EMEC21

21st European Meeting on Environmental Chemistry
November 30 – December 3, 2021, Novi Sad, Serbia

Association of Chemistry and the Environment

Serbian Chemical Society

Matica Srpska

www.emec21.rs

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BOOK OF ABSTRACTS

ASSOCIATION OF CHEMISTRY AND THE ENVIRONMENT

21st European Meeting on Environmental Chemistry

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EMEC 21

November 30 – December 3, 2021

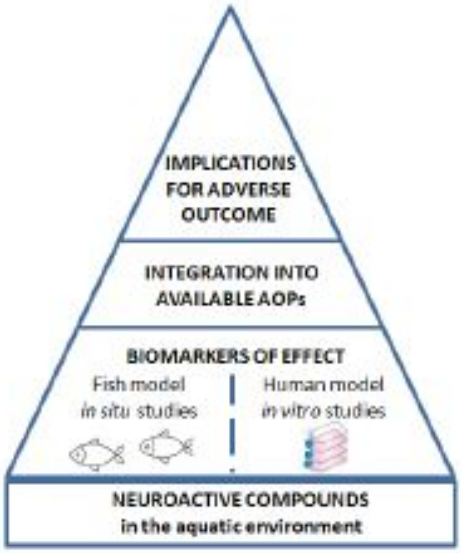
Novi Sad, Serbia

Thursday, December 2nd, 2021

Time	Type of presentation	Lecturer/ presenter	Title
08:00-	**Registration**		
Moderators	Jan Schwarzbauer/Maja Turk Sekulić		
08:30-09:00	Plenary lecture	Tatjana Ćirković-Veličković	Emerging food contaminants
09:00-09:20	Section lecture	Pierro Bellanova	Impact Of Tsunamis On Pollutants' Distribution
09:20-09:35	Oral presentation	Patricia Tarín-Carrasco	Study of the Impacts of Large Wildfires on PM10 and Human Mortality in Portugal
09:35-09:50	Oral presentation	Jan Schwarzbauer	Emission and dispersion of organic pollution by the Summer 2021 extreme flood in Germany
09:50-10:05	Oral presentation	Emira Hukić	Do Freezing and Heating Cycles Influence Differently on Soil Elements Leaching?
10:05-10:35	**Coffee break**		
10:35-10:50	Oral presentation	Milica Stefanović	The response of badland materials from Spain with different mineralogical content on seasonal changes
10:50-11:05	Oral presentation	Luisa Bellanova	Chemostratigraphic distribution of harmful organic contaminants in flood affected (sub-)tropical urban river sediments (Chennai, India)
11:05-11:20	Oral presentation	Filipe Rocha	Studying the Behaviour and Fate of Volatile Methylsiloxanes and Synthetic Musk Compounds in Soil
11:20-11:35	Oral presentation	Dragana Vidojević	Inadequate municipal solid waste management and soil pollution in Serbia
11:35-11:50	Oral presentation	Ioanna Pantelaki	Occurrence and fate of organophosphate esters in a municipal wastewater treatment plant
11:50-12:05	Oral presentation	Maria Krishna de Guzman	Comparative Profiling of Microplastics in Differently sized Manila Clams from South Korea by Nile Red Staining and µFTIR
12:05-12:35	**Sponsor presentation – ANNAFER d.o.o. (LECO)**	Pavel Jiros	MS Technology Diversity to Provide Enhanced GC Separation, Detection and Identification Solutions
12:35-14:00	**Lunch break**		
Moderators	Aleksandra Tubić/Vladimir Beškoski		
14:00-14:30	Plenary lecture	Albert Lebedev	Mechanisms of formation of disinfection by-products in water treatment
14:30-14:50	Section lecture	Lydia Niemi	Pharmaceuticals in the aquatic environment: A rural perspective and cross-sector partnership addressing the issue in Scotland
14:50-15:05	Oral presentation	Taja Verovšek	Wastewater Analysis Assessment: Prevalence of Drugs of Abuse in Educational Institutions
15:05-15:20	Oral presentation	Christina Alina Schwanen	Structural Diversity of Organic Contaminants in a Meso-Scaled River System
15:20-15:35	Oral presentation	Fábio Bernardo	Monitoring Volatile Methylsiloxanes Levels in Wastewater Collected from a Portuguese Wastewater Treatment Plant
15:35-15:50	Oral presentation	Polonca Trebše	Transformations of resveratrol, antioxidant component of sunscreen, under disinfection conditions
15:50-16:20	**Coffee break**		
16:20-16:35	Oral presentation	Urška Šunta	Adsorption of three pesticides onto different polymer types of microplastic particles in alluvial soil
16:35-16:50	Oral presentation	Mojca Bavcon Kralj	Determination of microplastics in environmental samples by simply applicable method
16:50-17:05	Oral presentation	Franja Prosenc	Method for Extraction, Quantification, and Identification of Microplastics from Soil and Compost
17:05-17:25	Section lecture	Sonja Kaišarević	Neuroactive Compounds in the Aquatic Environment: Biomarkers of Effect and Their Integration into Adverse Outcome Pathways (AOPs)
17:25-17:40	Oral presentation	Karla Jagić	Exposure to polybrominated diphenyl ethers associated with car dust
19:00-23:00	**Conference dinner at Restaurant "Wine House Kovačević"** (Kralja Petra 221, Irig) Transfer at 18:00h (the corner of Temerinska Street and Marija Trandafil Square, 5 min. walk from Matica Srpska)		

