GDAŃSK UNIVERSITY OF TECHNOLOGY

Introducing ecotoxicology into a curriculum of the GUT - future perspectives

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Please consider this lecture as an introduction to future advanced cooperation…

Toxicology…

- …is a science about factors that due to their chemical or physical properties may exert negative? impact on functioning of living organisms or cause their death. There is no universal definition of toxicity, but it can be considered that toxicity is feature of chemical ? compounds that cause disturbance in the physiology of the organism, which can result even in death. This definition may apply to the whole organism but also to individual organs, tissues and cells.

- Phillippus von Hohenheim (better known as **Paracelsus**) is considered to be the father of toxicology and in the 16th century he formulated his famous words "*All things are poison and nothing (is) without poison; only the dose makes that a thing is no poison*" which can be understood that the dose "makes" a toxin. Nowadays as a toxin are considered chemicals that quickly and in small doses, cause the death of the organism and according to the definition adopted from the United States for a poison are considered substances with lethal dose of 50 mg or less per kilogram of body weight.

- Toxicology as scientific discipline deals with the study of toxic properties of chemical substances against living organisms. Living organisms absorb both negative and positive effects of chemicals. The basis of toxicological studies is the dose-response dependence however, the observed effect is a component of many factors like species and individual differences, age and gender.

Ecotoxicology…

- …also known as environmental toxicology is a branch of science coming from toxicology. The term itself has been introduced in 1969 by Truhaut as combination of words toxicology and ecology.

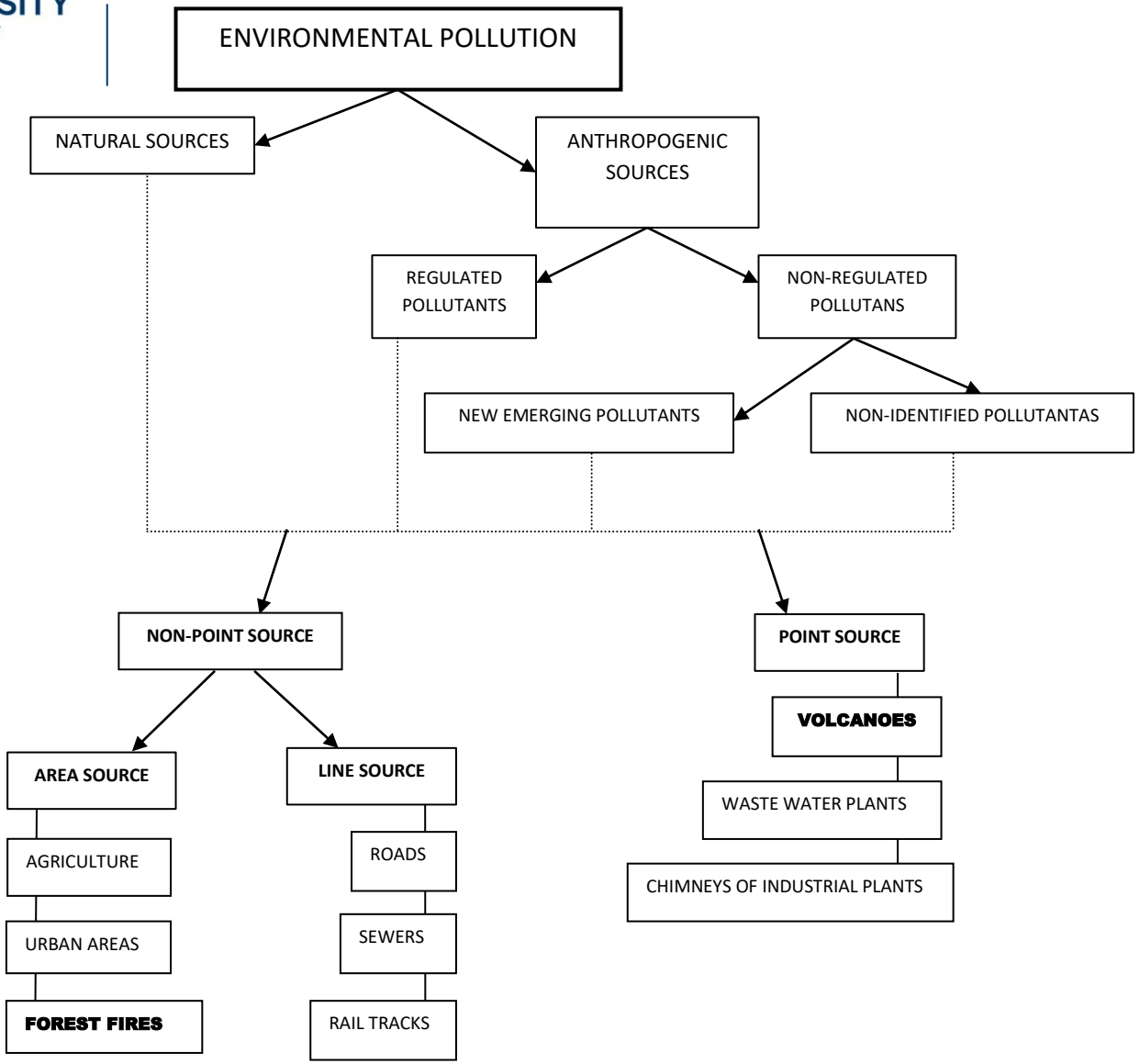
- Ecotoxicology deals with studies connected with detection and impact of harmful chemicals on living organisms. More widely it deals with studying impact of factors on ecosystems. Due to this it can have wider applications in determining processes at organisms and population levels.

There are many definitions describing ecotoxicology:

1. It is branch of science that studies impact of substances present in the environment on the organisms in prolonged, systematic and low-dosage manner.
2. It deals with protection of ecological systems from harmful action of synthetic compounds.
3. It deals with studying harmful impact of chemicals on the entire ecosystems.
4. It is branch of science that integrates ecological and toxicological effects of chemical pollutants and their environmental fate.

One can distinguish:

- **Clinical toxicology**, that studies impact of poisons and their treatment;
- **Pharmaceuticals' toxicology**, assessing impact of drugs of abuse or exhibiting negative site-effects to human beings;
- **Industrial toxicology**, studying impact of chemicals used in industry on human well-being;
- **Toxicology of plant protection chemicals**, determining toxicity of plant protection compounds to human beings;
- **Toxicology of foodstuffs**, studying natural products and chemicals added to food at different technological processes;
- **Toxicology of cosmetics** (e.g. of plastic masses);
- **Forensic toxicology**, that deals with detection and determination of poisons, mainly in living patients and dead bodies.


ENVIRONMENTAL POLLUTION
NATURAL SOURCES
ANTHROPOGENIC SOURCES
REGULATED POLLUTANTS
NON-REGULATED POLLUTANS
NEW EMERGING POLLUTANTS
NON-IDENTIFIED POLLUTANTAS
NON-POINT SOURCE
POINT SOURCE
AREA SOURCE
LINE SOURCE
VOLCANOES
AGRICULTURE
ROADS
WASTE WATER PLANTS
URBAN AREAS
SEWERS
CHIMNEYS OF INDUSTRIAL PLANTS
FOREST FIRES
RAIL TRACKS

Measures of toxic effect

Impact of environmental stressors on different organisms is a reason of introducing so called **toxic effects**.

Toxic effect is reversible or irreversible change occuring in the living organism having negative impact on health status.

As far as time of effect appearance is considered two basic types of toxicity can be observed:

acute toxicity - adverse effects on test organisms from exposure to a chemical compound (or to a mixture with relatively high concentrations) that may lead to disturbances of physiological activity and death after a short time of exposure.

chronic toxicity - adverse effects on test organisms from exposure to a chemical compound (or to a mixture with relatively low concentrations) over a long period of time – normally, 1/10 of a life cycle until the first offspring generation is produced. Sublethal concentrations are used in model tests. Changes in physiological activity, e.g. alimentary, reproductive functions, genetic disorders and organ disturbances are observed and assessed.

Let’s have some examples…

Measures of toxic effect

EC – effective concentration – the concentration of a factor (or of a mixture) that induces observable changes in test organisms, e.g. immobilization, inhibition of biochemical processes or growth. Test result is expressed as concentration that affects given physiological process by 50% after a specific time of exposure – EC_{50} in relation to control one.

