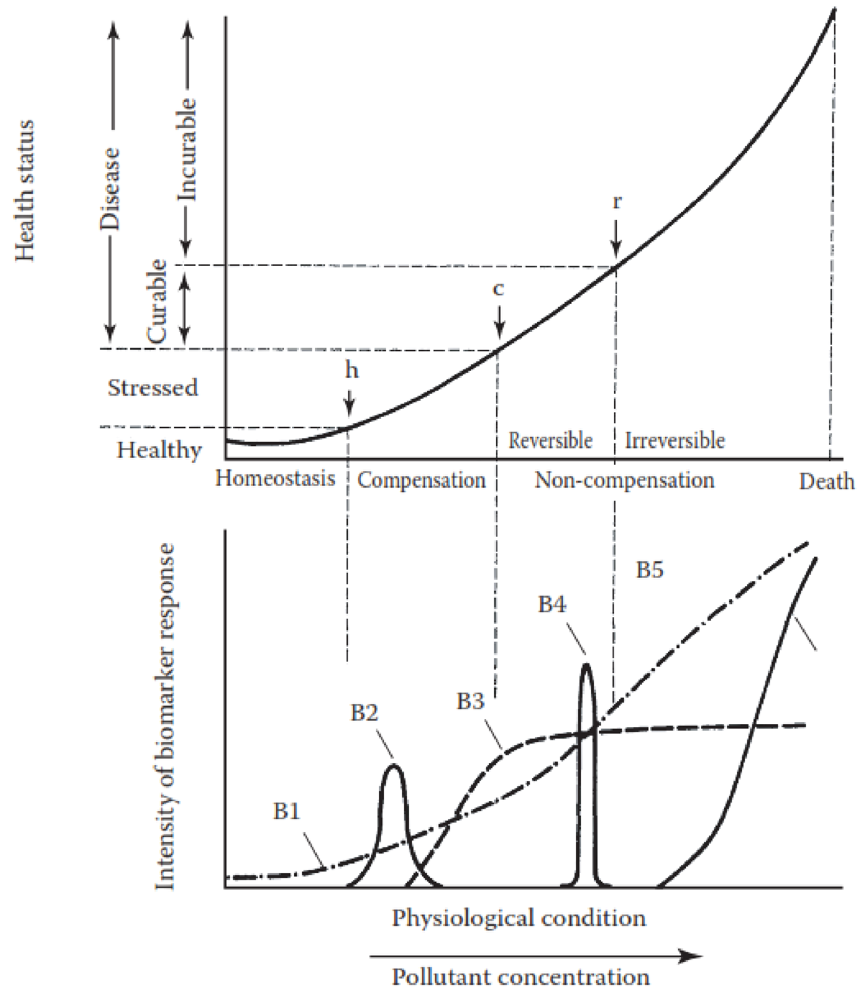


FIGURE 8.1 The relationship between toxicokinetics, toxicodynamics, and biomarker response.

FIGURE 10.2
Relationship of exposure to pollutants, health status, and biomarker responses. Upper curve shows the progression of the health status of an individual as exposure to pollutant increases. *h* = Point at which departure from normal homeostatic response range is initiated; c = limit at which compensatory responses can prevent development of overt disease; r = limit beyond which the pathological damage is irreversible by repair mechanisms. The lower graph shows responses of five hypothetical biomarkers used to assess the health of the individual. (*Source:* Depledge. M.H. et al. (1993). In *Biomarkers: Research and Application in the Assessment of Environmental Health*. Springer-Verlag. With permission.)



BIOMARKERI

TABLE 10.2

Biomarkers Listed by Decreasing Specificities to Pollutants

Biomarker	Pollutant	Comments and References
ALAD inhibition	Lead	Sufficiently reliable to replace chemical analysis (Wigfield et al., 1986)
Metallothionein induction	Cadmium	More difficult to measure than cadmium levels (Hamer, 1986)
Eggshell thinning	DDT, DDE, dicofol	Degree of eggshell thinning is easily measured (Ratcliffe, 1967)
AChE inhibition	OPs, carbamates	Easier and more reliable than chemical analysis (Fairbrother et al., 1991)
Anticoagulant clotting proteins	Rodenticides	Measurements similar in complexity to chemical analysis (Huckle et al., 1989)
Monooxygenase induction	OCs, PAHs	Dioxin equivalent more easily measured than chemical analysis (Murk et al., 1997)
Porphyrin profiles	Several OCs	Separation by high-performance liquid chromatography well developed (Kennedy and James, 1993)
Retinol profiles	OCs	Demonstrates exposure to specific chemicals (Shugart, 1994); considerable natural variations; ratios more reliable than absolute values (Spear et al., 1986)
DNA and hemoglobin adducts	Largely PAHs	Several tests available; complicated by repair mechanisms
Vitellogenin induction	Estrogenic chemicals	Induction in male fish is sensitive indicator of estrogenic chemicals (Harries et al., 1997)
Other serum enzymes	Metals, OCs, PAHs	Several enzyme systems have been studied (Fairbrother, 1994)
Stress proteins	Metals, OCs	Wide range of stress proteins have been studied (Sanders, 1993)
Immune responses	Metals, OCs, PAHs	Many tests available (Wong et al., 1992)

FKITMCMXIX

Table 1. Interactive effects between climate change and contaminants on biomarkers.

Outcome biomarkers	Environmental threats & Anthropogenic contaminants	Ref.
Endocrine disruption (polar bears)	OH-PCBs + Climate change	Gustavson et al. 2015
Endocrine disruption causes interference on adaptation capacity to environmental stress in Artic marine mammals and seabirds	EDCs + Climate change	Jenssen 2006
Changes in sexual proportions (vertebrates) Skeletal deformities (fishes)	Thermal stress	Eissa and Zaki 2011 Guidini et al. (under review)
Impairment in somatic and reproductive fish growth (deficit in metabolic requirements)		Sogard and Spencer 2004
Increase in mosquito population vectors of diseases and agricultural pests	Thermal stress	reviewed by Delorenzo 2015 Tadei et al. 2016
Loss of osmoregulatory function (fishes)	Atrazine + salinity	reviewed by Delorenzo 2015
Inhibit antioxidant defenses (bivalve mussel)	Herbicide + Temperature	reviewed by Delorenzo 2015
Changes on migratory behavior and breeding (birds)	Climate Change	Carey 2009
Human respiratory allergy	Urbanization (CO_2 vehicle) + Diverse Climate Change Factors	D´Amato et al. 2016
Osmoregulation effects (fishes)	Cu + Salinity	Adeyami et al. 2012
Reproductive effects (birds)	Environmental temperature + Hg	Hallinger and Cristol 2011
Effects on enzymes of biotransformation process (fishes)	Temperature + OH-PCBs	Buckman et al. 2007
Changes in phenology (amphibian)	Glyphosate + Climate Change	Lötters et al. 2014
Changes in the relative abundance of algae species	CO_2 + nutrients	Russell et al. 2009, Falkenberg et al. 2012
Physiological effects on flowering plants and pollinating insects	Climate warming	Scaven and Rafferty 2013
Decline fisheries biomass	Climate effects	Ainsworth et al. 2011

BIOMARKERI

TABLE 12.1 Biomarker Responses of Animals Commonly Used in Aquatic Toxicology

Biomarker	Biomarker Type	Remarks
ALA-D activity	Exposure and effect	Δ-aminolevulinic acid dehydratase catalyzes one enzymatic reaction in heme production. Only lead exposure is known to inhibit the reaction. Thus, observed inhibition indicates that the organism has been exposed to lead and that the exposure causes problems in oxygen transport and in all the reactions where heme groups are involved (i.e. all cytochrome enzymes)
cDNA (complementary DNA) microarrays	Exposure	As a large number of transcripts are targeted, virtually all contaminants give different signatures. However, it must be borne in mind that the transcript information does not give the mode of action, since one does not know if any toxicant-responsive gene products are formed and their activities are changed as a response to the environmental change
Bile composition	Exposure	The exposure to specific organic toxicants can be evaluated from their appearance, their breakdown, or conjugation products in the bile of exposed animals
Spiggin	Effect	One of the few biomarkers for the detection of androgenic effects in fish. The protein is the glue protein that is used by stickleback males to build nests where the offspring are reared. Androgenic contamination affects spiggin levels in males. In heavily contaminated sites, can even be secreted by females
Vitellogenin	Effect	The protein is produced in the livers of fish as a precursor of vitellins that form the yolk proteins of eggs. Specific antibodies for the detection of the protein have been developed. In an environment contaminated by estrogen agonists, males also often produce the compound. The production is species-specific, and although vitellogenin production in invertebrates has been used as biomarker of estrogenic effects, the actual regulation of vitellogenin production in different invertebrates is poorly known
Acetylcholinesterase activity	Effect	The activity of the enzyme indicates the presence of contaminants affecting synaptic function
Retinol profile	Exposure	Retinol is vitamin A. Organochlorine compounds affect the proportions of different retinols. Since the exact functions of vitamin A in animals are poorly known, the effects of an altered profile are likewise not clear
Porphyrin profiles	Exposure	Porphyrins are integral parts of heme (of globins and cytochromes). Different porphyrins can be characterized by liquid chromatography. The profiles change as a response to organochlorine contamination