Neuroactive Compounds in the Aquatic Environment: Biomarkers of Effect and Their Integration into Adverse Outcome Pathways (AOPs)

S. Kaišarević, I. Vulin, D. Tenji, T. Tomić, I. Teodorović. Laboratory for Ecophysiology and Ecotoxicology - LECOTOX, Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovica 2, Novi Sad, Serbia; sonja.kaisarevic@dbe.uns.ac.rs.*



Neuroactive compounds (NCs) represent a large group of chemicals with the ability to affect the activity of the nervous system of target organisms via different primary modes of action (MoA). They include neuroactive pharmaceuticals, illicit drugs, stimulants and neuroactive pesticides. The global use of NCs is increasing worldwide which results in their constant release in the aquatic environment, making these compounds an emerging hazard with possible risk to aquatic ecosystems [1]. Lack of well characterized and widely accepted biomarkers of effect of NCs, clearly related to adverse effects in the exposed organisms, represent a challenge in the development of a biomarker-based strategy for impact assessment of NCs in the aquatic environment [2].

In our study, we are applying the holistic approach in search for sensitive biomarkers of effect of NCs, integrating three research avenues: (a) mechanistic *in vitro* study on human neuroblastoma cells treated with environmentally relevant NCs with various primary MoA, (b) *in situ* study on fish caged at the pollution hot spot in Danube and (c) integration of the responsive biomarkers in the adverse outcome pathway (AOP) framework database for prediction of adverse effects of NCs.

At the moment, our results point out the promising candidates for sensitive biomarkers of effect of NCs: synaptotagmin 10 (SYT10) - protein involved in exocytosis of neurotransmitters, myelin basic protein (MBP) – responsible for myelination of axons and neuroprotection, as well as several elements of serotonin, dopamine and GABA neurotransmitter pathways. While SYT10 and MBP are still not included as molecular targets in the AOP frameworks, observed changes in the expression of elements of the neurotransmitter pathways might imply to depression, agitation and epilepsy as possible adverse outcomes in affected organisms.

Using the presented research approach, we will continue in contributing to the development of biomarker-response patterns for NCs. Also, we stress the necessity of synchronisation of database on promising and most responsive biomarkers with events described in the existing AOPs for successful establishment of a strategy for impact assessment of NCs. Moreover, overall findings will contribute to the knowledge on neurotoxicity patterns which could be used in characterisation of environmental contaminants and mixtures of compounds with unknown primary MoA.

Acknowledgements

This research was supported by the Science Fund of the Republic of Serbia, PROMIS, Grant No. 6061817, BIANCO. The abstract content is the responsibility of the Faculty of Sciences University of Novi Sad, and it does not reflect the opinion of the Science Fund of the Republic of Serbia.

References

[1] W. Busch, S. Schmidt, R., T. Schulze, M. Krauss, R. Altenburger. *Environmental Toxicology and Chemistry*, 35(8) (2016) 1887.

[2] S. Kaisarevic, I. Vulin, D. Tenji, T. Tomic, I. Teodorovic. *Environmental Sciences Europe* (2021) https://doi.org/10.1186/s12302-021-00557-0.

