Toxicology course
Principles of Toxicology

Environmental exposure and biomonitoring

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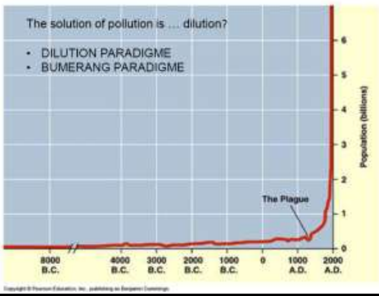
April 4-8, 2016, Zagreb, Croatia

Environmental exposure and biomonitoring
Environmental Toxicology

A relatively new science that grew out of three phenomena:

- Dramatic pressure/presence of human species on the environment
- Awarenes of environmental degradation (either real or potential)
- The importance of a healthy environment in maintaining the quality of life



Environmental degradation/improvement

	1970	1986	2002
World pop'n (10^9)	3.7	5.3	6.2
World carbon emissions (10^9 T)	3.9	5.2	6.6
Solid waste in U.S. /year (10^6 t)	100	130	369
Land in urban develop. (10^6 ac)	35	47	63
U.S. ac. treated w/herbicides (10^6)	158	362	194
Coal use (10^6 t)	613	890	1065
Lead emissions (10^3 T)	204	21	6.2
Sulfur dioxide emissions (10^6 T)	27	21	10.2
Fish kills in US by pollution (10^6)	22	10	10
Accidental oil spilled at sea (10^3 T)	84	6	116
DDT in human adipose tissue (ppm)	8	2	0.03

Ecology vs Toxicology vs Environmental Toxicology

Definitions:

Ecology – the study of the interactions among organisms and between organisms and the environment

Toxicology – study of biological effects of toxins

Environmental toxicology

- Truhart, 1969 - Study of adverse effects of chemicals and physical agents on living organisms
- Duffus, 1980 – Study of effects of toxic substances in both natural and human-created environments
- Moriarity, 1985 – Study of the fate and effects of toxic compounds on ecosystems
- Calow, 1993 – Protection of ecological systems from adverse effects by synthetic chemicals

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Difference between Toxicology and Ecotoxicology

Pharmacology/Toxicology	Ecotoxicology
To protect humans against toxic exposure	Protect the ecosystem
Target organism known Single organisms Homogenous population, contr. conditions	Susceptible species not known, variable Organisms, population, communities... Natural population in var. conditions
Mammalian models as human proxy Only direct effects measured	Direct Experiments on indicator species Both direct and indirect effects measured
Models are homoeothermic and static	Most are poikilothermic and many conformers
Administered dosage via a number of routes can be measured accurately. Dosimetry available.	Toxin identity, dosage and duration often known but many additional variables can alter dosimetry
Basic research: to understand mechanisms	Less basic research but more empirical to estimate threshold concentrations necessary for regulation
Test methods well developed standardized	Methods new and are just now being standardized

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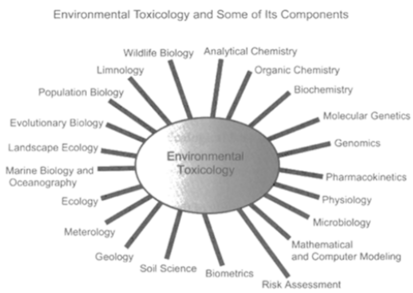
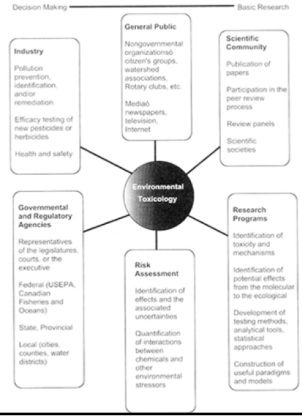
Environmental toxicology is highly interdisciplinary field



Areas interacting within Enivronmental Toxicology



Environmental Science is driven by protective legislation (USA)

A) Federal Food, Drug and Cosmetic Act
B) Toxic Substances Control Act (TOSCA)
C) Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)
D) Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, Superfund)
E) Clean Water Act (CWA) 1974
F) Clean Air Act (CAA) – last amended in 1990

Note:
Can often have overlap of regulations with different standards.
USA Ex. EPA reg. for Hg in wild fish is 10X less than that allowed by FDA in supermarket fish…