BIOMARKERI

Mixed-function oxidases	Exposure	Mixed-function oxidases are the major enzymes of phase 1 in biotransformation. They usually contain cytochrome P450. In the case that only mRNA of cyp enzymes is determined, the increase mostly indicates contamination by PAHs. Antibodies against some major cytochrome P450s in fish have also been generated.
EROD (ethoxyresorufin-O-deethylase) activity	Effect	The enzyme activity is used to show changes in the activity of phase 1 of biotransformation, mainly by PAHs and other compounds that activate the aryl hydrocarbon receptor pathway
Metallothionein levels	Exposure	Levels are measured especially to indicate exposure to cadmium, copper, zinc, and mercury (and other metals). Problems in interpretation are caused by the facts that metallothionein induction is not always observed; that there are marked differences in induction between different species; that in addition to being induced by metals, metallothioneins are involved in redox regulation; and that their levels and induction are cell-cycle-stage specific.
DNA adducts	Exposure	DNA repair makes interpreting the results difficult. DNA adducts are formed especially by bulky aromatic compounds
Lipid peroxidation	Effect	Measured as MDA (malonyl dialdehyde), one of the final products of lipid peroxidation. The most common method used is evaluating TBARS (thiobarbituric acid reactive substances) levels, the most important of which is MDA. All conditions and chemicals that cause oxidative stress cause an increase in TBARS
Comet assay	Effect	Indicates the formation of DNA fragments. Caused by various genotoxicants
Micronucleus test	Effect	Caused by various genotoxicants. Results either from disturbances in the function of the mitotic spindle during cell division or fragmentation of DNA so that daughter cells have a main nucleus and a smaller micronucleus
TUNEL assay	Effect	The TUNEL assay is a common method for determining DNA fragmentation characteristic of apoptotic cell death. Many contaminants cause apoptosis, and so the specificity of the response is low
Stress protein (heat shock protein, HSP) levels	Effect	Stress protein levels increase after all treatments that affect the normal three-dimensional structure of proteins. Thus, virtually all toxicants can affect HSP levels

The biomarkers are arranged according to their specificity. In addition to the presented biomarkers, many other are used, but typically either their specificity is low (e.g. if redox parameters are used as biomarkers, many compounds, from metals to PAHs, can cause changes in them) or what the response actually means for the function of organisms is not known.

BIOMARKERI

- PREDNOSTI biomarkera:

- Potvrđuju apsorpciju u organizam
- Mjere integriranu izloženost iz svih izvora
- Zbog vrlo osjetljivih analitičkih metoda mogu se detektirati u jako niskim koncentracijama
- Služe za validaciju modela izloženosti kada predviđene doze odgovaraju izmjerenim
- Pomažu u praćenju izloženosti kada se pojedinac ili grupa ispitanika prate tijekom vremena
- Pomažu u procjeni javnozdravstvenih intervencija gdje se može pratiti je li se smanjila izloženost ako se onečišćujuće tvari ograniče ili povuku iz upotrebe

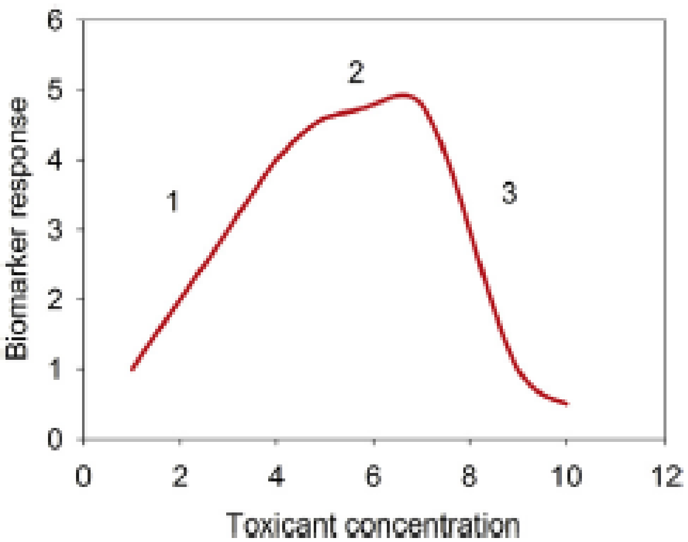
BIOMARKERI

- NEDOSTACI biomarkera:

- Ne mogu definirati izvor – prikazuju samo trenutno stanje bez informacija o podrijetlu ili načinu apsorpcije
- Ne mogu definirati toksičnu dozu – sama prisutnost tvari u organizmu ne govori puno, treba provesti toksikološke i epidemiološke studije koje bi odredile krivulju doze i učinka
- Osjetljivi su na lošu laboratorijsku analizu – spojevi koji se detektiraju su ubikvitarni – nalaze se svuda u okolišu i svakodnevno se koriste, pa su, zbog prisutnosti i u laboratorijskoj opremi, moguća onečišćenja tijekom skupljanja i skladištenja uzoraka
- Nedostatak referentnih vrijednosti – za neke od njih još se ne zna je li izmjerena doza štetna ili ne
- Nedostatak toksikoloških i epidemioloških informacija o većini kemijskih spojeva – u upotrebi je preko 90 000 umjetnih kemijskih spojeva, a manje od polovice ih je testirano na bilo kakvu toksičnost – nemoguće je znati jesu li izmjerene razine markera sigurne ili opasne

BIOMARKERI

FIGURE 12.3 **The reason why biomarker responses do not follow a concentration–response relationship.** (1) Initially, an increasing toxicant concentration causes a linear increase in the biomarker response. The increase may be due both to an increase in protein production and to an increase in the maximal activity of the protein(s) involved in the response. (2) The increase slows down and a maximal response is reached, whereafter (3) the chemical becomes acutely toxic to the response and may decrease the maximal activity of the protein molecule and/or decrease the production of the protein (possibly both transcription and translation are inhibited).



BIOMARKERI

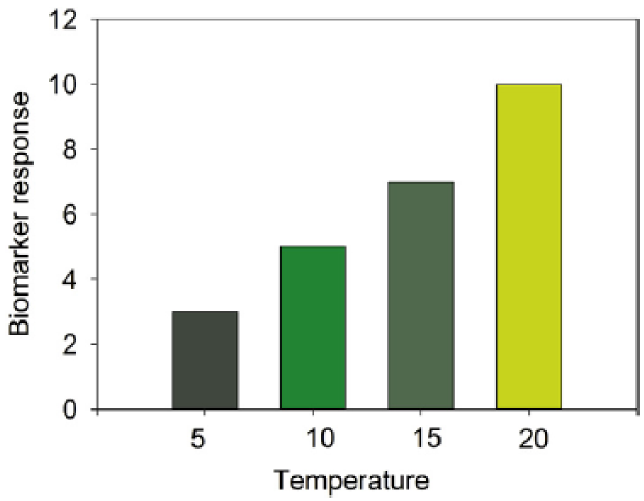


FIGURE 12.4 A hypothetical example of how a natural environmental variable (temperature) affects a biomarker response. With an increase in temperature, the value of the response increases markedly.

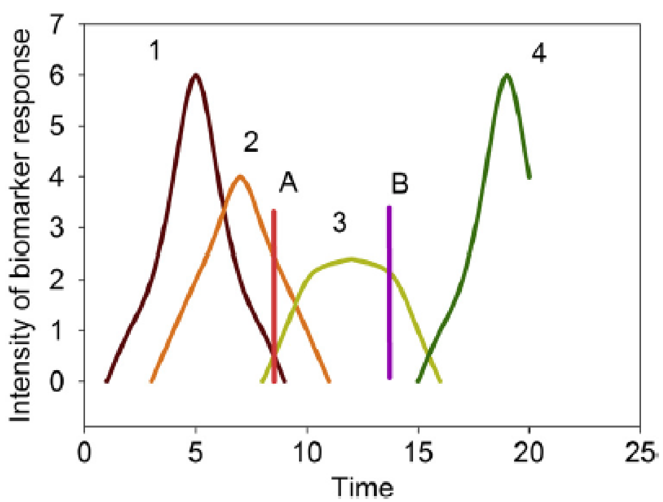


FIGURE 12.5 A schematic example of temporal differences in biomarker responses to a toxicant. The *y* axis gives the relative change in the biomarker value in comparison to the control. At 0 the control values and those measured from exposed organisms are the same. The different biomarkers (1–4) each have a unique time profile of responses. If sampling can be done only at one point, biomarkers 1–3 are all altered in comparison to the control at time point A, but only biomarker 3 at time point B. All the different biomarker responses cannot be measured at a single time point. Even at time point A, where three biomarkers show alterations, no values are close to the maximal response of any of the biomarkers.