NEUROACTIVE COMPOUNDS IN THE AQUATIC ENVIRONMENT: BIOMARKERS OF EFFECT AND THEIR INTEGRATION INTO ADVERSE OUTCOME PATHWAYS (AOPs)

Sonja Kaisarevic, **Irina Vulin, Dina Tenji, Tanja Tomic & Ivana Teodorovic**

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Novi Sad, Serbia

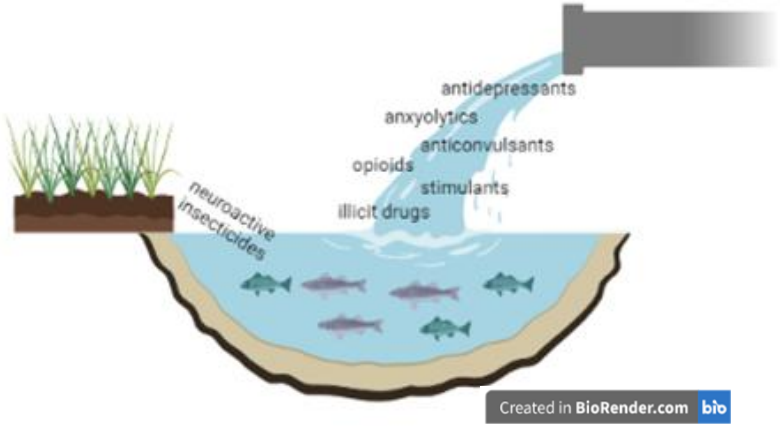
21st European Meeting on Environmental Chemistry – EMEC21, 30.11-03.12.2021., Novi Sad, Serbia

Why a study on neuroactive compounds?
(pharmaceuticals, stimulants, illicit drugs, neuroactive pesticides)

- **Intensive use worldwide**
- **Constant release in the environment through municipal wastewater effluents or agricultural surface runoff and leaching**
- **Concentrations in effluents and surface waters:**

  ng/L - µg/L

- **NOT INCLUDED into mandatory official surface water and WWTP effluent monitoring programs**



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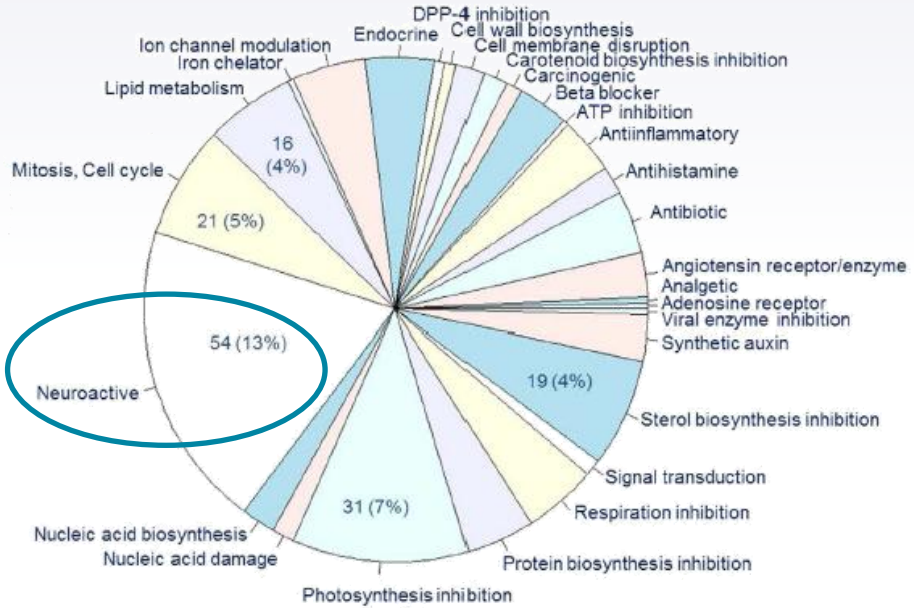
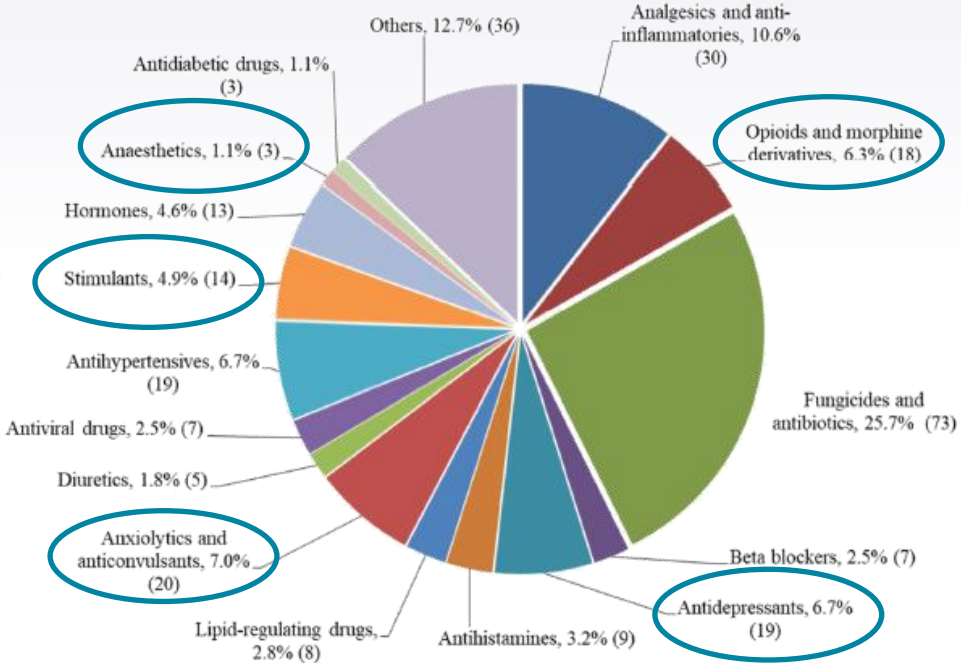