IC – inhibitory concentration (inhibiting the growth, chlorophyll generation, etc.) – the concentration of a factor (or of a mixture) that in sub-lethal tests (where the organisms are not killed) inhibits physical and biological activity of test organism by a specific percent in relation to control one (e.g. IC_{25}).

LC – lethal concentration – the concentration of a factor (or of a mixture) that is lethal to a specified number (expressed as %) of population members after a specified time of exposure, e.g. LC_{50}, LC_{100} in relation to control one.

LOEC – lowest observed effect concentration – the lowest concentration of a factor (or of a mixture) which (in a specified time of exposure) has an effect causing observable changes in test organisms in relation to control ones.

NOEC – no observed effect concentration – the highest concentration of a factor (or of a mixture) which (in a specified time of exposure) has no effects causing observable changes in test organisms in relation to control ones.

[mol/dm^3] or [mol/kg]

DOSES…

Interactions of environmental factors

Toxicity of pollutants depends on numerous factors, just to mention:

- physical and chemical properties of substances,
- composition and condition of popultaions,
- concentration and bioavailability of pollutants.

Not always determination of continuously decreasing concentration levels makes sense because one have to consider environmental samples as a mixture of different chemical compounds, which not remain neutral to each other. There are three fundamental interactions, which can occur between chemicals:

- additivity occurs, when the total effect, which can produce chemical compounds present in sample is a sum of the effects of compounds separately;

- synergism is potentiating of the effects of the substance, the total effect is greater than the effects produced by the individual compounds alone;

- antagonism means decreasing of total effect, the total effect is smaller than the effects caused by the individual compounds alone.

Interactions between the compounds may result in decrease or increase in toxicity against the exposed organisms inhabiting a given ecosystem therefore, substances even at low levels of the concentration can cause adverse reactions.

BIOTEST (greek *bios* – life + latine *testari* – testify)

is an analytical procedure, performed in laboratory under standardized biotic and abiotic conditions to quantitatively determine effects like e.g.:

- ✓ **growth inhibition**,
- ✓ **mobility change**,
- ✓ **change of bioluminescence**,
- ✓ **change of mortality** *etc*.,

observed at bioindicating organisms after contact with toxic substance of sample.

Define: biotest or bioindicator?

Features of biotests

- universal – detection of wide range of toxic factors;
- high sensitivity – ability to respond to presence of toxic factors at low concentration levels 10^{-12} – 10^{-15} mol/l;
- Rate of measurement – short time of reaction (few miliseconds in case of bioluminescence);
- Simplicity and availability of reagents;
- Low cost of devices;
- High S/N ratios – no noise signal at all in some cases;
- Easyness to collect signal – process can be quite easily automated;
- No need to use toxic materials (neither radioactive or explosives/flamable).

Biotests

Biotests are more and more popular in environmental analytics and monitoring.

Biotesting has now 3 leading directions:
1. *ex-situ*, in laboratory. It can be divided into two subcategories:
    a) pollutants and their mixtures are introduced to water and sediments. It can be used to certify bioassays or get information on toxicity of substances under given precise conditions;
    b) studies conducted against samples coming from natural habitats. Always reference material/sample must be used.

2. *in-situ*, under natural conditions.

Furthermore biotests can be divided into way of performing studies:
1. static conditions – sample is not changed during performing the research,
2. semi-static conditions – sample is periodically changed during performing the research,
3. dynamic conditions – sample is continuously changed during performing the research.

Battery…

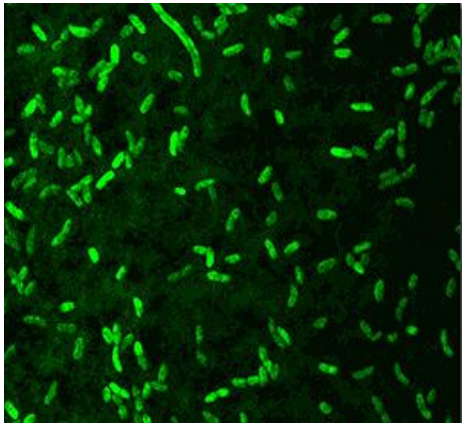
One of goals of performing bioassaying is supplementing classical instrumental studies. Still, the greatest issues may appear at the stage of data treatment. Different organisms are characterized with different sensitivity, range of inhabiting, metabolism *etc*.

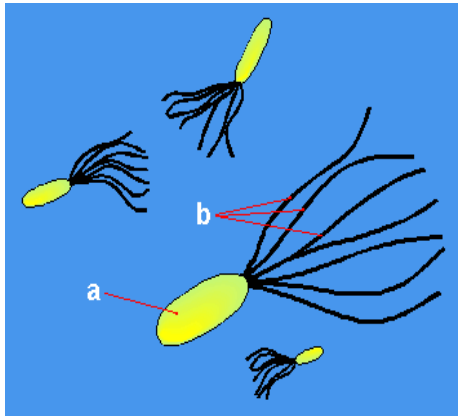
Utilizing 1 biotesting organism rises threat of underestimating or overestimating danger coming from presence of pollutants present in given sample.

This risk can be reduced by using several biotests/organisms of different trophic levels – such a system is called a battery of bioassays. It also enables studying toxicity of complex mixtures of factors present in sample but having different Modes of Action (MoA).

Bacterial tests

Bacterial species used in bioassaying:
- *Escherichia coli,*
- *Pseudomonas fluorescens,*
- *Pseudomonas putida,*
- *Lactobacillus casei,*
- *Lactobacillus brevis*, etc..





Endpoints assessed include:
- Growth inhibition,
- Metabolism of basic materials/substrates,
- Change of bioluminescence/fluorescence.

Microtox patent 1968

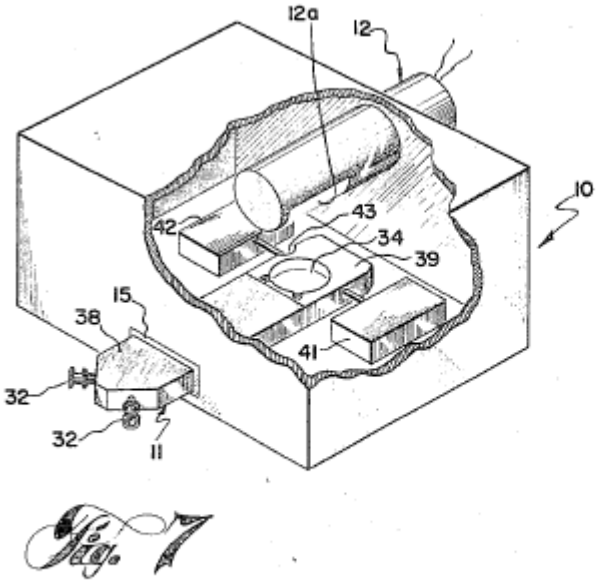


Fig. 7

INVENTORS
ALBERT L. JORDAN
EDWIN R. SCHNAUSS
EDWARD H. SIE
ANDREW THANOS
EARL V. WAGONER, JR.
BY Richard D. Seibel
ATTORNEY

United States Patent Office

3,370,175
Patented Feb. 20, 1968

1

3,370,175
TOXICANT DETECTOR
Albert L. Jordon, West Covina, Edwin R. Schnauss and Edward H. Sie, Los Angeles, Andrew Thanos, Redondo Beach, and Earl V. Wagoner, Jr., San Pedro, Calif., assignors to North American Rockwell Corporation, a corporation of Delaware
Filed Jan. 28, 1965, Ser. No. 428,744
24 Claims. (Cl. 250—217)

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preferred embodiment there is provided a drift normalizing system including means for repetitively generating voltage ramps that converge on a value of voltage corresponding to light intensity. Coincidence of one of these ramp voltages with the voltage from a photodetector monitoring a culture of luminous organisms provides either a charge or discharge of a capacitor depending on which of the converging ramps first reaches the photodetector amplifier for adjusting the gain thereof to compen-

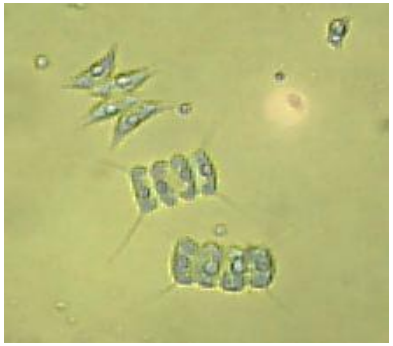
Plant tests - phytotests

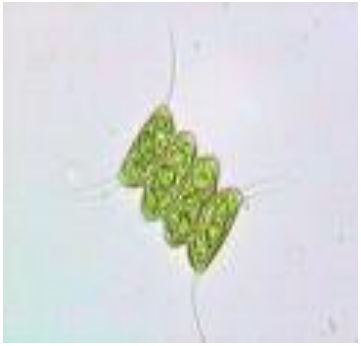
The most often assessed endpoints include:
- Growth inhibition,
- Change of breathing and metabolism,
- Total chlorophyllle content,
- Biomass increment,
- Number and surface of cells,
- fluorescence.