BIOMARKERI

Karakteristike dobrog biomarkera

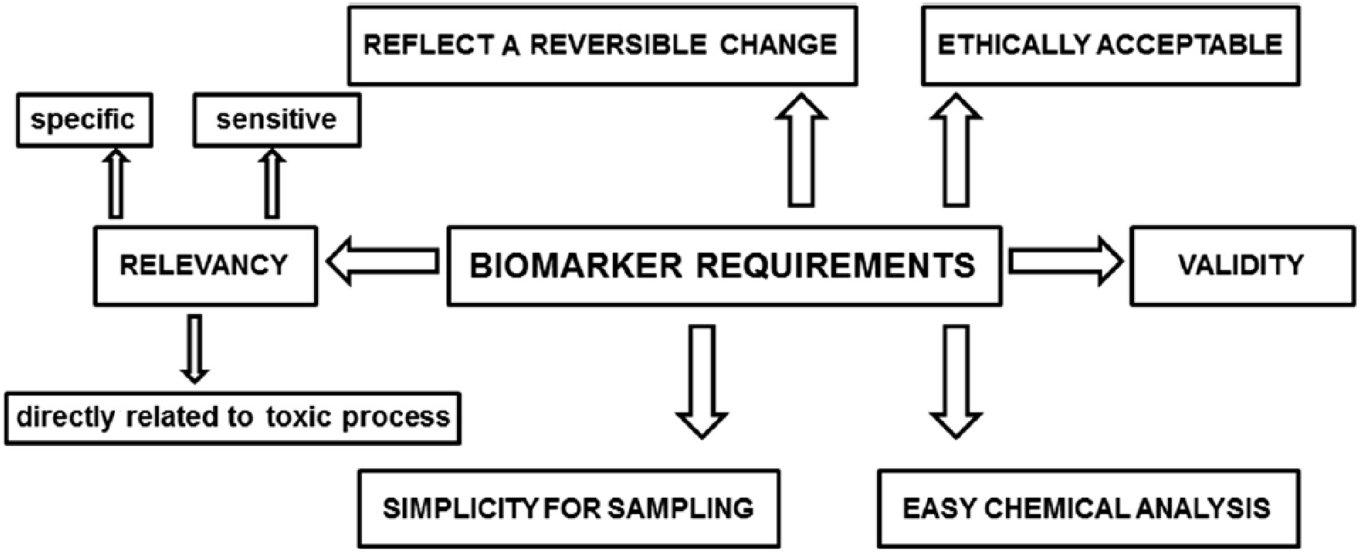


FIGURE 57.2 Requirements of a good biomarker in toxicological testing and biomonitoring of xenobiotics exposure.

BIOMARKERI

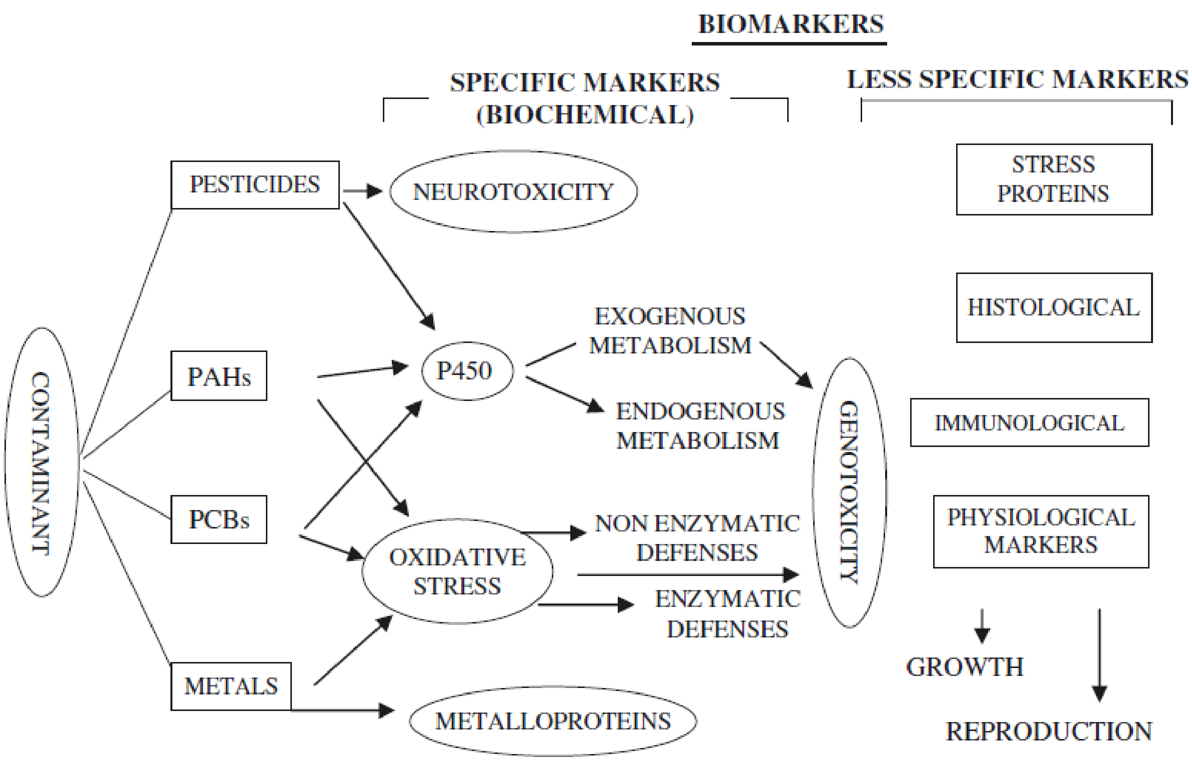


Figure 3: Interactions between contaminants and biomarkers (modified from Narbonne and Michel, 1993 [1]).

PODJELA BIOMARKERA

- Prema PROCJENI IZLOŽENOSTI i RIZIKA od utjecaja okolišnih čimbenika:

  BIOMARKERI IZLOŽENOSTI

  BIOMARKERI UČINKA

  BIOMARKERI VJEROJATNOSTI

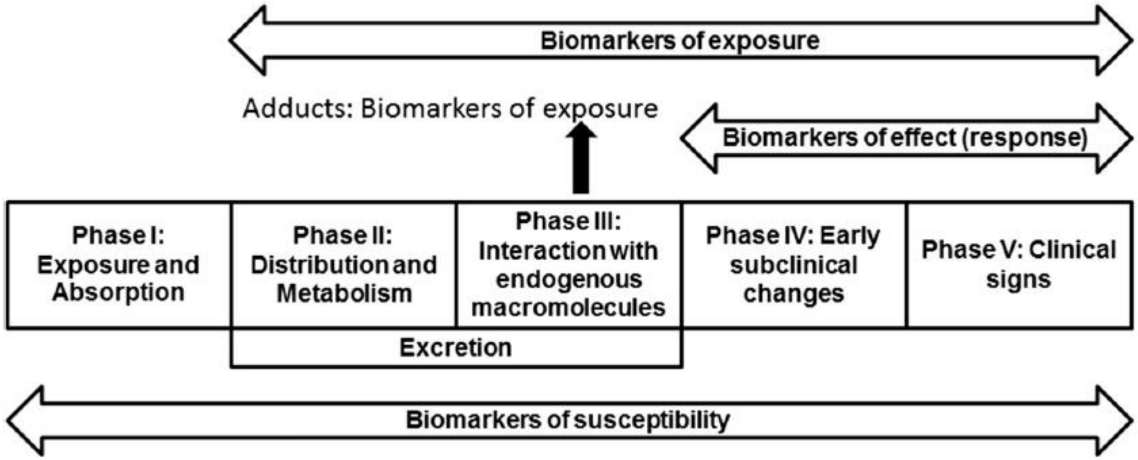


FIGURE 57.1 Stages of toxic phenomena and timing of the three types of biomarkers.