Busch et al. (2016) Environ Toxicol Chem 35 (8):1887-1899



Zhou et al. (2019) Environ Int 128:1-10

Neuroactive compounds (NCs) – an emerging hazard in the aquatic environment!

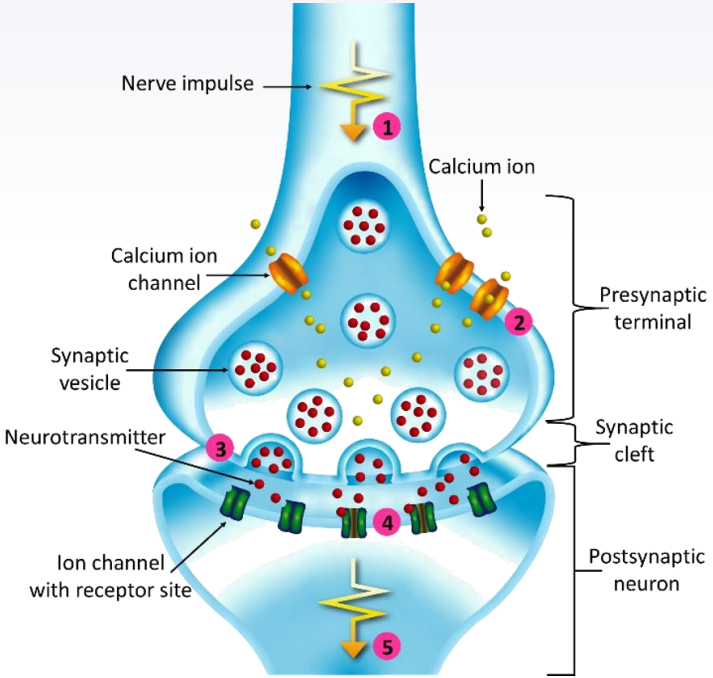
Do NCs present an emerging risk to aquatic ecosystems?

- **High level of evolutionary and functional conservation of molecular targets of NCs**
- **Experimentally-derived evidence on unintended adverse effects in non-target organisms**

However…

- **Prospective risk assessment (RA) of chemicals does not adequatly address the potential risk of NCs in aquatic environment and does not provide reliable data for impact assessment of NCs**

Universal mechanism of synaptic transmission in neurons in animal kingdom: each element as a potential target of NCs



https://www.oist.jp/news-center/photos/diagram-synaptic-transmission

Can we assess, identify and confirm the adverse effects and ecological impacts of NCs in the aquatic environment?

According to Backhaus et al. (2019) Environ Sci Eur 31:98: WoE approach based on four LoE to assess ecological impact of overall chemical pressure and identify the drivers of mixture risks

CBM – COMPONENT-BASED METHODS

Predicitive mixture risk modelling

EMB – EFFECT-BASED METHODS

Battery of MoA-specific bioanalytical tools

Caged or free-living animals; battery of MoA-specific biomarkers of effect measurable on various levels of biological organization

***IN SITU* TESTS AND ANALYSES**

Aquatic communities structure and composition as an ultimate measure of ecosystem integrity

BIOMONITORING DATA

Limitations and challenges to be addressed:

LoE 1:

chronic or subletal effect data

not available

LoE 2 – LoE 3:

identification of sensitive neurochemical biomarkers and establishment of biomarker response patterns which could serve as indicators of adverse effects of NCs at lower levels of biological organization

Kaisarevic *et al. Environ Sci Eur* (2021) 33:115
https://doi.org/10.1186/s12302-021-00557-0

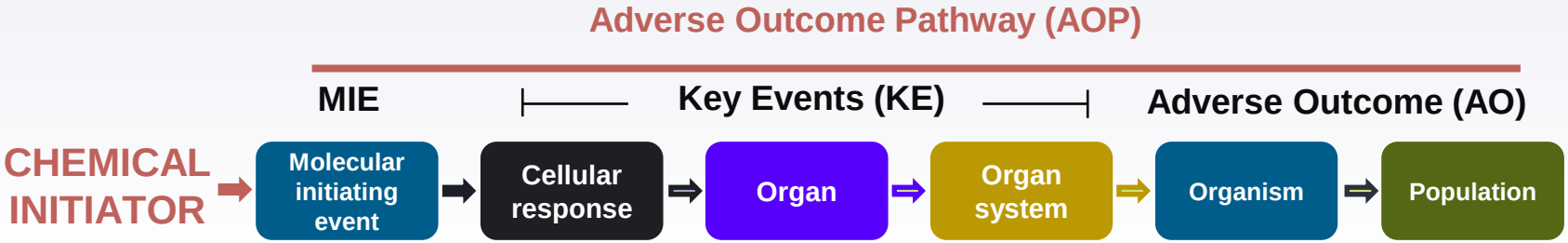
Environmental Sciences Europe

DISCUSSION Open Access

Approaches, limitations and challenges in development of biomarker-based strategy for impact assessment of neuroactive compounds in the aquatic environment

Sonja Kaisarevic*, Irina Vulin, Dina Tenji, Tanja Tomic and Ivana Teodorovic

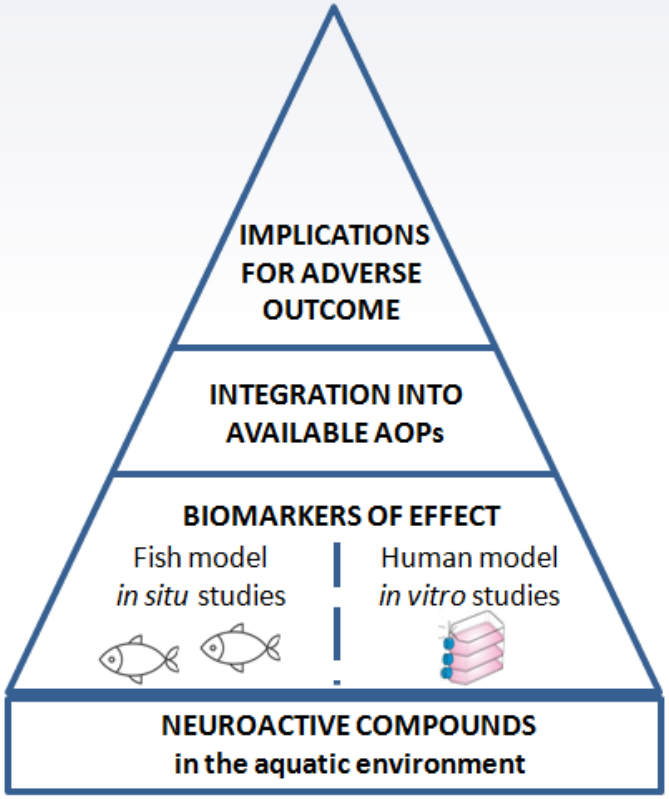
Relevance of Adverse Outcome Pathway (AOP) framework in impact assessment of NCs



- AOPs available in an open-source platform AOP-Wiki (http://aopwiki.org)

- Matching events described within AOPs with responsive biomarkers observed for NCs can contribute to the strengthening of the WoE for causal relationships between chemical exposure and adverse health outcomes and identification of novel biomarkers of effect of NCs.