Higher plants are less often used for liquid samples studies.

Plant tests - phytotests

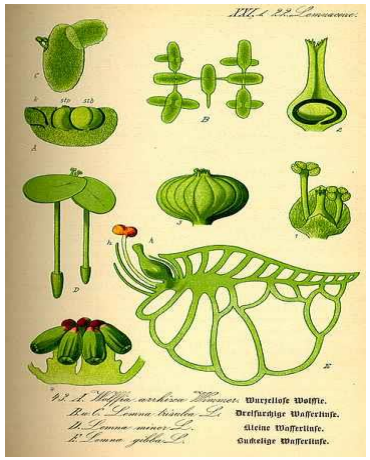




Plant tests - phytotests





Lemna minor

Plant tests - phytotests

ALGALTOXKIT F – utilizing *Selenastrum capricornatum*.

Test ALGALTOXKIT F

Plant tests - phytotests

PHYTOTOXKIT F^{TM}– using *Sorghum saccharatum*, *Lepidium sativum* and *Sinapis alba*

Test PHYTOTOXKIT F

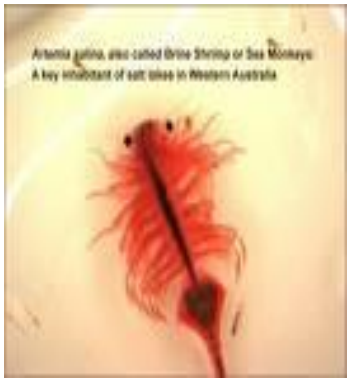
Animal tests

- Used more often than phytotests.
- Used to determine toxicity of all types of samples: gas, liquid, solids.

Most often assessed endpoints are:
- Growth inhibition,
- Mortality,
- Length and mass of particular individuals,
- Number of next generation individuals and their sex ratio,
- Number of cells,
- Lost of apetyte or mobilty.

Animal tests





Artemia salina



Rotifera

Animal tests

Daphnia magna

Animal tests

DAPHTOXKIT F^{TM}– utilizing crustaceans *Daphnia magna* and *D. pulex*

Test DAPHTOXKIT F

Animal tests

ARTOXKIT M- crustaceans *Artemia salina*

Test ARTOXKIT M

Animal tests

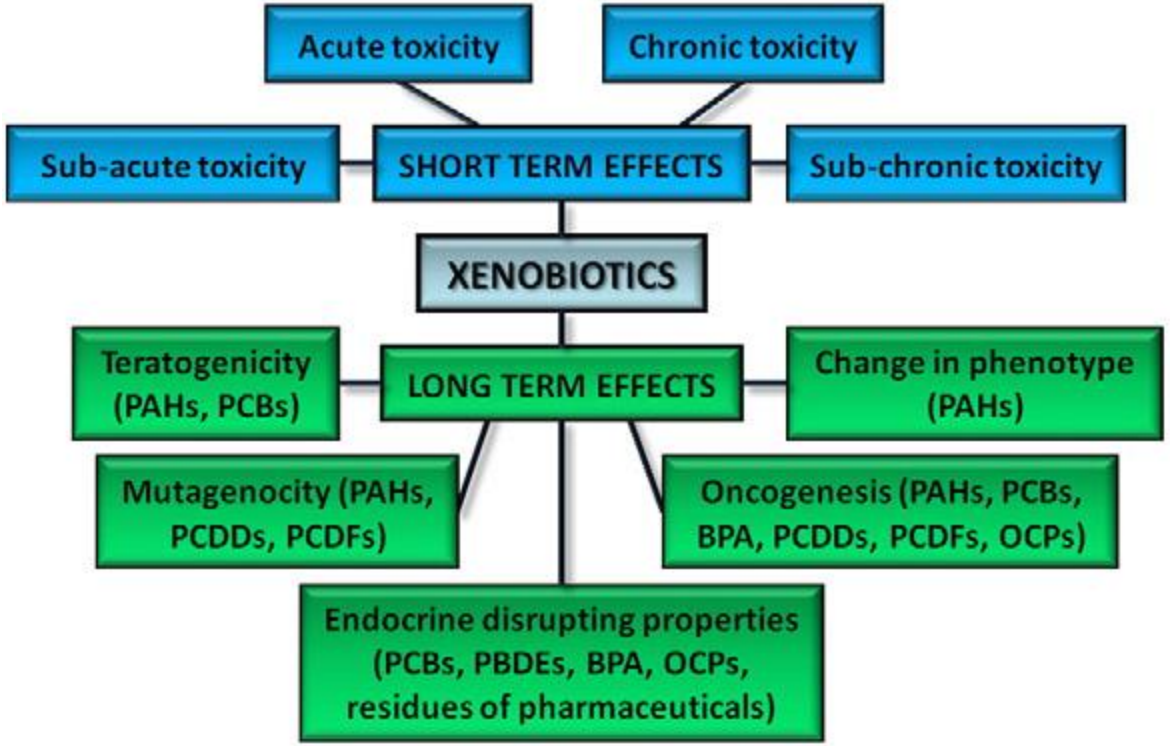
Test *Helix aspersa* - test with land animals

Animal tests



Heterocypris incongruens

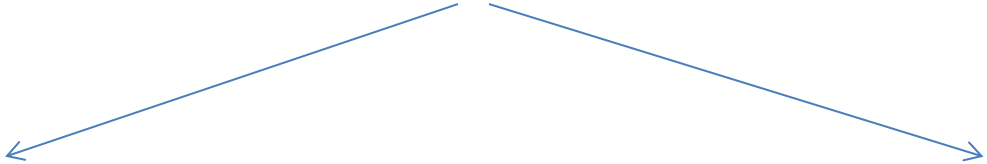
Aims…



…but to reach them it is ecessary to apply universal holistic approach.

Main scientific directions

Toxicological and chemometric studies of:



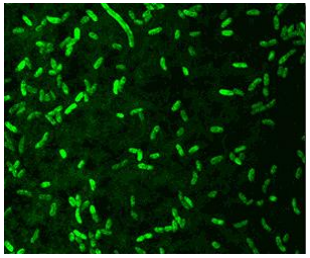
A. Environmental samples

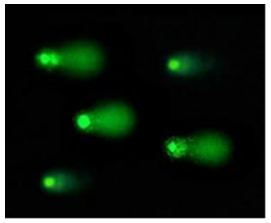
B. Model samples

1. WWTP (*Poland*)*

Object studied:

- Samples of water from 116 locations (prior to and after WWTP release point)





Research performed:

1. Microtox®
2. Ostracodtoxkit F™
3. Xenoscreen YES/YAS
4. comet assay (HT29)
5. instrumental studies (e.g. *TOC, selected heavy metals and inorganic ions – over 30 parameters*)
6. statistical and chemometric treatment (PCA, neuron nets, clusters, Hasse diagrams)

Aim:

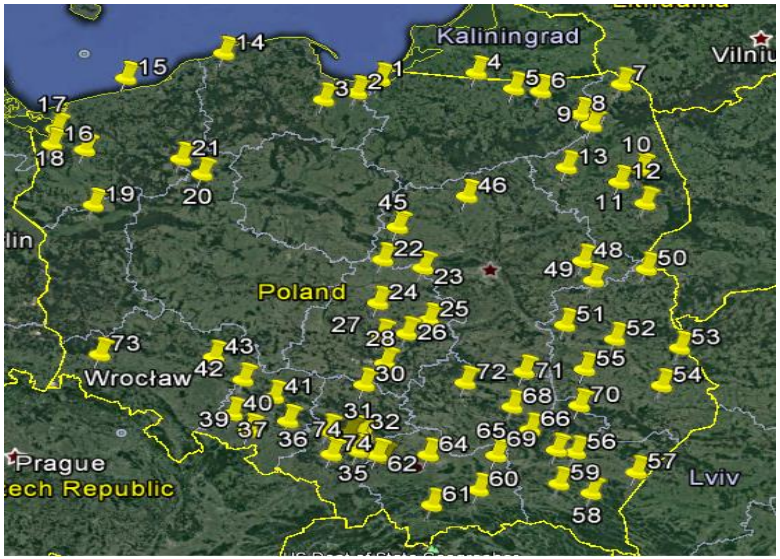
- Ecotoxicological assessment of condition of water bodies receiving WWTP effluents

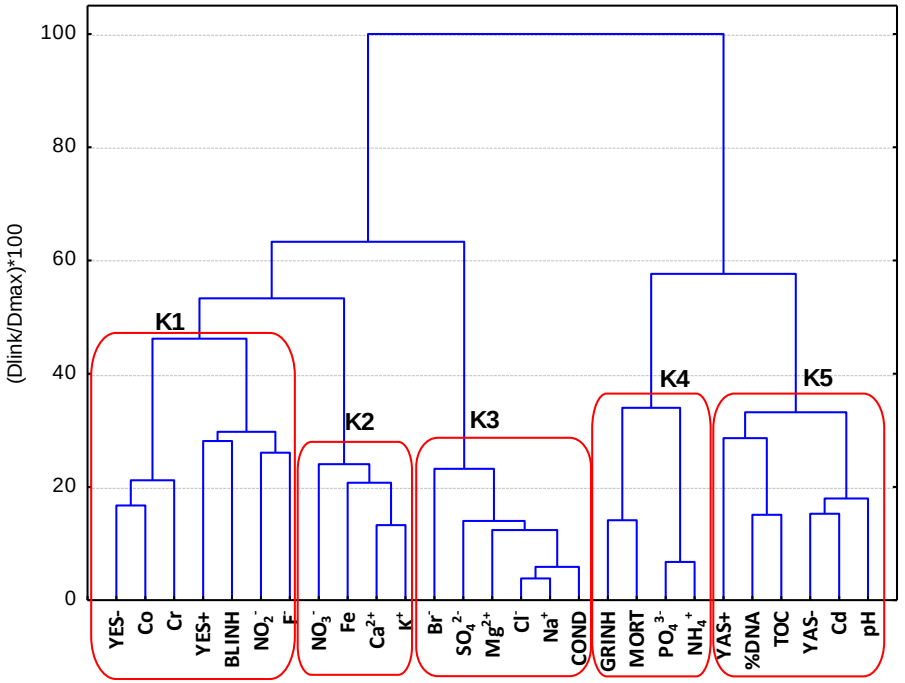
*B. Kudłak, M. Wieczerzak, G. Yotova, S. Tsakovski, V. Simeonov, J. Namieśnik, Chemosphere **160,** 181 (2016), project cofinanced by MNiSzW, IP2011-028071.

1. WWTP (*Poland*)

Selected conclusions:

- Biotests and chemometric data treatment enable discrimination between degradation and improve of ecotoxicological status of WWTP impacted water bodies.





1. WWTP (*Poland*)

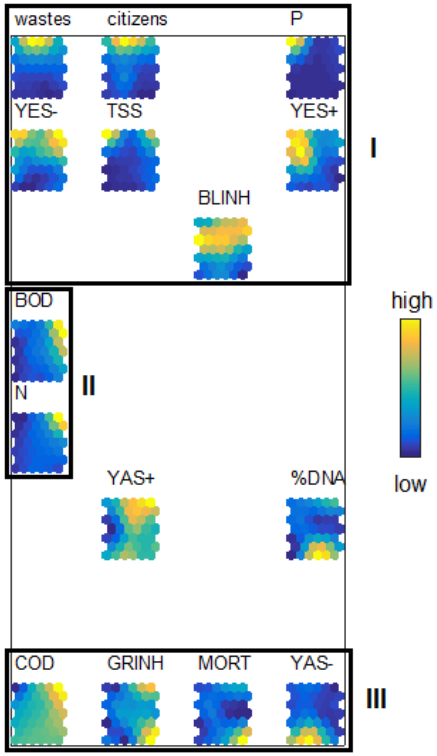


Fig. 6. Grouping of toxicological and operational parameters of WWTP as a results of SOM analyses

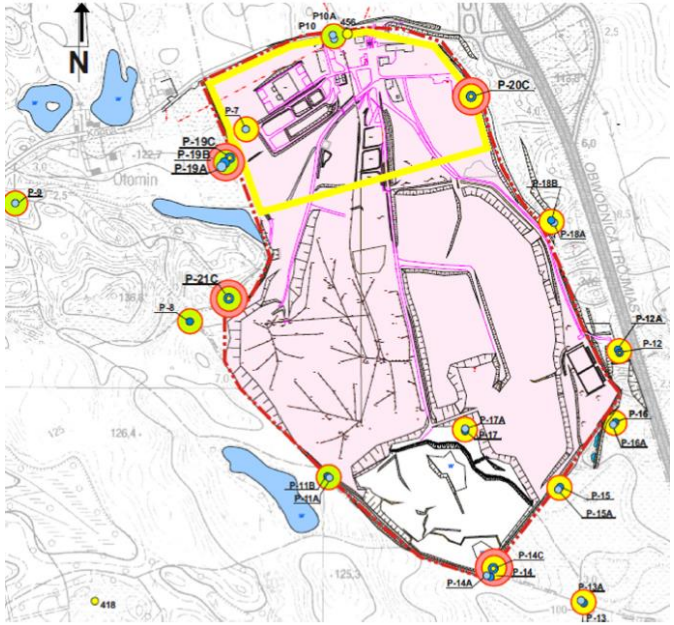
Table 2. List of locations where ecotoxicological negative/positive impact was confirmed with PCA

Negative impact	Positive impact
PC1: Sosnowiec, Mielec, Grajewo, Żory, Wrocław, Bielsk Podlaski, Oświęcim, Łódź, Staszów Połaniec, Ciechanów	PC1: Zdzieszowice, Radomsko, Kętrzyn, Elbląg, Katowice, Kielce, Kraków, Białystok, Zielona Góra, Chorzów, Police, Ostrowiec Świętokrzyski
PC2: Katowice, Mielec, Grajewo, Oświęcim	PC2: Elbląg, Stargard Szczeciński, Giżycko, Białystok, Radomsko, Kołobrzeg
PC3: Katowice, Ciechanów, Siedlce, Bielsk Podlaski, Wrocław, Sosnowiec	PC3: Żory, Zdzieszowice, Oświęcim, Kołobrzeg, Mielec, Kętrzyn
PC4: Katowice, Zdzieszowice, Mielec, Sanok, Radomsko, Chorzów	PC4: Sosnowiec, Ciechanów, Elbląg, Staszów Połaniec, Białystok

2. Communal landfill (*Szadółki, Gdańsk*)*

Object studied:

- Water samples from piezometers located in and around landfill



Studies performed:

1. Microtox®
2. Daphtoxkit F™
3. Phytotoxkit F™
4. Instrumental studies (m. in. like turbidity, hardnes, pH, heavy metals, inorganic ions, COD),
5. Chemometric studies

Aim:

- Ecotoxicological and chemical assessment of impact of landfill on status of water bodies surrounding it.

*B. Kudłak, S. Tsakovski, V. Simeonov, A. Sagajdakow, L. Wolska, J. Namieśnik, Chemosphere **95,** 17 (2014),), project cofinanced by MNiSzW project no. 3468/B/P01/2007

Main scientific directions: environmental

Selected conclusions:

- plant tests (germination inhibition and root growth inhibition) appeared to be the most sensitive endpoints of bioassays used. They correlated with consent of calcium carbonate, ammonium, dissolved matter, nickel, chlorides, total nitrogen, hardness and turbidity.