PODJELA BIOMARKERA

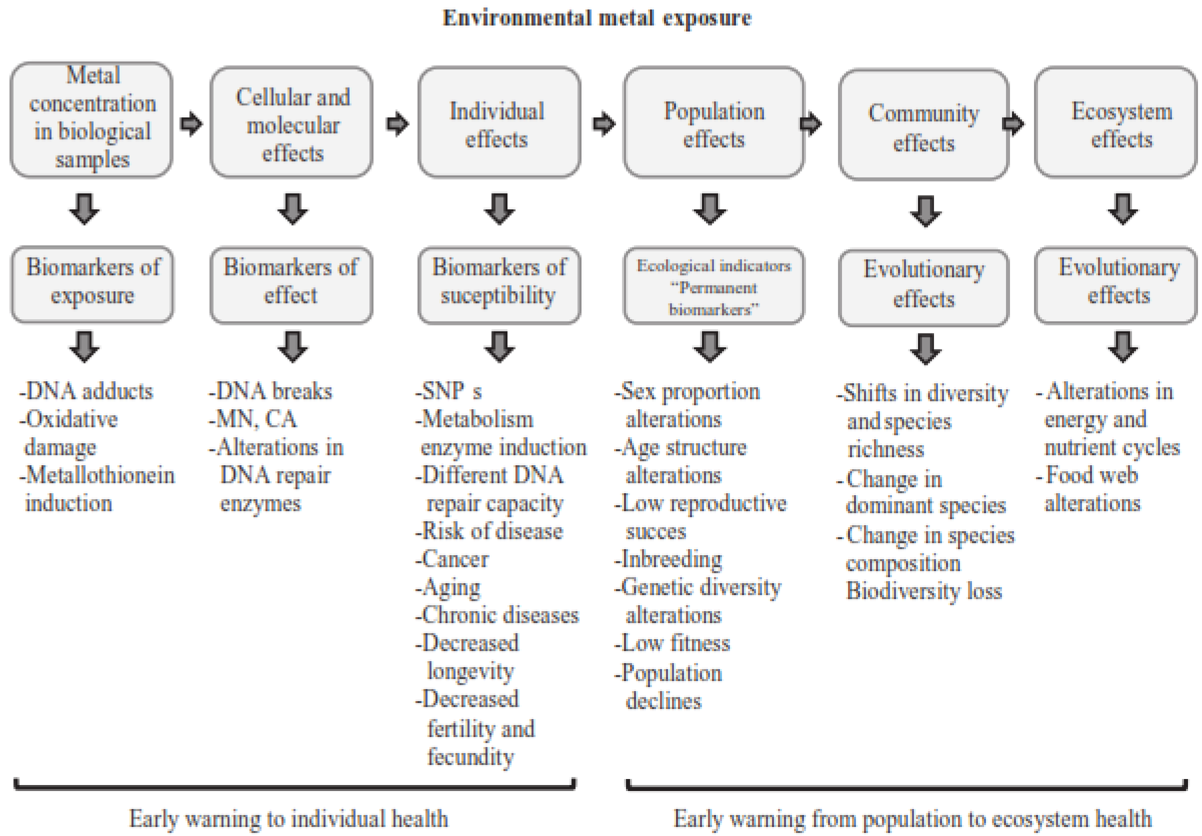


Fig. 1. Environmental pollutants –such as metals– can exert their effects at all levels of biological organization. Most used biomarkers for assessing toxic responses are listed in each level. MN= micronuclei, CA= chromosome aberrations, SNPs= single nucleotide polymorphisms.

FKITMCMXIX

PODJELA BIOMARKERA

GEN

Protein - Cytochrome

Protein

Iz obitelji cisteina, ima mogućnost vezanja na sebe metale, ksenobiotike kroz tiolnu grupu

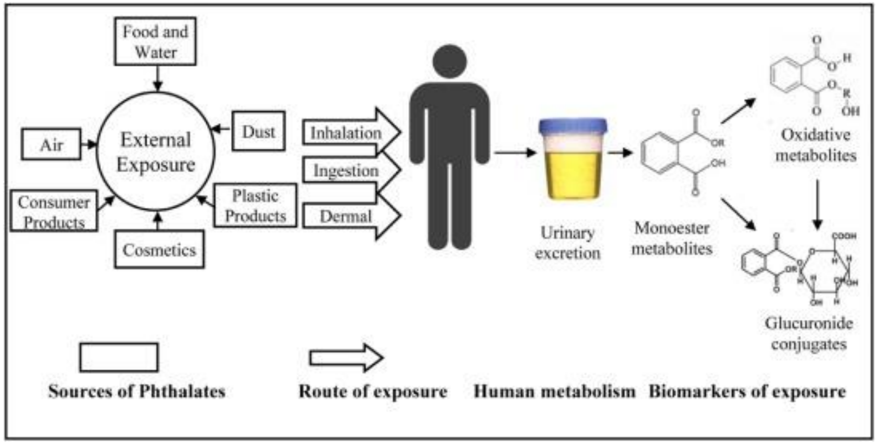
Aquatic Biomarkers, Table 1 Examples of biomarkers of exposure, effects, and susceptibility and their links with different classes of pollutants

Type of biomarker	Type of stressor
Biomarkers of exposure	
CYP1A	PAHs, PCBs, dioxins, pesticides
Metallothionein	Metals, oxidative stress
HSP	Thermal shock, metals, salinity, anoxia, oxidative stress
MXR	Organic pollutants, metals, algal toxins, organic matter, pesticides, perfluorochemicals
GST	Organic pollutants, PCBs, organochlorine pesticides
Antioxidant defenses	PAHs, PCBs, organochlorine pesticides, carbamates, organophosphates
Vitellogenin	Xenoestrogens
Biomarkers of effect	
Acetylcholinesterase	Organophosphates, carbamates, neurotoxins
Lysosomal system	Environmental pollutants, metals
Lipid peroxidation	Environmental pollutants, metals
DNA damage	Mutagens, clastogens, genotoxic xenobiotics
Biomarkers of susceptibility	
Paraoxonase	Organophosphates
Ah receptor (AhR)	PAHs, PCDDs, PCDFs

BIOMARKERI IZLOŽENOSTI

- Koriste se kod procjene količine kemijske tvari u tijelu
- Mnogi od njih se mogu izmjeriti u urinu, krvi, slini, i ako su liposolubilni u masti ili majčinom mlijeku

- Pružaju informacije o:
  - Izloženosti kemikalijama u pojedinaca
  - Promjenama njihovih razina u vremenu i varijabilnosti među različitim populacijama
  - Različitim načinima izloženosti i njima pridruženom riziku
- Detekcija kemikalija u organizmu – ne znači da su štetne



BIOMARKERI IZLOŽENOSTI

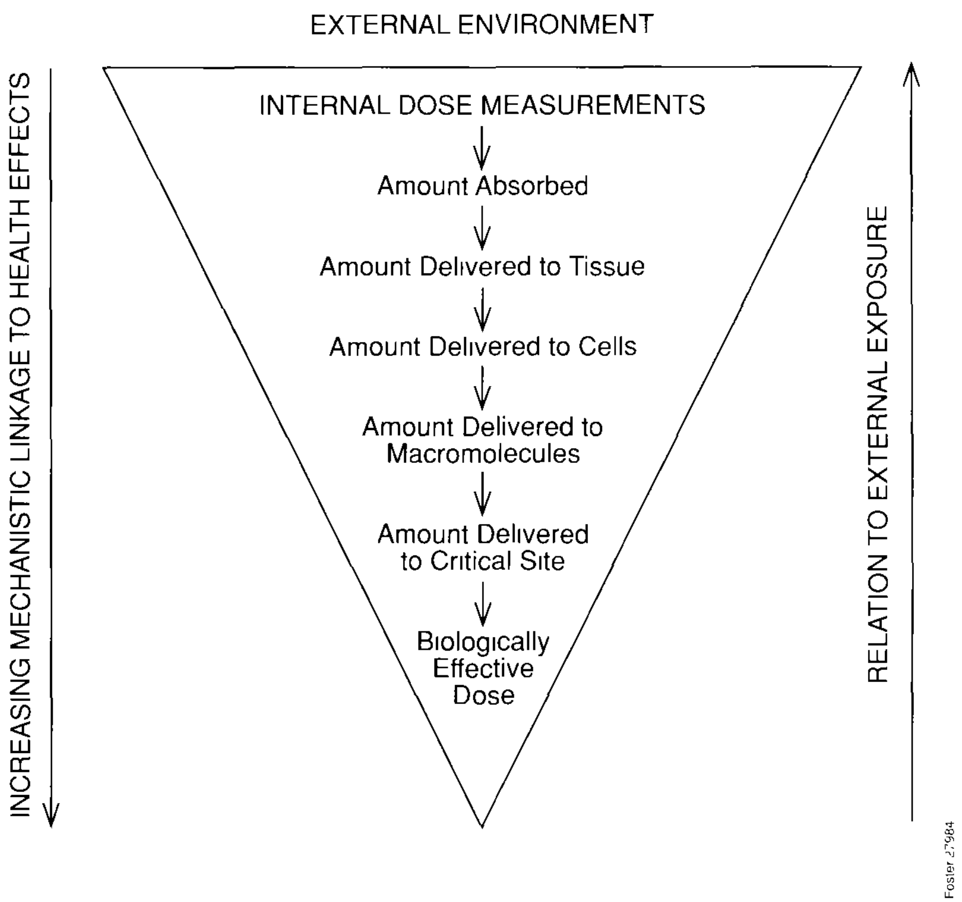


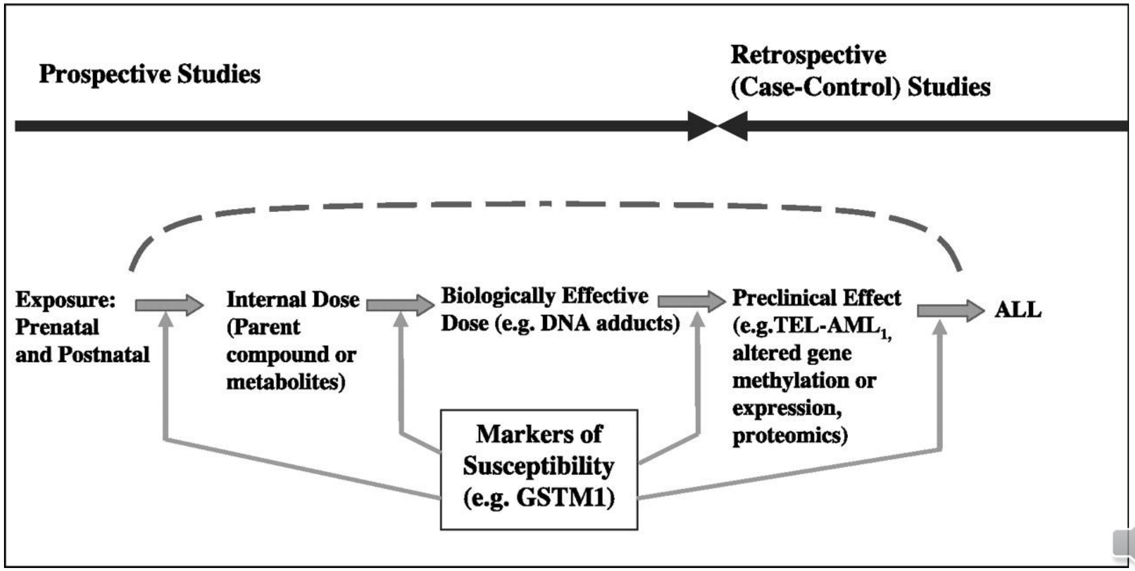
Fig 2 Biomarkers for internal dose for chemicals for which the major mechanism of action occurs through molecular interaction