Scientific concept of the project PROMIS - BIANCO
(Science Fund of the Republic of Serbia, Grant No. 6061817, 2020-2022)



- **Transferring mechanistic data on (neuro) toxic potency of NCs to their specific biomarker response patterns**
- **Integration into proposed AOP frameworks and definition of Key Event Relationships (KERs) between NCs and AO**
- **Implications to AO resulting from the NC exposure**

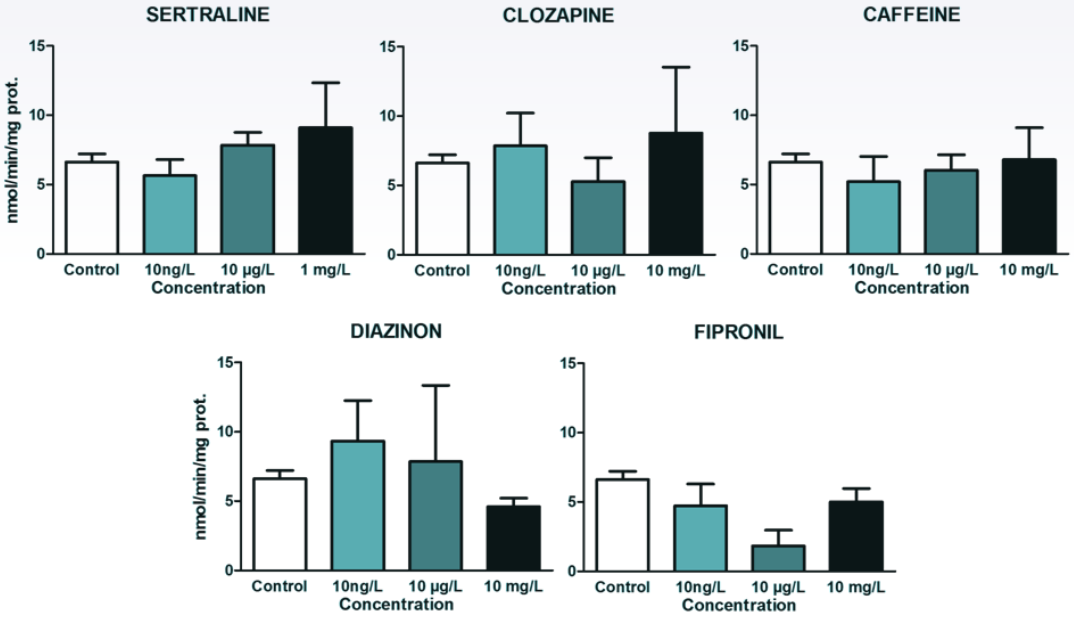
Biomarker responses in human *in vitro* model

Mechanistic studies on human neuroblastoma SH-SYHY cells treated with environmentally relevant NCs with different primary MoA

COMPOUNDS	BIOMARKERS		METHODS
	BIOMARKERS OF GENERAL TOXICITY		
✓ **SERTRALINE** antidepressant ✓ **CLOZAPINE** antypsychotic drug ✓ **CAFFEINE** stimulant and pharmaceutical ✓ **DIAZINON** organophosphate pesticide ✓ **FIPRONIL** biocide (tested concentration range: 10 ng/L – 10 mg/L)	TOTAL PROTEIN LEVELS	SRB assay	SRB assay
	DISTURBANCE OF MITOCHONDRIAL ACTIVITY	Activity of mitochondrial dehydrogenase	MTT assay
		Mitochondrial membrane potential	TMRE assay
	BIOMARKERS OF NEUROTOXICITY		
	KEY ELEMENTS OF NT PATHWAYS (Ach, serotonin, dopamine, GABA)	Receptors, NT catabolic enzymes	RQ-PCR; enzyme activity assays
	DISTURBANCE OF EXOCYTOSIS	Synaptotagmine 10	RQ-PCR
	MYELINATION OF AXONS AND NEUROPROTECTION	Myelin basic protein	RQ-PCR
	NEUROENDOCRINE REGULATION OF REPRODUCTION	Tachykinin 3	RQ-PCR
	DISTURBANCE OF MEMBRANE RESTING POTENTIAL	Na+/K+ ATPase	RQ-PCR