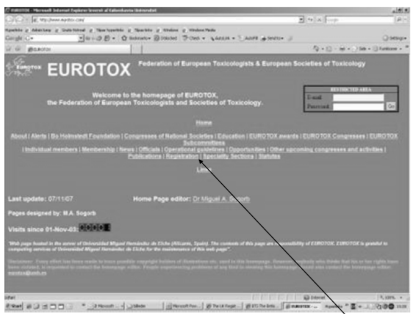
Good Environmental Status 2020 (GES)(EU)

Good Environmental Status 2020 (GES)
- Water Framework Directive (2000/60 / EC) (WFD)
- Marine Strategy Framework Directive (2010) (MSFD)
- Set of detailed criteria and indicators

National implementation:
- AZO List of indicators

Eurotox (www.eurotox.com)

EUROTOX



Education

Some Definitions:

Toxicant: Agent that cause deleterious perturbations and responses outside of the "normal" range for a healthy non-perturbed organism.

Pollution: Introduction of foreign material/substances/energy/organisms into the aquatic environment (freshwater/marine) by humans

Pollutant: Introduced foreign toxicants and substances together with physical changes that decrease the quality of the environment.

Xenobiotic: Normally refers to a synthetic, non-natural, man-made chemical that can cause deleterious effects

The Medium: all bodies of water, freshwater, brackish, saline, permanent, transient

Principles of Toxicology

Paracelsus (1492 – 1541)
„All substances are poisons; there is none which is not a poison. The Right dose differentiates a poison for a remedy."

An Individual View

"The sensitivity of the individual differentiates a poison from a remedy. The fundamental principle of toxicology is the individual's response to a dose."

S. G. Gilbert (1997)

What Constitutes a Toxicant

Assumptions Underlying Toxicity:
1) Causality: Cause-Effect relationship exists (direct or indirect)

2) A dose-response or concentration -response relationship exists
- The effect or response in question results from the toxic agent interacting with sites of toxic action in the organism
- The dosage of toxin is related to dosage and possibly duration of exposure
- above a statistically based threshold value the magnitude of effect is proportional to amount of toxin reaching the sites of action
- dose and concentration relate more to potency at target site of interaction than administered external concentration

3) Effects can be quantified in an accurate and precise manner

However toxic response is complex and predicated upon a number of biological, physical and chemical factors that are often hard to measure and control.

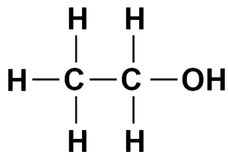
Keywords

Toxicology Definitions:
The study of poisons or the adverse effects of chemica and physical agents on living organisms.

Toxicant
Concentration, Dose
Uptake
Exposure & Adsorption
Frequency of Exposure
Duration of Exposure (Acute, Sub-chronic, Chronic)
Effects (NOEC, LOEC)
Distribution, Accumulation
Metabolism, Detoxification
Excretion

Dose / Response
Risk =
Hazard X Exposure
Individual Sensitivity

What Is This?



(CH_3-CH_2-OH)

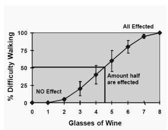
Concentration, Dose, Effects, Response

Ethanol as a Medicin/remedy?

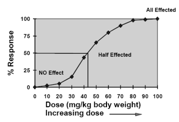
Effects of Amount on Response

Effects of Size on Response

Glasses of Wine - Dose Response



Dose Response Function



Toxicity

Agent	**LD-50 (mg/kg)**
Ethyl alcohol	10 000
Salt (sodium chloride)	4 000
Iron (Ferrous sulfate)	1 500
Morphine	900
Mothballs (paradichlorobenzene)	500
Aspirin	250
DDT	250
Cyanide	10
Nicotine	1
Tetrodotoxin (from fish)	0.01
Dioxin (TCDD)	0.001
Botulinum Toxin	0.00001

What about: - Plastic, Phthalates free?
- Dihydrogen Monoxide (DHMO)?