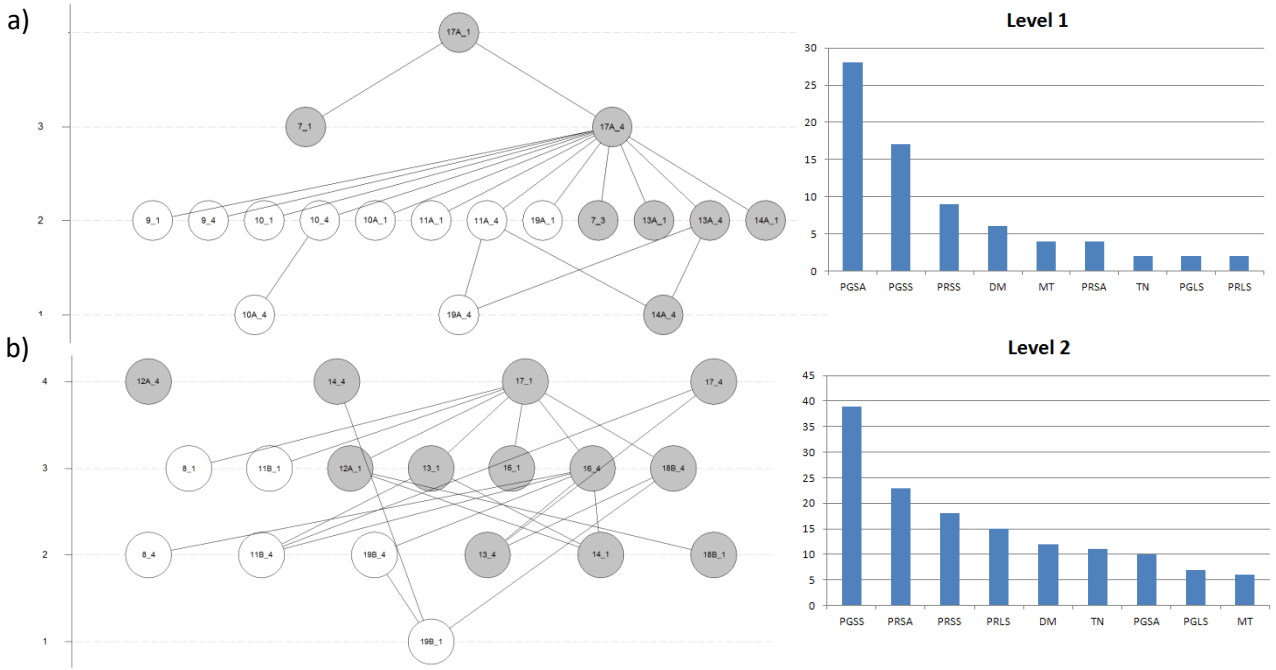


Fig. 7Hasse diagrams of piezometrs toxicity studies:
(a) First water bearing level, (b) second water bearing level and sensitivity of particular bioassays

3. Ecotoxicity of toys and products for kids (+ *model studies*)*

Objects studied:

- Aqueous and simulation media extracts (artificial saliva and sweat) of presented objects (teethers, pacifiers, decorative rubbers)

Studies performed:

1. Microtox®
2. Ostracodtoxkit F™
3. Xenoscreen YES/YAS®
4. NIR
5. Chemometric treatment

Aim:

- Assessing ecotoxicity and endocrine potential of extracts and impact of time/temperature on rate of extraction.

*B. Kudłak, S. Tsakovski, V. Simeonov, A. Sagajdakow, L. Wolska, J. Namieśnik, Chemosphere **95,** 17 (2014), cofinanced by MNiSzW project no. 3468/B/P01/2007

Main scientific directions: environmental

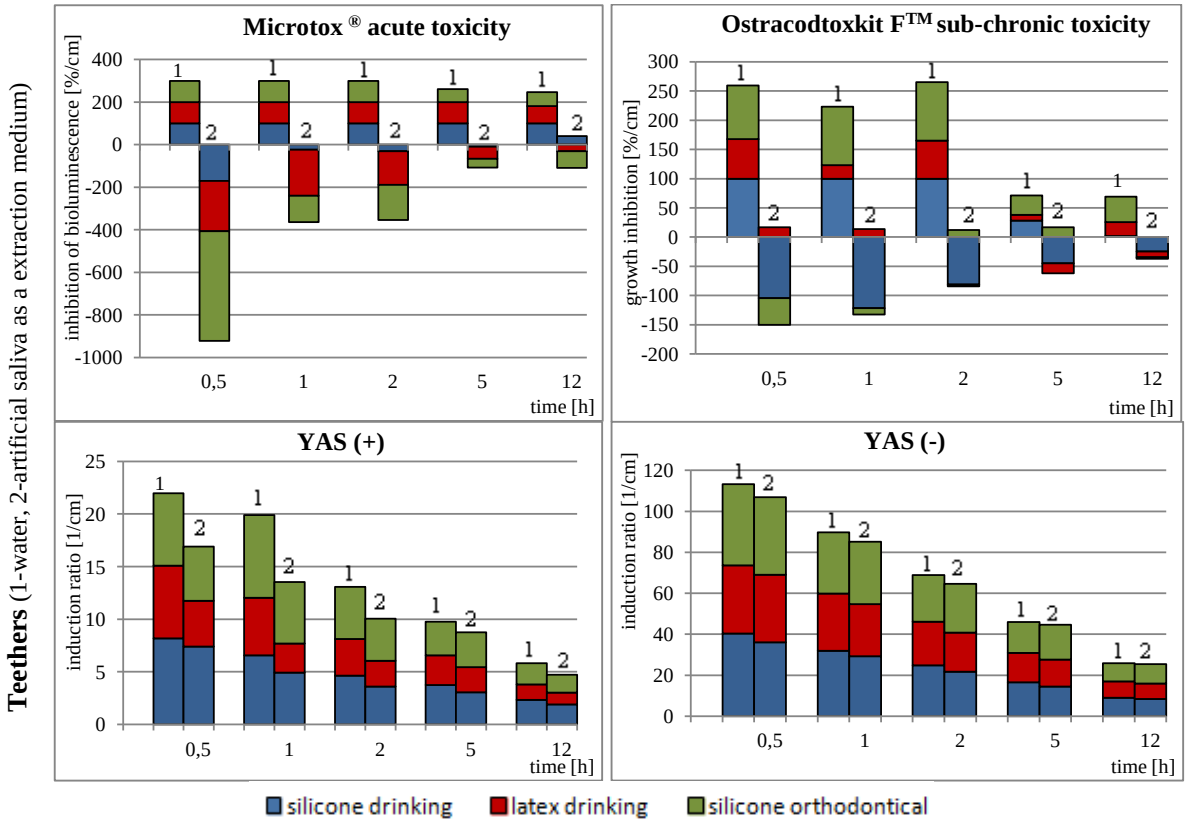
Selected conlusions:

- Microtox®, Ostracodtoxkit F™

Utilizing battery of bioassays enable concluding that as a result of action of simulation media the chemicals of elevated biological potential are released. Their toxicity depends greatly on simulation media used.

- XenoScreen YES/YAS®

Substances released from objects studied show mainly agonistic endrogenic potential.