BIOMARKERI IZLOŽENOSTI

- Biomarkeri izloženosti okolišnim čimbenicima dijele se na:

- UNUTARNJU DOZU BEZ UČINKA
- BIOLOŠKI EFEKTIVNU DOZU
- DOZU KOJA IMA SUBKLINIČKE UČINKE
- VJEROJATNOST

The "Meet-in-the-Middle-Approach":
The Example of Childhood Acute LymphoblasticLeukemia



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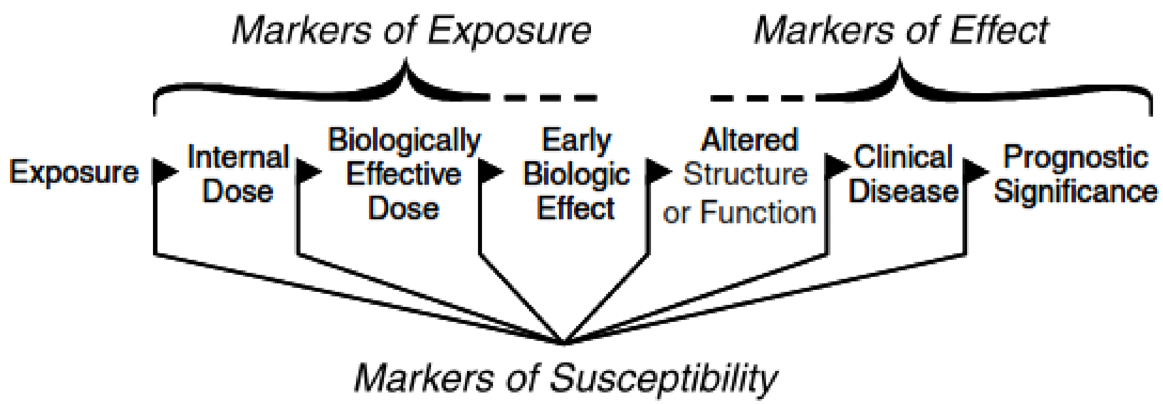


FIGURE 1-1 Simplified flow chart of classes of biomarkers. Source: NRC 1987.

- Unutarnja doza (ID – *internal dose*) – količina štetne tvari ili metabolita nađena u biološkom mediju
- Biološka efektivna doza (BED) – količina štetne tvari ili metabolita koji reagiraju sa kritičnim mjestima stanice i tkiva (npr. DNA, enzim)
- Biološki učinci (EBE) – događaj povezan s učinkom na zdravlje

BIOMARKERI IZLOŽENOSTI

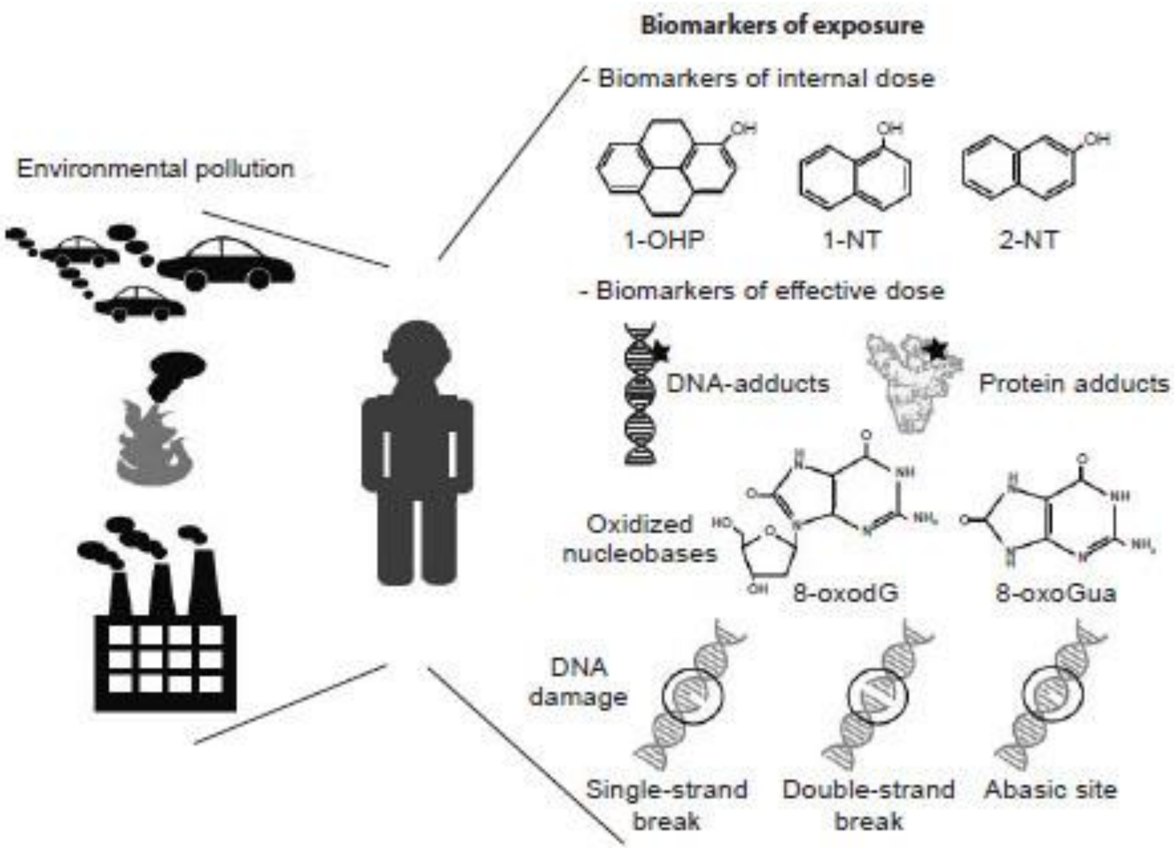
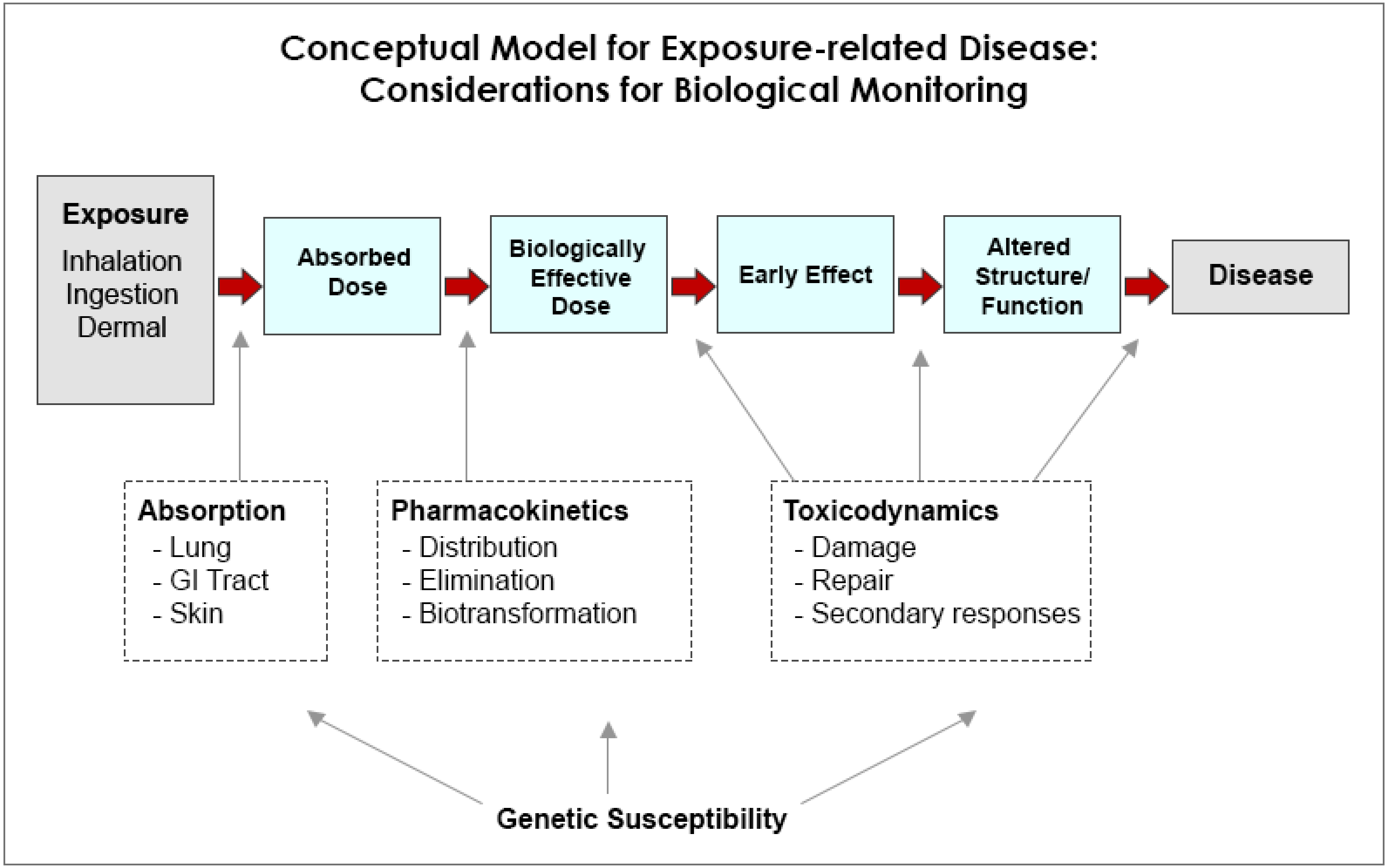