Many chemicals, limited toxicity data

Chemicals are natural, biological, or synthetic origin:
- Natural (food, metals, minerals)
- Biological (toxins from bacteria)
- Synthetic (manufactured throught chemical processes)

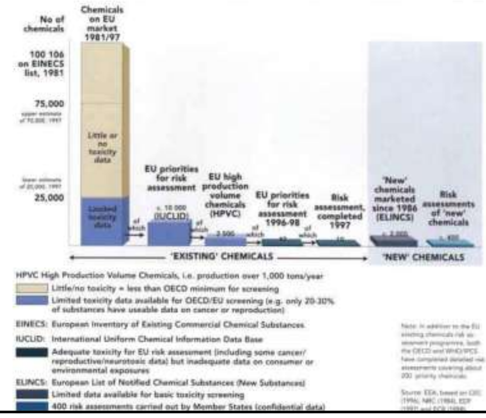
Approximately 100 000 chemicals are currently in use worldwide.

500 new formulations enter the marketplace annually.

Estimated Number of Chemicals in Commercial Substances during the 1990s

Type of chemical	Estimated number
Chemicals in commerce	100,000
Industrial chemicals	72,000
New chemicals introduced per year	2,000
Pesticides (21,000 products)	600
Food additives	8,700
Cosmetic ingredients (40,000 products)	7,500
Human pharmaceuticals	3,300

Many chemicals, limited toxicity data



Many chemicals, limited toxicity data

CAS-system: ~ 10 000 000
(Chemical Abstracts Number)

EU 2006
EINECS+ELINCS: ~ 104 525
(EINECS - European Inventory of Existing Chemical Substances)
(ELINCS - European List of Notified Chemical Substances)

TOSCA: ~ 73 757
(Toxic Substances Control Act USA - 2001)

~10 % (of total 100 000) chemicals with detailed toxicity data
(US National Academy of Sciences)

~43 % (of total 2 800) HPV chemicals without basic toxicity data
(Environmental Protection Agency US, EPA 1998)

HPV (high production volume) chemicals = production ≥ 1000 t/g
4843 chemicals on OECD HPV list (2006)

REACH - new EU system

Registration, Evaluation, Authorisation and Restriction of Chemicals
Directive EC 1907/2006 and 2006/121/EC

since 2007 (annex V TD 67/548/EEC)

- Registration (ECHA - European Chemicals Agency) 10 years cca 30 000 chemicals
- Registration more rigorous and with detailed testing = liability of the Industry!
- REACH is applying for all HPV chemicals (including import) and all chemicals that represent serious threat for human and environment
- REACH is intending to minimize animal testing and development of alternative testing methods

Principles of Toxicology
Environmental exposure and biomonitoring

Environmental exposure and biomonitoring

- Definition : XENOBIOTIC
  - a chemical that is foreign to the biosphere i.e. is not produced by a natural biological or abiotic source
  - Also called anthropogenic, man-made, synthetic, pollutant, contaminant, recalcitrant, persistent, and toxicant
  - Distinguishes between quantity and scale – Gordon Gribble e.g. studied the natural occurrence of organohallogens (chlorbenzoates in fungi) – this is different from large scale chemical processes for the production of PCBs
  - **BTEX** is an acronym for benzene, toluene, ethylbenzene, and xzylene – natural ??
  –

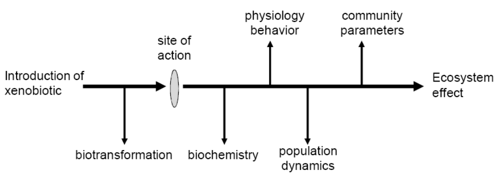
Environmental exposure and biomonitoring

- Environmental toxicology depends on
  - Lab work
    Effects of toxicants on biochemistry and physiology
  - Field work
    Field observations of reproduction and survival in polluted vs. non-polluted sites
  - Modeling of fate and transport of toxicants in the environment i.e. exposure and risk assessment

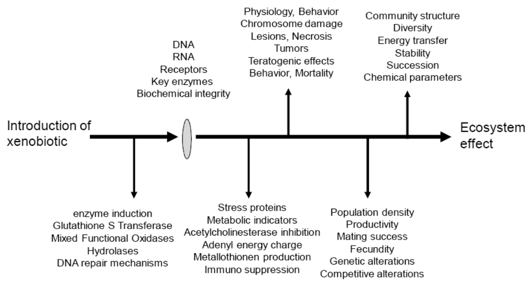
Static models	: short term modeling of ecosystems
Strategic models	: model of a specific aspect of a system
Testable models	: model makes predictions that can be tested in the field or laboratory