*B. Kudłak, S. Tsakovski, V. Simeonov, A. Sagajdakow, L. Wolska, J. Namieśnik, Chemosphere **95,** 17 (2014), cofinanced by MNiSzW project no. 3468/B/P01/2007

4. Ecotoxicity of packaging*

Object studied:

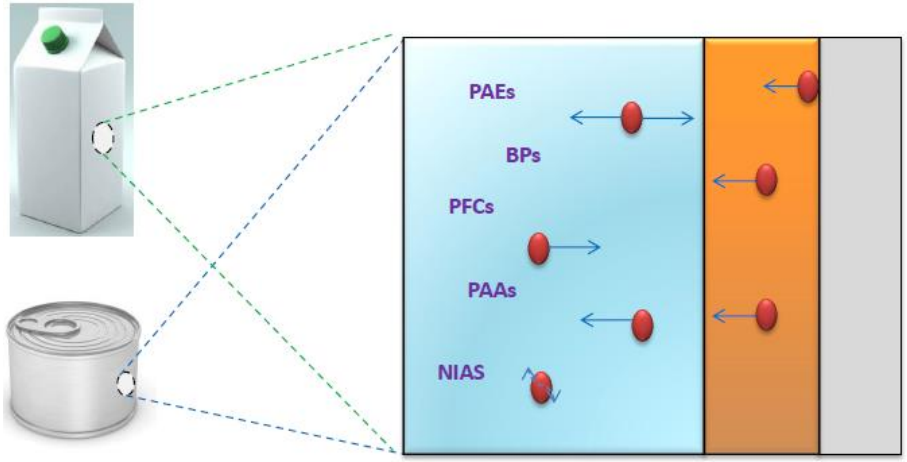
- Samples collected from packaging materials (cans, composite, cups) and treated with different regimes.

Studies conducted:

1. Microtox®
2. Ostracodtoxkit F™
3. Xenoscreen YES/YAS
4. comet assay (HT29)
5. instrumental studies (*LC-MS/MS, FTIR, ASV*)
6. chemometric treatment

Aim:

- Estimating impact of type of food packaging, processing at production and storage at release rate of EDC.



*N. Szczepańska, B. Kudłak, G. Yotova, S. Tsakovski, J. Namieśnik, 2017, Assessing acute toxicity of selected packages internal layers extracts using Microtox®, Packag. Technol. Sci., 30:347-357.

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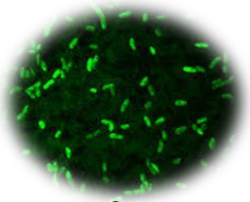
FACULTY OF CHEMISTRY

Migration studies with simulation media

Surface studies

sampling

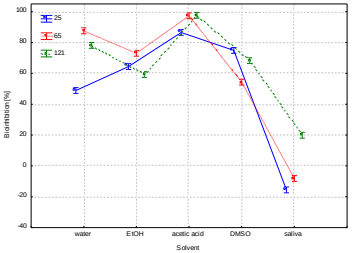


Microtox ®

XenoScreen YES/ YAS

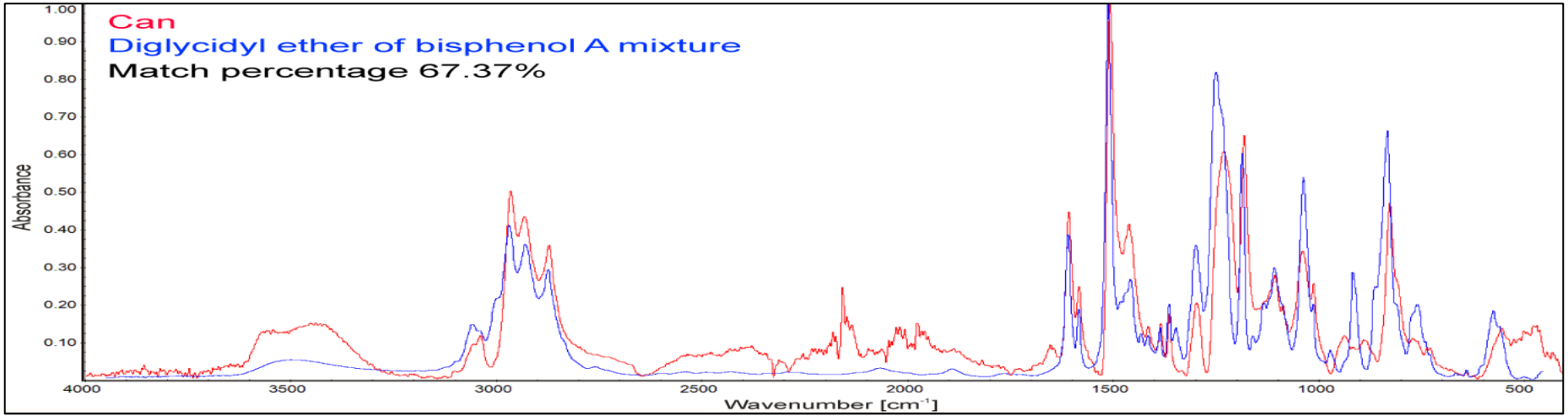
Acute toxicity

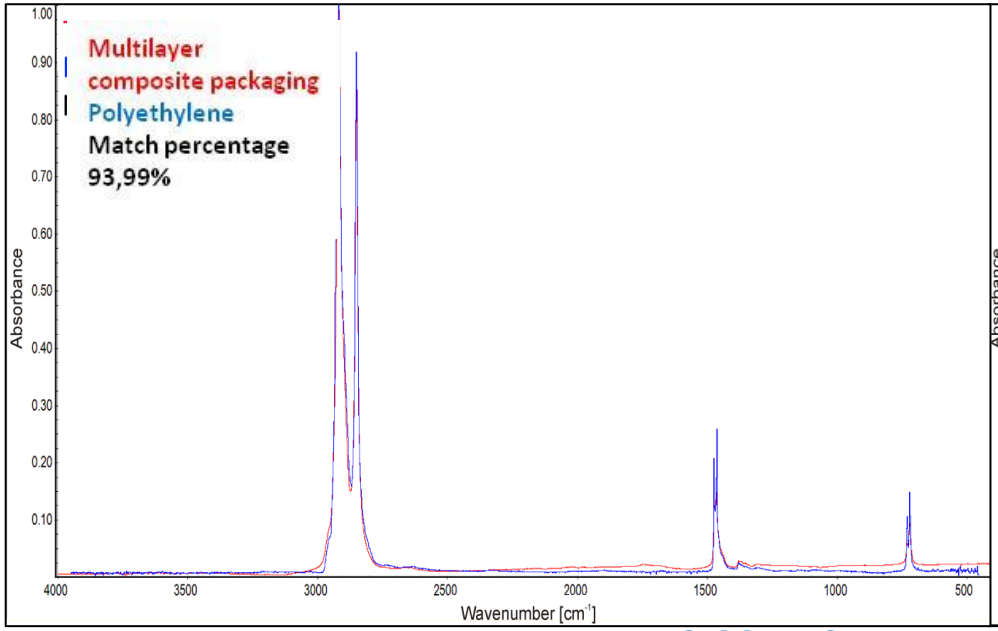
Endocrine potential

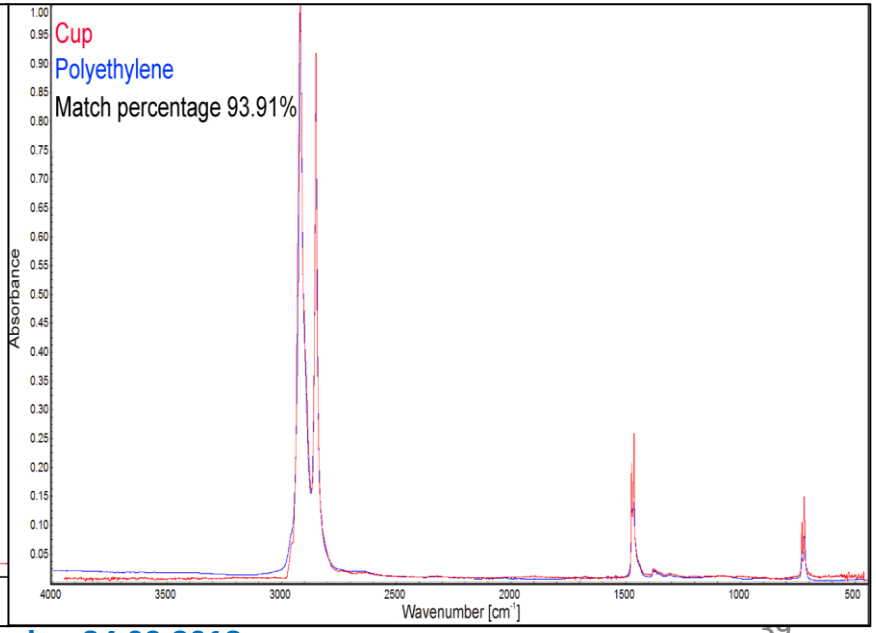


Chemometric studies

RESULTS OF FTIR ANALYSIS





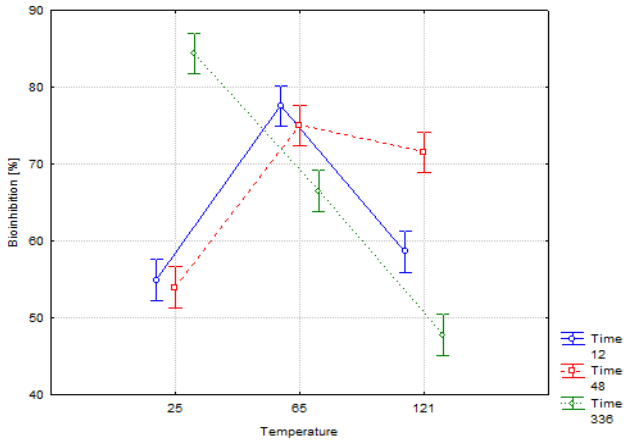


4. Ecotoxicity of packaging

Selected conclusions:

- Bioassays justify statement that internal layer of metal cans releases much more low molecular toxic substances than composite materials.

- Results of chemometric studies prove that every factor studied has greatly contributed to the observed ecotoxicological responses and stream of xenobiotics migrating from packaging.



Main conclusions: model

The role of widely understood packaging will be growing in modern societies so actions are needed to:

- develop reproducible and repeatable methods of extraction of packaging material and to use biological simulant media (e.g. artificial saliva, GI and stomach fluid) for this purpose,
- introduce bioassays into routine assessment of impact of packaging on food quality,
- elaborate and develop certified reference materials and international standards where bioassays could play crucial and indispensable role in packaging quality and aging studies,
- extending biological studies to create tailored batteries of bioassays,
- introduce common practice of performing parallel instrumental and biological studies on newly engineered packaging candidates.

5. First steps… - ecotoxicity of mixtures of pharmaceuticals

1: studies conducted:

- Determining toxicity of selected compounds (diclofenac, ketoprofen, progesteron, estron, chloramfenicol, oxytetracycline, diazepam, fluoxetine, androstendion, gemfibrozil)
- Mixtures of these compounds to :
  a) Microtox®
  b) Ostracodtoxkit F™
  c) Phytotoxkit F™
  d) Xenoscreen YES/YAS
  e) Comet assay

Aim:

- Determining interactions under real environmental conditions (also impact of pH, Cl^-, Br^-, F^-, NH_4^+, Na^+, K^+)

2:

- Determining toxicity of mixture of selected compunds (example matrix of results)

		Substance A			
	EC_{50}	0 %	33 %	66 %	100 %
Substance B	0 %	T1	T6	T10	T14
	33 %	T2	T7	T11	T15
	66 %	T3	T8	T12	T16
	100 %	T4	T9	T13	T17

3:

- Modelling and MDR (*Model Deviation Ratio*)

$$MDR = \frac{Expected}{Observed}$$

$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

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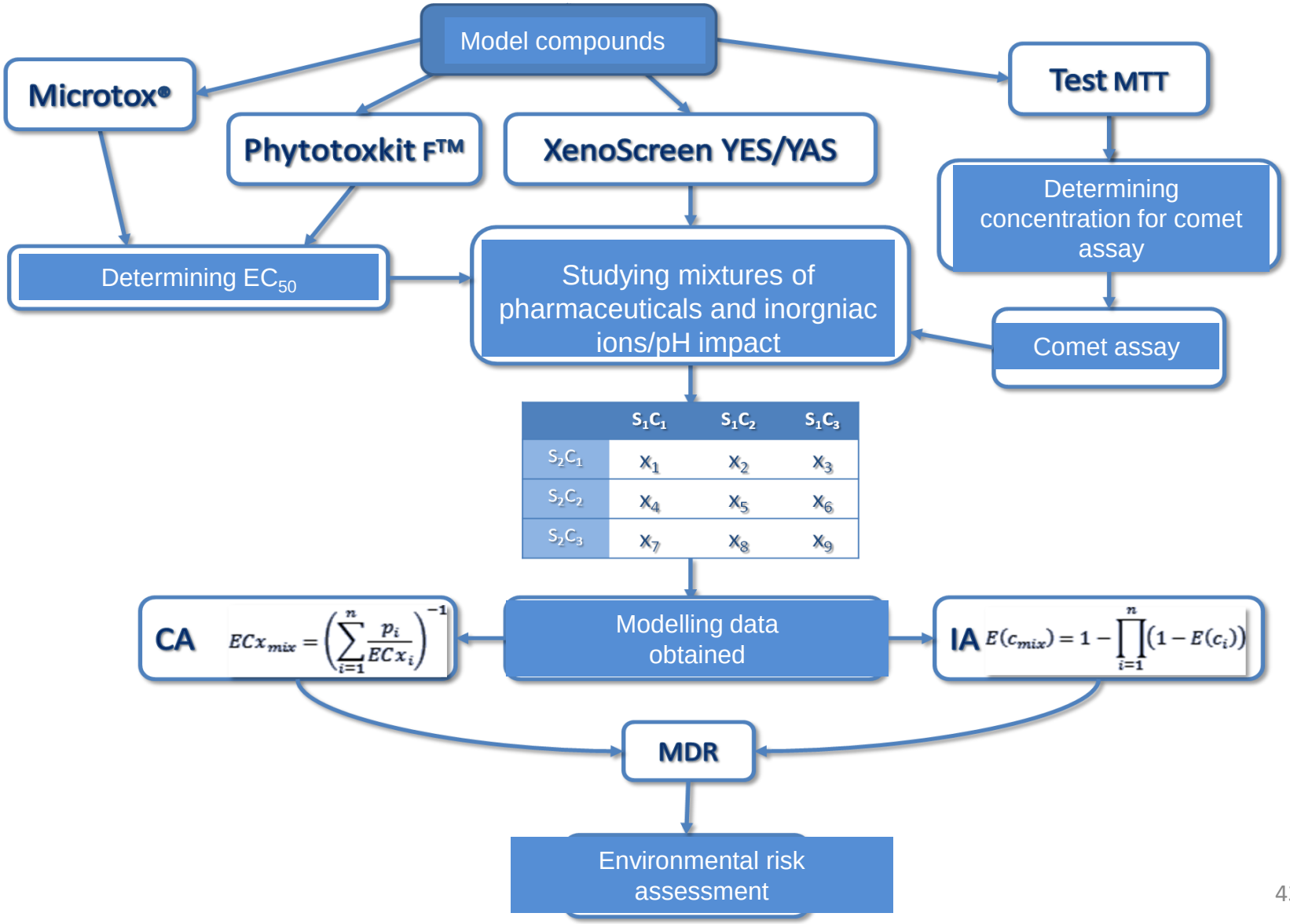
Main scientific directions: model