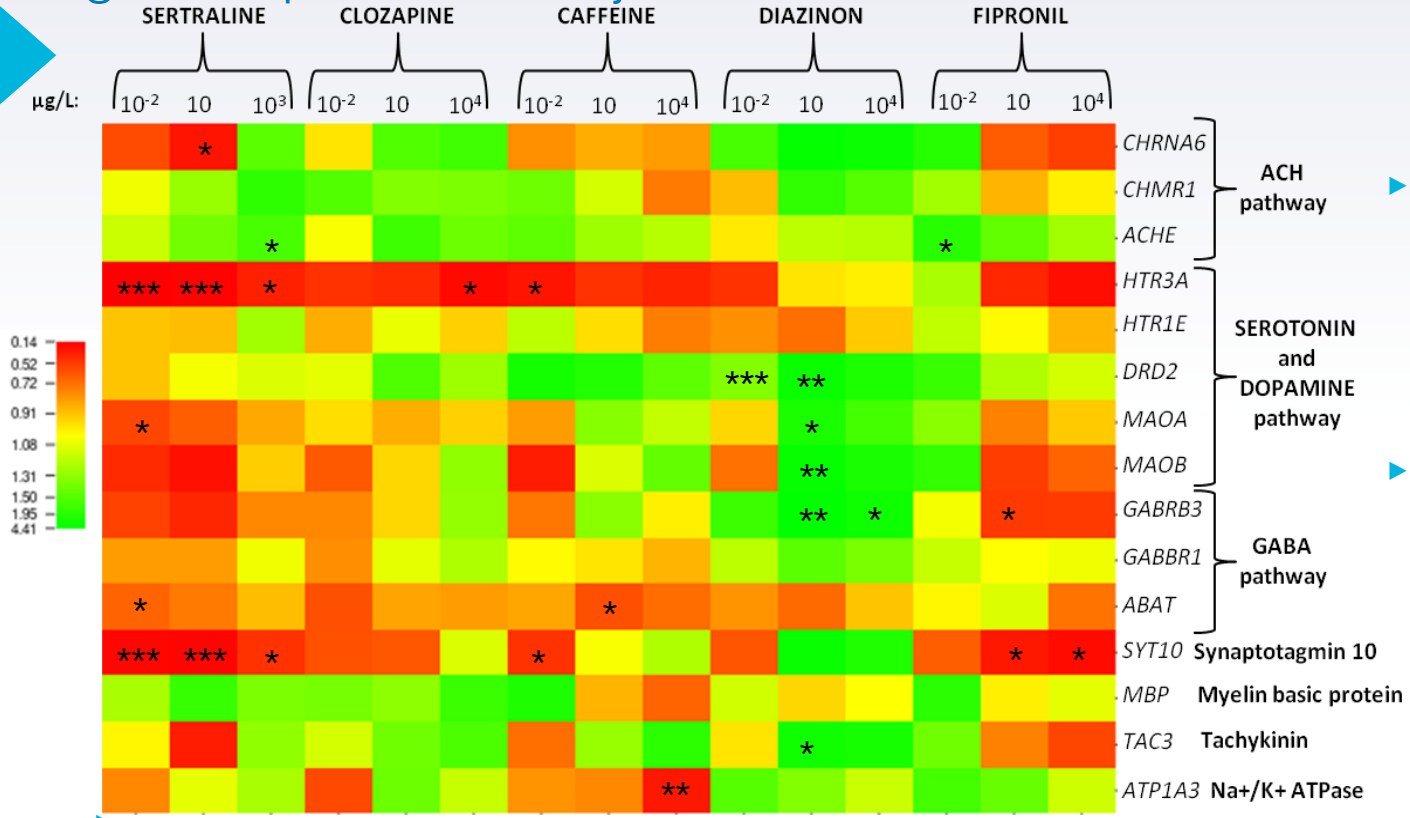
Biomarkers of neurotoxicity

Acetylcholinesterase (AChE) enzyme activity



- **Inhibition of AChE activity – the most well known and the only commonly used biomarker of effect for neurotoxicity**
- **Tendency of disturbance of AChE activity**

Biomarkers of neurotoxicity
(gene expression analyses – RQ-PCR)



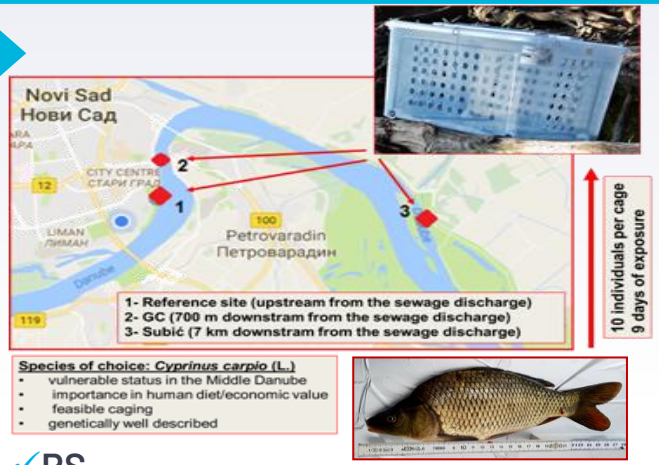
- Pharmaceuticals – inhibitory effect
- Organophosphate pesticide diazinon – stimulatory effect
- Differential effects of various groups of NCs, with possible relevance in development of biomarker-based strategy for NCs.