Environmental exposure and biomonitoring

- Purpose/function of environmental toxicology is to
  - Identify the mode / site of action of a xenobiotic
  - FATE and TRANSPORT / interaction of a xenobiotic with the biosphere (including specific organisms) after it is released / pollution occurs
  - identify the effect the xenoboitic has on an ecosystems / higher level organisation e.g. loss of fertility

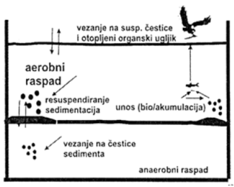


Parameters of xenobiotic interaction with the ecosystem



Variable Environments

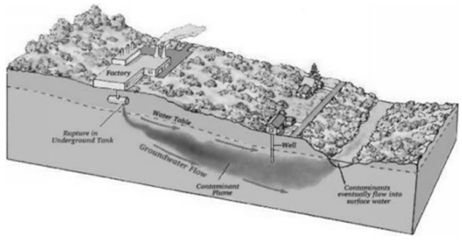
1. Air
2. Water
3. Soil



Called environmental media/environment

Environmental exposure and biomonitoring

- Abiotic environmental fate
- Partitioning
- Adsorption
- Transport/advection



Environmental exposure and biomonitoring

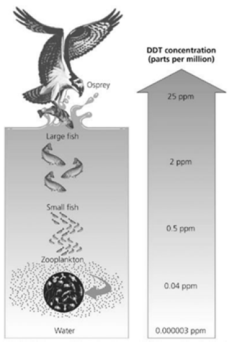
- **Biotic environmental fate**
  - The interaction of a xenobiotic at the site of action in an organism is often 'molecular happenstance' i.e. xenobiotic mimic compounds which are naturally found in species that they affect – hormone mimics
  - **Bioaccumulation**

    The storage of a compound in fatty tissue of an animal
    Result of food chain / trophic levels
  - **Biotransformation**

    Metabolic processes, mainly by environmental bacteria, that alter the structure and toxicity of a compound
  - **Biodegradation**

    Breakdown of a xenobiotic to CO_2 and water

Poisons move up the food chain



- At each trophic level, chemical concentration increases: **biomagnification**.
- *DDT concentrations increase from plankton to fish to fish-eating birds.*

Understanding the three basic functions in environmental toxicology

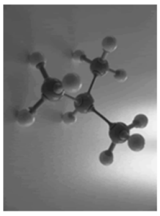
1. Interaction of toxicant (xenobiotic) with the environment
   - Determines amount (dose) of toxicant available to living organisms
   - Time component
2. Interaction of toxicant with site of action
   - Usually receptor on/in a cell
   - Receptor often a cell protein
3. Interaction of toxicant at the molecular level, leading to all higher level ecological effects
   - Line from molecular to ecological effects is poorly understood

Classification of toxicological effects

- Chemical/physical-chemical characteristics
- Bioaccumulation/biotransformation/biodegradation
- Site of action
- Biochemical monitoring
- Physiological and behavioral effects
- Population parameters
- Community parameters
- Ecosystem effects

Chemical/Physical-Chemical Characteristics

- Interactions of xenobiotic compound with biological compounds determines toxicity
- The degree of effect that is due to the physico-chemical characteristics of the compound is called the Structure-Activity Relationship (QSAR).
- Has potential for allowing prediction of toxic effects based only on structure of xenobiotic.
- Could save lots of time, money, effort while allowing a greater degree of protection



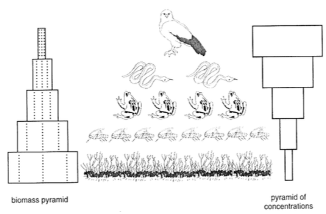
Bioaccumulation/Biotransformation/Biodegradation

…

- Many things can happen to chemical between release to environment and arrival at the biological site of action
  1. <u>**Bioaccumulation**</u> – increase in concentration of chemical in tissue relative to concentration in environment
     - More likely in lipid solubule/lipophilics
  2. <u>**Biotransformation**</u> – chemical change in toxicant caused by biological tissue
     - May decrease [usually] or increase toxicity
  3. <u>**Biodegradation**</u> – breakdown of a xenobiotic into a simpler chemical form
     - Could be the result of biotransformation
- All above processes dependent on site specific conditions so direction and degree hard to predict

Environmental exposure and biomonitoring

Dilution Paradigm



Boomerang Paradigm

DDT, MeHg

Receptor (site) and Mode of Action

- Active site extremely important on determining mode of action
  - May cause very specific or very general effect
- Active site may be on specific nucleic acids, enzymes, cell membranes or non-specific

Covered much more in Xenobiotic Metabolism part of course

Biochemical/Molecular Effects

- Broad range of possible effects
- Could be general (ex. general effect on DNA) or specific (ex. effect of specific portion of DNA)
- Includes effects on chromosomes, enzyme systems, immunological system, etc.