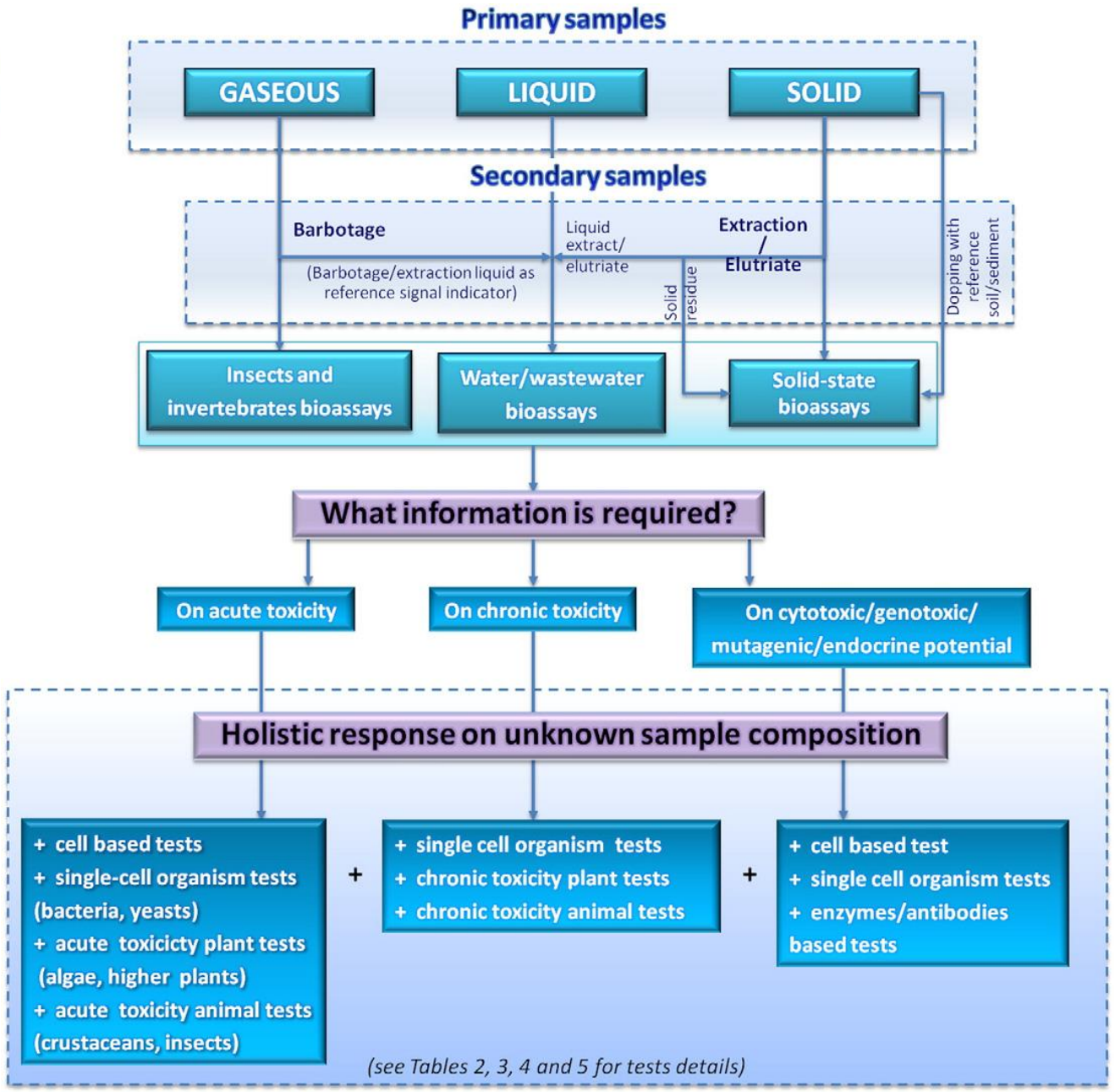
GŁÓWNE NURTY BADAWCZE: modelowe

Table 1. MDR of selected pharmaceuticals for Microtox® depending on pH, presence of anions/cations at environmentally levels (3 concentrations for each pharmaceutical) (**red - synergism, blue - antagonim, green- overestimation, yellow – underestimation**)

	Diazepam			Progesteron			Oksytetracycline			Flueoxetine			Diclofenak			Androstendion			Estron			Chloramfenicol			Ketoprofen			Gemfibrozil		
	Cl^-	F^-	Br^-	Cl^-	F^-	Br^-	Cl^-	F^-	Br^-	Cl^-	F^-	Br^-	Cl^-	F^-	Br^-	Cl^-	F^-	Br^-	Cl^-	F^-	Br^-	Cl^-	F^-	Br^-	Cl^-	F^-	Br^-	Cl^-	F^-	Br^-
C1	0,87	0,14	0,26	0,54	0,21	1,91	**2,75**	0,21	0,15	0,08	0,07	0,17	0,02	0,01	0,24	0,93	0,28	0,20	0,38	0,17	0,69	0,16	0,09	0,12	0,25	0,35	0,32	0,23	**2,66**	0,18
C2	0,66	0,18	0,11	0,16	0,16	0,49	0,62	0,12	0,09	0,05	0,03	0,15	0,01	0,01	0,08	0,53	0,30	0,12	0,24	0,13	0,40	0,14	0,07	0,13	0,23	0,54	0,30	0,25	0,21	0,10
C3	0,30	0,12	0,08	0,09	0,16	0,41	0,44	0,08	0,06	0,05	0,03	0,14	0,01	0,01	0,08	0,51	0,29	0,14	0,24	0,10	0,20	0,12	0,06	0,09	0,25	0,29	0,25	0,08	0,13	0,09

	Na^+	K^+	NH_4^+	Na^+	K^+	NH_4^+	Na^+	K^+	NH_4^+	Na^+	K^+	NH_4^+	Na^+	K^+	NH_4^+	Na^+	K^+	NH_4^+	Na^+	K^+	NH_4^+	Na^+	K^+	NH_4^+	Na^+	K^+	NH_4^+	Na^+	K^+	NH_4^+
C1	1,06	0,63	0,49	**2,95**	0,47	0,36	1,86	0,76	0,46	0,43	0,36	0,19	0,07	0,08	0,15	1,12	0,16	0,33	0,61	0,02	0,29	0,11	0,26	0,08	0,15	0,49	0,30	0,03	0,41	0,16
C2	0,61	0,98	0,55	0,45	0,81	0,16	1,48	0,83	0,26	0,32	0,21	0,21	0,03	0,06	0,13	0,87	0,03	0,75	0,47	0,02	0,48	0,05	0,18	0,06	0,16	0,31	0,34	0,03	0,32	0,08
C3	0,45	0,24	0,33	0,29	0,39	0,13	0,66	0,27	0,31	0,15	0,17	0,15	0,03	0,04	0,05	0,45	0,06	0,53	0,25	0,01	0,38	0,05	0,14	0,04	0,07	0,10	0,24	0,02	0,18	0,06

pH										
5,5	0,93	**2,77**	0,90	1,23	1,04	0,99	1,15	1,17	1,00	0,89
6,5	0,71	**2,76**	1,07	1,11	0,96	0,73	1,15	1,17	0,90	0,84
7,5	1,13	**6,99**	0,92	1,27	0,70	0,45	1,00	1,14	1,07	0,53
8,5	1,09	1,25	0,68	1,18	0,70	0,19	0,52	1,22	0,91	0,55


Primary samples
GASEOUS
LIQUID
SOLID
Secondary samples
Barbotage
(Barbotage/extraction liquid as reference signal indicator)
Liquid extract/ elutriate
Extraction / Elutriate
Solid residue
Dopping with reference soil/sediment
Insects and invertebrates bioassays
Water/wastewater bioassays
Solid-state bioassays
What information is required?
On acute toxicity
On chronic toxicity
On cytotoxic/genotoxic/ mutagenic/endocrine potential
Holistic response on unknown sample composition
+ cell based tests
+ single-cell organism tests (bacteria, yeasts)
+ acute toxicicty plant tests (algae, higher plants)
+ acute toxicity animal tests (crustaceans, insects)
+
+ single cell organism tests
+ chronic toxicity plant tests
+ chronic toxicity animal tests
+
+ cell based test
+ single cell organism tests
+ enzymes/antibodies based tests
(see Tables 2, 3, 4 and 5 for tests details)

CONCLUSIONS

As a result of numerous research projects finished we can state that:

- Ecotoxicological studies and modeling of toxicity of both environmental and model samples constitute an important tool for decisive administrative bodies to understand and predict impact of wide range of pollutants on living organisms as many chemicals show synergic activity when present in mixtures.

- Risk of increasing toxicity of numerous analytes may appear as a result of accidental events (e.g. leaks during transportations, natural disasters) what will result in local or regional pollution and ecotoxicological risk increment.

- More research is needed to study mixtures of xenobiotics of higher order e.g. 3x3, 4x4 etc. Being now performed in our unit.

- Ecotoxicological studies are now quickly developing tools in environmental risk assessment studies.

- Utilizing bioassays with instrumental studies and chemometric data treatment enables obtaining deep conclusions on condition of object studied and its impact in surroundings.

- Bioassays can be considered as screening tools prior to performing widened instrumental studies being very often very expensive and „not-green”.

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