Figure 3. Biomarkers of exposure. The biomarkers of exposure are classified as markers of internal dose and markers of effective dose. 1-OHP: 1-hydroxypyrene; 8-oxodG: 8-oxo-7,8-dihydro-2'-deoxyguanosine; 8-oxoGua: 8-oxo-7,8-dihydroguanine; NT: naphthol

BIOMARKERI IZLOŽENOSTI



BIOMARKERI IZLOŽENOSTI

- Biomarkeri izloženosti se dijele u tri kategorije:

- KEMIJSKI SPOJ – direktni i najspecifičniji marker izloženosti nekom spoju je mjerenje tog samog spoja u dostupnim biološkim uzorcima (krv, urin)
- METABOLITI – mnogi kemijski spojevi se brzo metaboliziraju i teško mjere;

  - u tom slučaju mjeri se stabilniji i trajniji metabolit da bi se procijenila izloženost

  - ako je pronađeni metabolit moguće povezati s više ishodišnih spojeva, treba učiniti dodatne testove i utvrditi o kojem spoju se radi
- SUROGAT – u nekim slučajevima kemijski spoj u organizmu izaziva odgovor koji je patognomoničan (glavni, tipični simptom bolesti) za taj spoj, pa mjerenje tog odgovora služi kao zamjena (surogat) umjesto mjerenja samog spoja ili njegovog metabolita;

  - najmanje siguran i točan marker jer postoji mnogo čimbenika koji mogu utjecati na odgovor organizma

BIOMARKERI IZLOŽENOSTI

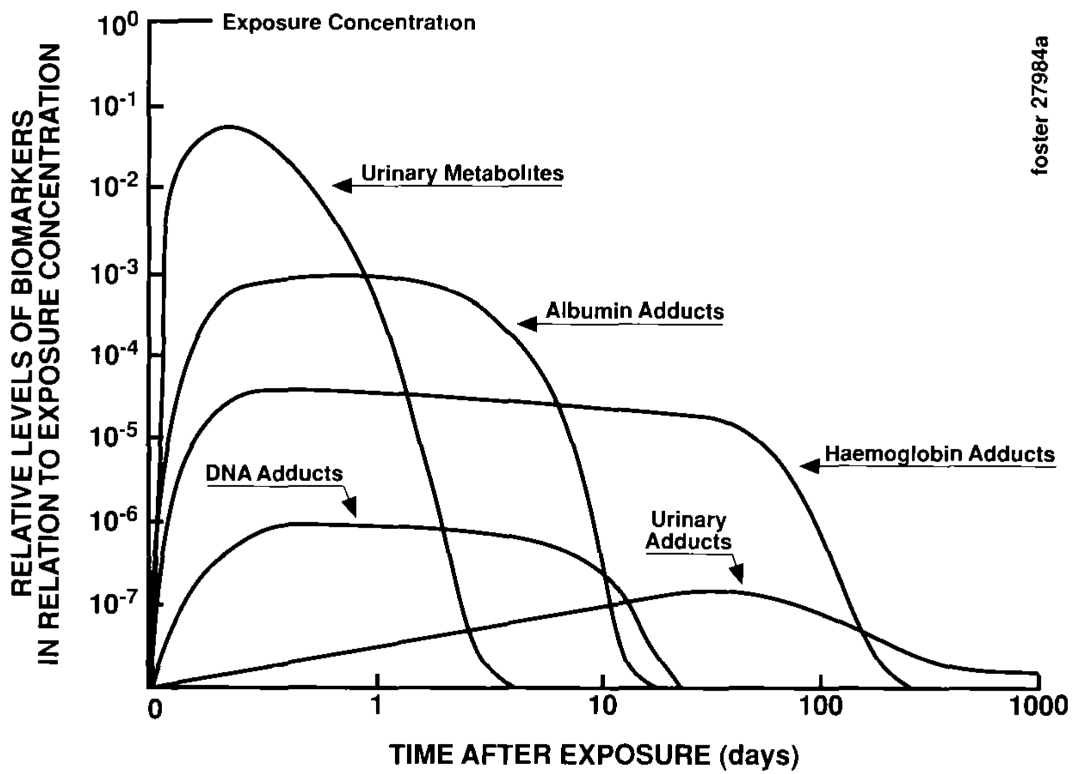


Fig. 3. Hypothetical relationships among different biomarkers of exposure with respect to their relative levels and time of appearance after a single dose (Henderson et al., 1989)

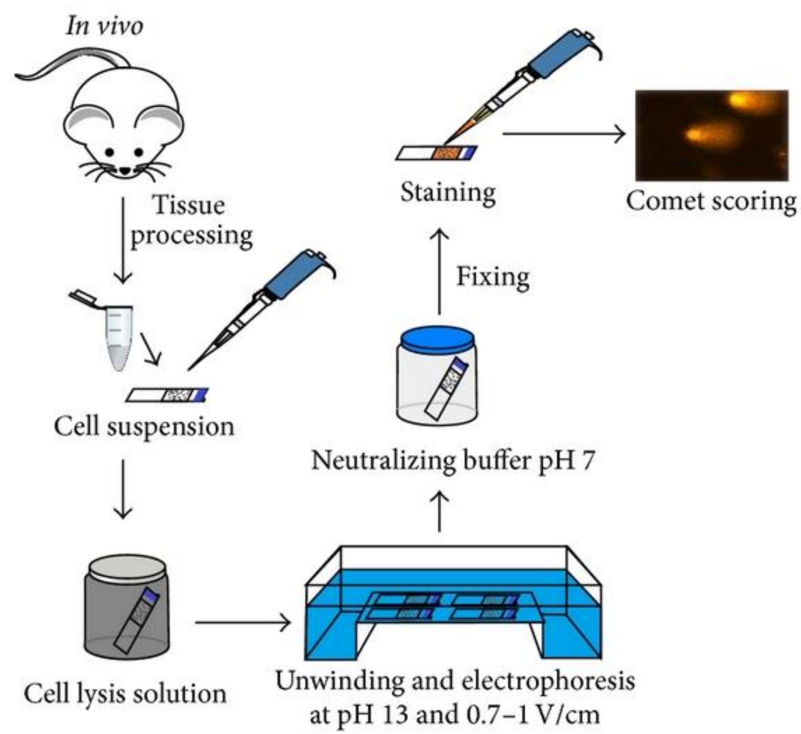
BIOMARKERI IZLOŽENOSTI

TABLE 57.2 Some biomarkers of exposure

Exposure to:	Biological Sample	Chemical
Dioxins	Fat	Dioxins
Lead	Blood	Lead
Aluminum	Bone	Aluminum
Toluene	Exhaled air	Toluene
Methylmercury	Hair	Methylmercury
Aniline	Urine	p-Aminophenol
Hexane	Urine	2,5-Hexanodione
Styrene	Exhaled air and urine	Styrene
Styrene	Urine	Mandelic acid
Ethylene glycol	Urine	Oxalic acid
Benzene	Exhaled air and blood	Benzene
Benzene	Urine	Phenol
Acetone	Urine and blood	Acetone