Physiological and Behavioral alterations

- Biochemical/molecular effects manifested at higher organismal level
- Classical means by which population health is assessed
- Major drawback → extrapolation from individual effect to population and ultimately ecosystem effect
- Can include pathology, oncogenesis, reproduction, **mortality**, osmo- and ionoregulation, behaviors (fish respiration, cough response), temperature preference, predator avoidance or prey detection

Community effects

Principles of Toxicology

- Evaluation of community structure extensively used in field studies
- Many indices developed to quantify species composition
- Most widely used → species diversity (biodiversity)
  - Most dramatic impact that can be observed
  - Decrease in species diversity usually = impact but sometimes reverse is true
  - Diversity can be misleading → can have same diversity after exposure but be the result of a completely different set of species

Note: most of what we call communities are really assemblages because we do not understand most of the interactions among populations in a "community"

Ecosystem effects

- Most ecosystem-level changes indicate a serious problem
- Variables measured can include metabolism (energy capture, flow, loss), net productivity (gross productivity – respiration), biomass accumulation, rate of detrital breakdown, landscape alteration, species distribution, chemistry
- Evaluation of effects must be system-specific
- Most environmental regulations aimed at protecting ecosystem structure and function but these are rarely measured when determining compliance

Human Impact on Environment

INDUSTRIAL REVOLUTION

Before
- Agriculture (8000-7000)
- Deforestation
- Domestication of animals

After
- Intensive resource usage
- Intensive fossil fuel usage
- Sewage, industrial waste water release
- Synthesis of new compounds and materials (xenobiotics)
- Intensive agriculture and pesticide application
- Mass production
- Accidents (chemical, nuclear, oil)
- Release of green house gasses (CO_2, CH_4, CFCs)

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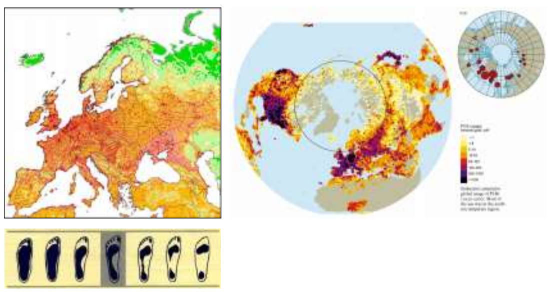
Need for Toxicology/Ecotoxicology and Biological Monitoring of Pollution

Map of Human Footprint



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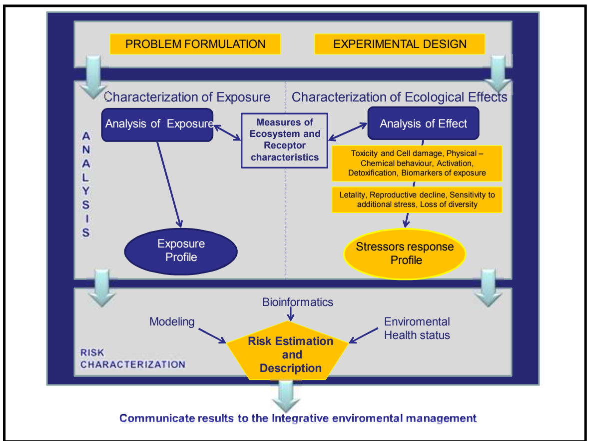
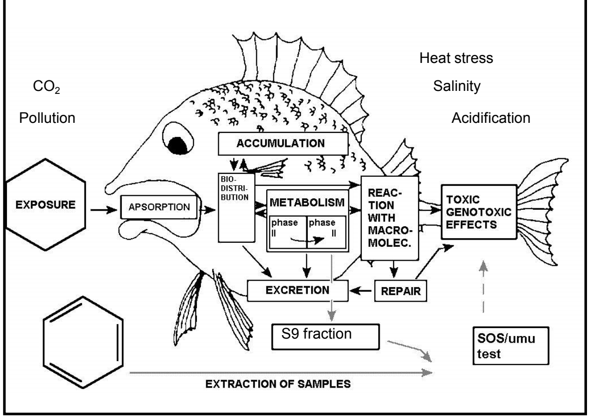
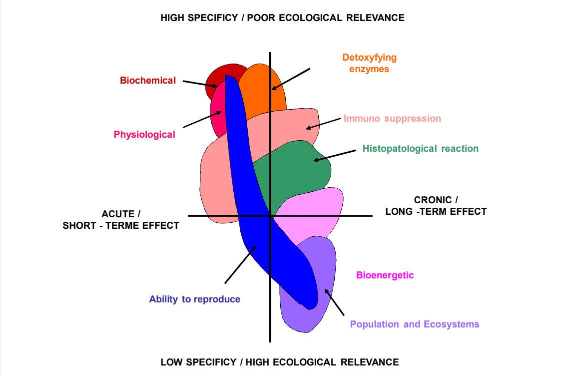