BIOMARKERI IZLOŽENOSTI

- **KOMET test**
- SCGE – *engl. single -cell gel electrophoresis* / gel elktroforeza pojedinačnih stanica
- Jednostavna, osjetljiva i kvantitativna metoda izučavanja DNA oštećenja (uključujući oksidacijska oštećenja) i popravka na razini pojedinačne stanice tj. određivanja genotoksičnosti
- Kada se provjerava neka tvar ili kemijski spoj u takvim testovima, zapravo se provjerava da li on izaziva kakve promjene u genomu stanice, tj. promjene na razini gena ili kromosoma - spoj toksičan za organizam ili ne
- Princip rada: neka stanica, tj. samo njena jezgra, izloži se nekom agensu i onda se stavlja u alkalne uvjete i pusti na elektroforezu. Jezgra putuje kroz elektorforezni gel i manji komadići putuju prvi jer lakše prolaze kroz gel. Ako ima puno manjih komadića znači da je agens potrgao jezgru i njen genetički materijal – toksičan za organizam
- Za komet test – bitno prirediti stanice koje će se obrađivati



BIOMARKERI UČINKA

- Indikatori su promjene u biološkoj funkciji kao odgovora na izloženost nekom kemijskom spoju
- Biomarkeri štete, biomarkeri toksičnog učinka, biomarkeri toksičnosti
- Daju precizniji i izravniji podatak o učinku na zdravlje od biomarkera izloženosti

TABLE 57.4 Some examples of biomarkers of effect

Exposure to	Biomarker
Organophosphorus and carbamate insecticides	Inhibition of acetylcholinesterase in red blood cells
Lead	Inhibition of δ-amino levulinic dehydratase and subsequent increases of δ-amino levulinic acid in blood
Oxidizing compounds	Induction of antioxidant enzymes such as superoxide dismutase and others
Heavy metals	Induction of metallothioneins

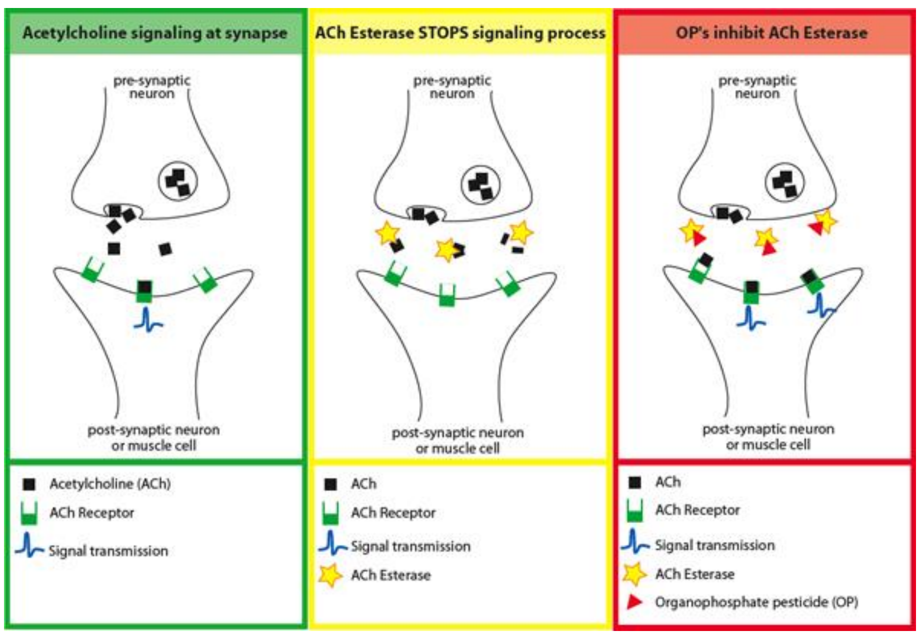
BIOMARKERI UČINKA

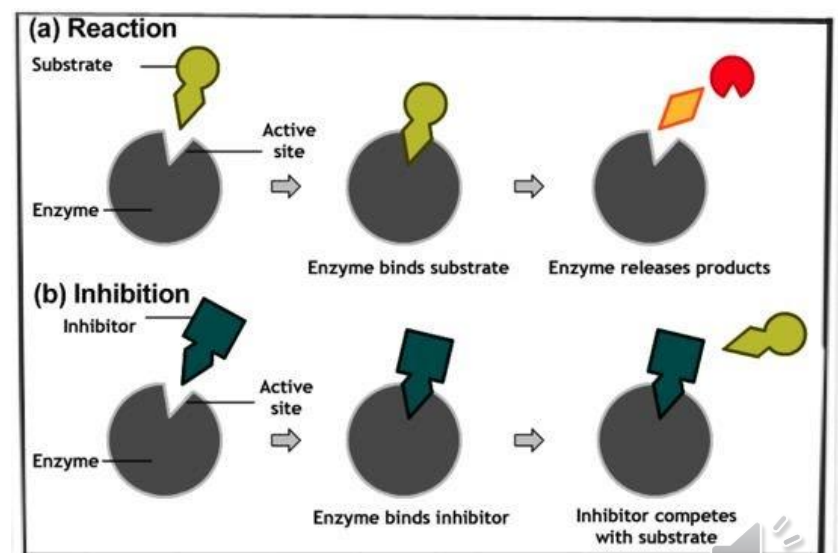
TABLE 57.5 Examples of biomarkers of effect in several matrices and targets

Target System	Matrix	Biomarker
Respiratory system	Bronchoalveolar fluid	Clara cell protein
Blood	Plasma	Alterations in hemosynthesis
Nervous system	Red blood cells	Inhibition of acetylcholinesterase
	Lymphocytes	Inhibition of neuropathy target esterase
	Platelets	Inhibition of monoamine oxidase
Kidney	Urine	Proteinuria (depending on the molecular mass of the excreted protein, it is possible to differentiate among loop of Henle, proximal tubule, and glomeruli)
Immune system	Blood	Immunoglobulin concentration Phenotypic analysis of lymphocytes Antibodies

BIOMARKERI UČINKA

- Acetylcholinesterase (acetilkolinesteraza) – AChE – poznati biomarker učinka
- Organofosfati i karbamati – inhibitorno djeluju na AChE
- AChE – enzim koji katalizira razgradnju acetilkolina na acetatni ion i kolin koji se vraća u presinapsni živčani završetak za ponovnu proizvodnju acetilkolina
- Uloga – sprječava kontinuirano otpuštanje živaca, prijenos živčanih impulsa
- Inhibicija AChE – dovodi do paralize, čak i smrti





BIOMARKERI UČINKA

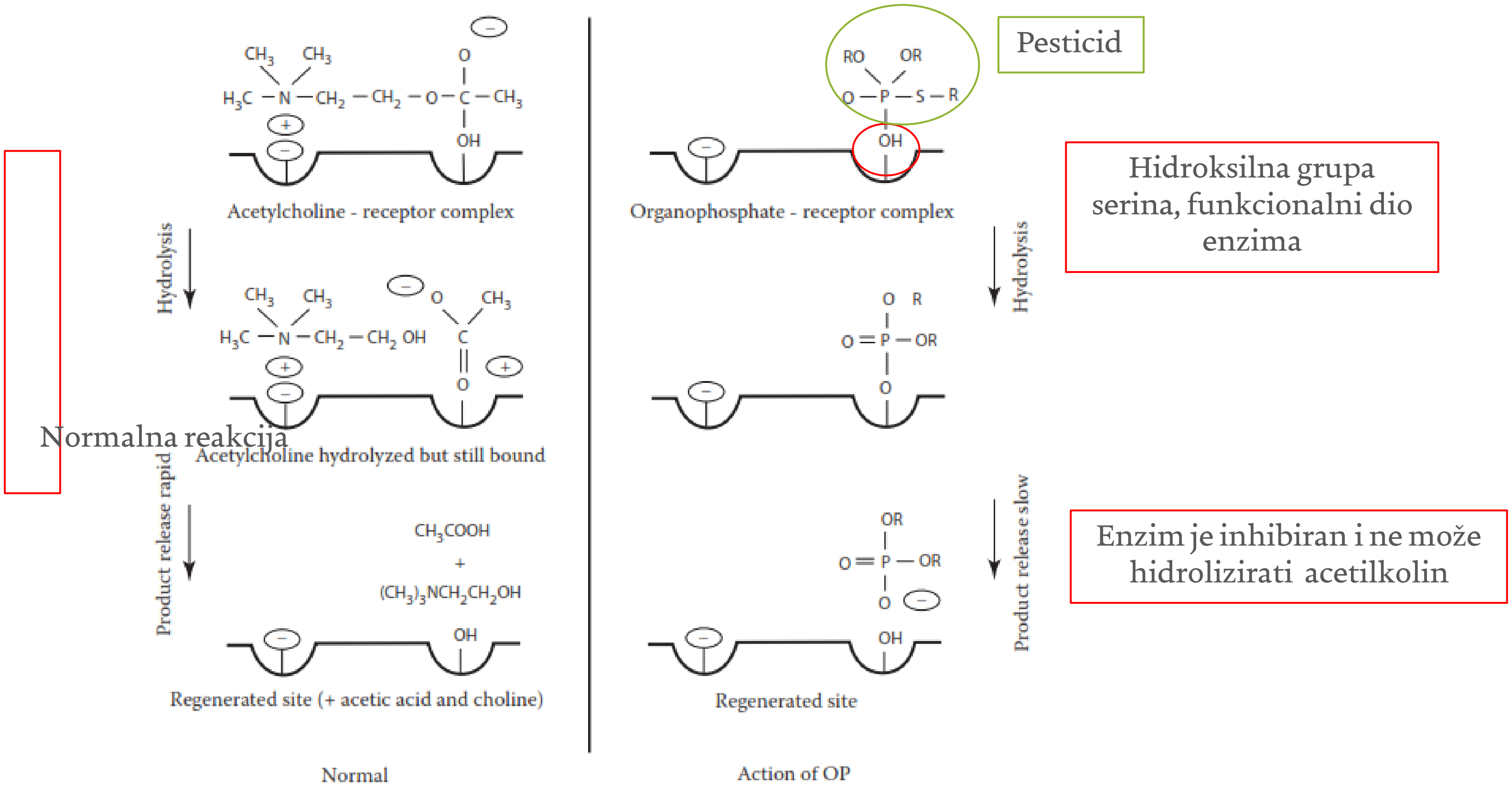


FIGURE 7.3
Mechanism of action of AChE. Under normal conditions, acetylcholine binds to acetylcholinesterase and is broken down (hydrolyzed) to yield acetic acid and choline that break away from the enzyme. Organophosphates bind to hydroxyl groups belonging to the serine amino acid, which is part of the binding site shown on the right side of the enzyme surface. When this happens, the enzyme is inhibited and can no longer hydrolyze acetylcholine.

BIOMARKERI UČINKA

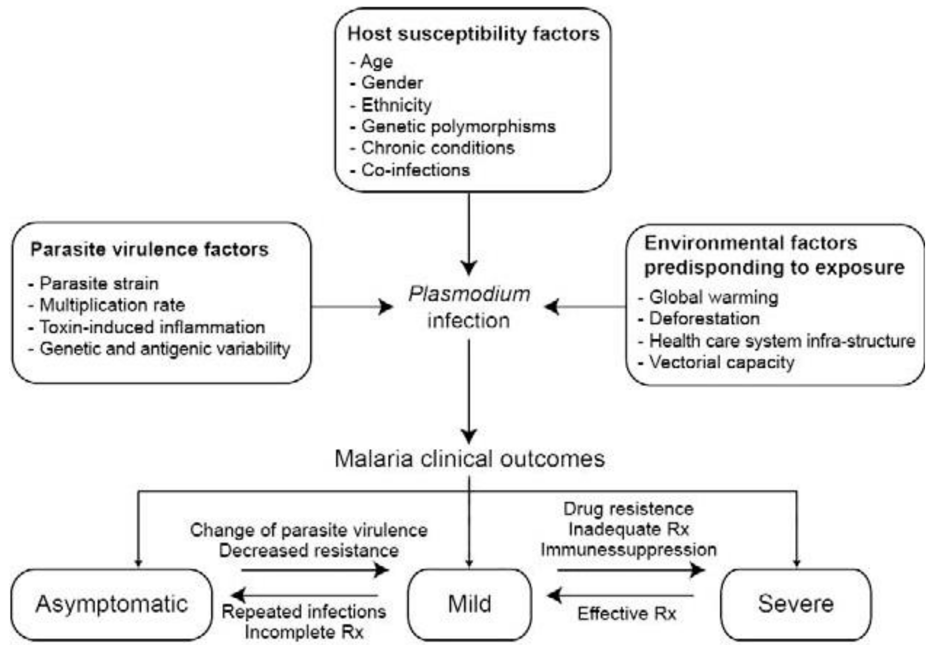
- Podjela biomarkera učinka:

  - BIOINDIKATOR
  - NEODREĐENA POSLJEDICA
  - EGZOGENI SURGAT

- Bioindikator – idelani biomarker učinka ima isključivo poznat mehanizam koji povezuje marker s ishodom
- Pružaju visoku razinu pouzdanosti u predviđanju potencijalnih negatinih učinaka kod jedinke ili populacije na temelju razine markera
- Neodređena posljedica – ne pruža toliko sigurne informacije o potencijalnim štetnim učincima jer su pojedini procesi ili veze između njih manje poznati
- Mogu se koristiti paralelno s drugim biomarkerima – da bi se povećala osjetljivost
- Egzogeni surogat – neki spojevi imaju dobro poznatne štetne učinke koji pak prate sekundarni učinci koji se onda koriste kao zamjenski pokazatelji

BIOMARKERI VJEROJATNOSTI

- Čimbenici koji neke jedinke čine osjetljivijima na izloženost kemijskim spojevima
- Čimbenici koji utječu na kinetiku (apsorpciju, distribuciju, metabolizam) i dinamiku (biološki učinak) kemikalija, veliki utjecaj imaju metabolički enzimi
- Oni uključuju genetske faktore koji bi mogli utjecati na interakciju organizma i kemijskog spoja, starost, zdravlje, stil života
- Koriste se moderne biološke tehnike, npr. određivanje razlike u genima
- Ovaj tip markera se primjenjuje za prevenciju, npr. genetsko testiranje – vjerojatnost izloženosti beriliju



BIOMARKERI – IZLOŽENOSTI, UČINKA I VJEROJATNOSTI (ORGANOFOSFATI)

TABLE 9.2 Biomarkers for organophosphates

Type of Biomarker	Example
Biomarker of exposure	Plasma cholinesterase (BuChE) Red blood cell acetylcholinesterase (ACHE) OP in blood OP metabolites in urine OP adducts to blood proteins
Biomarker of susceptibility	Paraoxonase 1 status Butyrylcholinesterase polymorphisms CYPs
Biomarker of effect	Red blood cell acetylcholinesterase Muscarinic receptors in lymphocytes Lymphocyte neuropathy target esterase (NTE)

BIOMARKERI – IZLOŽENOSTI, UČINKA I VJEROJATNOSTI

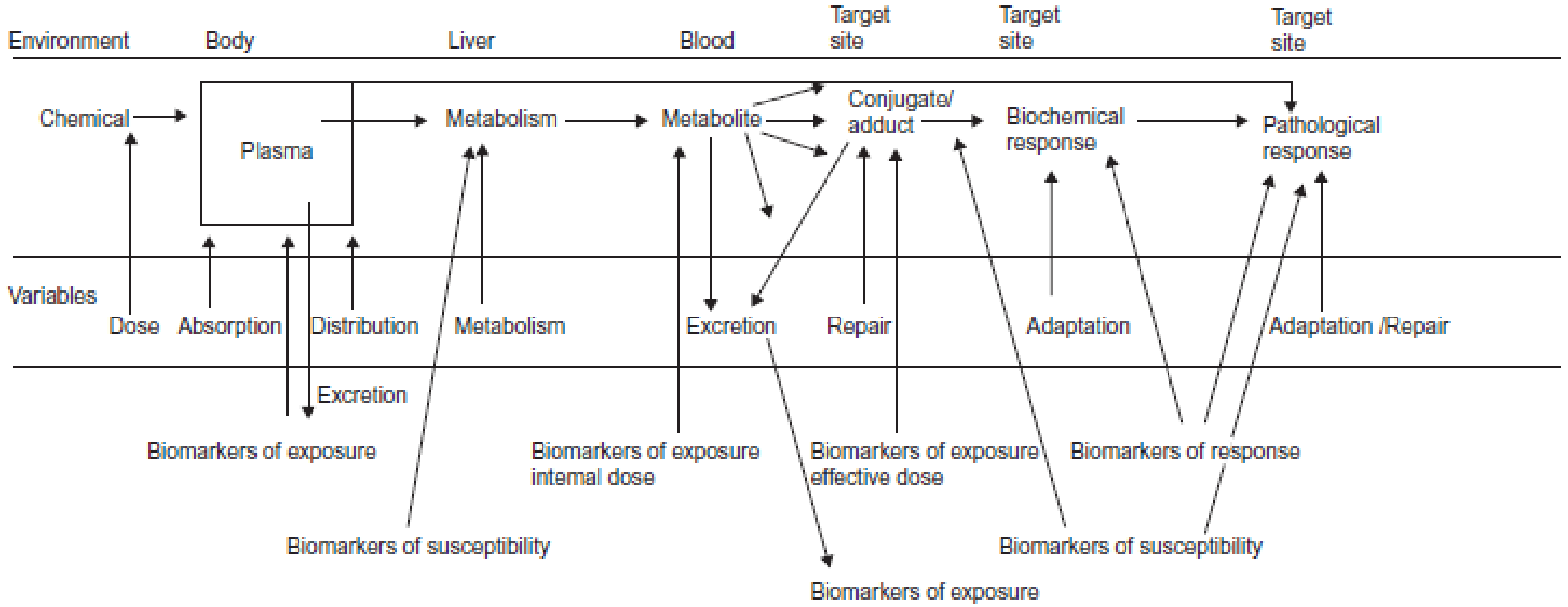


FIGURE 34.1 The biomarker paradigm for metabolism of drugs.