Natural and anthropogenic effects on marine organisms



BIOLOGICAL MONITORING OF POLLUTION - BIOMARKERS



Marine ecotoxicology

Explain toxic effects on marine organisms caused by stabile natural and anthropogenic chemical stressors

Based on 4 processes
- Release of compounds in the environment
- Transfer of compounds with or without chemical transformation
- Exposure of one or more target organisms to different substances
- Response of organisms to xenobiotics (pollution/contamination)

Goal
Distinguish between natural and anthropogenic factors that effects an organism by
- Determination of contaminant effect and its level in selected marine invertebrates
- Determination of proteotoxic, cytotoxic and genotoxic mechanisms involved in toxification of xenobiotics on cellular and molecular level

Application of obtained scientific knowledge in marine environmental risk assessment

Types of Pollutant

Major classes of pollutant can be broken down into different categories based either upon source, chemistry or effect:
- Oil
- Sewage
- Chemicals
- synthetic organic chemicals e.g. pesticides
- inorganic chemicals e.g. metals, fertilizers
- Non biodegradable, persistent solid wastes. e.g. plastics, tins, cans
- Thermal Pollution – power stations
- Nuclear Pollution - radioactive contaminants and irradiation
- Biological Pollution – introduction of non-native, alien species
- Atmospheric Pollution – particles, volatile compounds, greenhouse effect, ozone depletion and acid rain.

Problem and aim

Legislation and practices have highlighted the importance of the biomarker integrated approach in environment quality assessment.

The main difficulty for using biomarkers in a monitoring programs is the interference of natural environmental factors with the biological responses.

Laboratory studies using model substances (pollution) usually show potential of different biomarkers for environmental studies.

Animals *in situ* are exposed to a variety of natural stressors including hypoxia, salinity, temperature stress, etc.

However studies of combined effects of ecologically relevant environmental conditions variation and pollution are scarce and still missing.

Problem and aim

Field studies of biomarkers in marine organisms distinguished sites-areas influenced by industrial/urban mixed pollution, but also some of applied biomarkers indicated seasonal variation… (Hamer et al. 2004. Environ. Int., Hamer et al. 2008. Aquat. Tox)



Biological monitoring of Pollution using biomarkers in a monitoring programs

1998-2012 Croatian national programme for monitoring of the Adriatic Sea (“Systematic Research of the Adriatic Sea as a Base for Sustainable Development of the Republic of Croatia” - Project Adriatic

It consists of four subprojects including more than fifteen themes.

1.5. “The level and influence of pollution in selected areas - Hot spots”

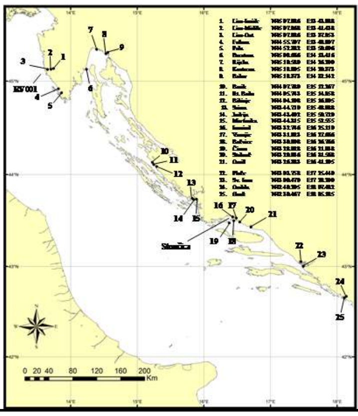
- “Impact of anthropogenic activities on the Croatian coastal sea”

Jadranski projekt II

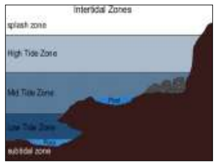
Jadranski projekt III

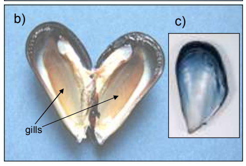
2015 National list of indicators – AZO

Investigated sites of the eastern coastal area of Adriatic sea



Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819 (Mollusca, Bivalvia)





a well-known and extensively used “sentinel” organism in marine environmental monitoring programs

wide distribution, easy to collect, filter-feeding nutrition, prone to accumulate very high concentrations of many pollutants…

Mussel shells as indicators of freshwater influx (hypoosmotic stress) and pollution along Eastern Adriatic Sea Coast

1999 ICES WGBEC meeting

WORKING GROUP ON BIOLOGICAL EFFECTS OF CONTAMINANTS

- Discussion regarding the effects of changing salinity on the responses of biomarkers used in monitoring programmes.

Conclusion:

- agreed that more experimental work is required to model the effects of fluctuating salinity on the susceptibility of organisms to contaminants.

2010 SETAC Call for Research

GLOBAL CLIMATE CHANGE AND ENVIRONMENTAL CONTAMINANTS

- Members of SETAC (environ. chemists, toxicologicst, and risk assessors) were challenged to work together to understand how global climate change will influence the mechanisms of contaminants on humans and the environment.

Hypothesis

Biomarkers are essential to assess the health risk of exposure to potentially toxic/harmful chemicals.

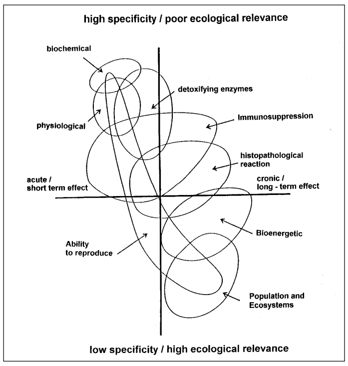
Interference of natural environmental factors with the biological responses to contamination/pollution - similar to Chemical Mixtures Studies?

- **ADDITIVE :**
  2+2 = 4
- **LESS THAN ADDITIVE (ANTAGONISTIC):**
  2+2 =3
- **GREATER THAN ADDITIVE (SYNERGISTIC):**
  2+2 = 6

Ecological response of biological markers

When external conditions are closely controlled, biological responses can be accurately assessed and related to exposure events (pollution).

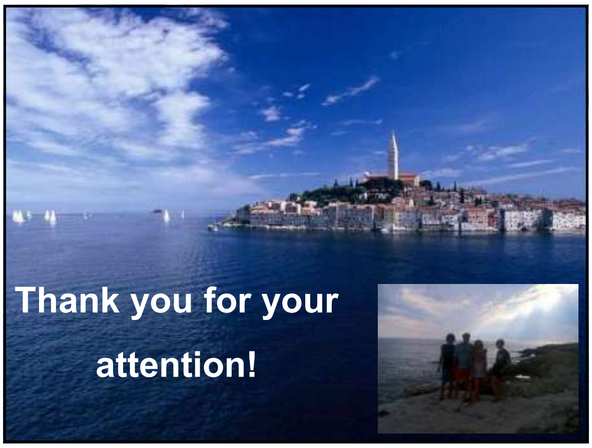
In such cases, the use of biomarkers to monitor the health of the environment can be proposed (McCarthy and Shugart, 1990).



Biomonitoring - Applied biomarkers and physiological indices of pollution exposure, environmental stress and organism health status

Chemical analyses of media and biota + biomarker and biotests

Sea Water Biotests	**Sediment Biotests**	**Mussel *Mytilus* spp. Biological effects**	**Fish Biological effects**
Toxicity (Microtox)	Toxicity (Microtox)	Toxicity (Microtox)	
Genotoxicity (Umu-test)	Genotoxicity (Umu-test)	Genotoxicity (Umu-test)	Genotoxicity (Umu-test) S9 Potential activation
			EROD
		General stress -Survival in air (SOS test) -Stability of lysosomal membranes (LMS)	Bile PAH metabolites
		Oxidative stress -Lipofuscine content (LF)	
		Organic pollution -Neutral lipids (NL)	
		Neurotoxins (OP) -Acetillkolin esterase (AChE)	
		Heavy metals -Metallothioneins (MT)	
		Genotoxins -DNA integrity (FAST Micromethod/ Commet assay) Single strand breaks, cell cycle, micronucleus	



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