- ***HTR3A*** and ***SYT10*** distinguish as the most sensitive parameters to tested NCs and promising candidates for biomarkers of effect of NCs.
- ***DRD2A***, ***MAOA***, ***MAOB*** and ***GABRB3*** might be promising candidates for sensitive biomarker of effects specific for the group of organophosphate pesticides.

Biomarker responses in fish model *in situ*

Common carp (*Cyprinus carpio*) caged at the pollution hot spot in Danube

EXPOSURE



✓**RS**
referent site, upstream discharge

✓**SD**
700m downstream sewage discharge;
exposure to NCs

✓**SD+ID**
7km downstream sewage discharge;
influence of industrial discharge, exposure also to industrial compounds

BIOMARKERS

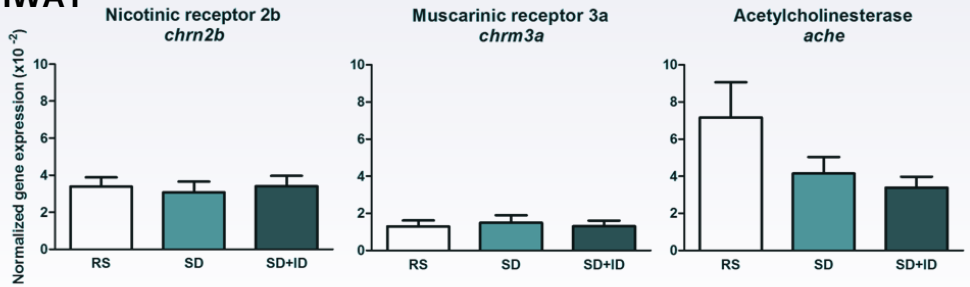
METHODS

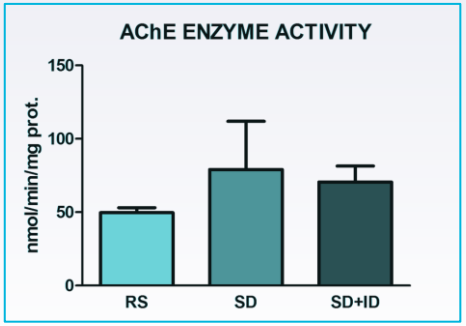
BIOMARKERS OF NEUROTOXICITY IN FISH BRAIN TISSUE

KEY ELEMENTS OF NT PATHWAYS (Ach, serotonin, dopamine, GABA)	Receptors, NT catabolic enzymes	RQ-PCR; enzyme activity assays
DISTURBANCE OF EXOCYTOSIS	Synaptotagmine 10	RQ-PCR
MYELINATION OF AXONS AND NEUROPROTECTION	Myelin basic protein	RQ-PCR
NEUROENDOCRINE REGULATION OF REPRODUCTION	Tachykinin 3	RQ-PCR
DISTURBANCE OF MEMBRANE RESTING POTENTIAL	Na+/K+ ATPase	RQ-PCR
NERVE IMPULSE CONDUCTION	Voltage gated ion channels	RQ-PCR

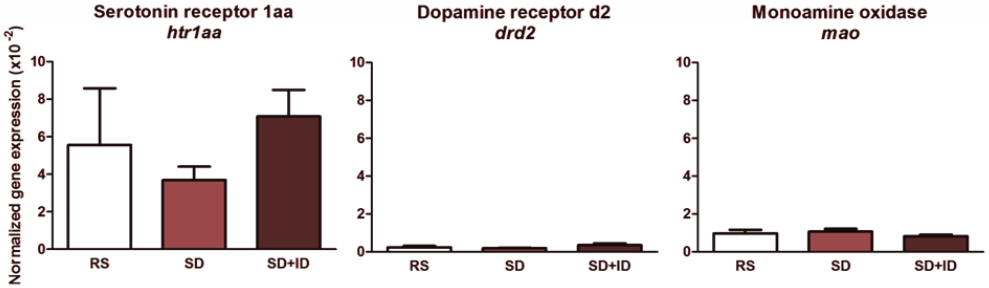
Biomarkers of neurotoxicity in fish brain tissue
(gene expression analyses – RQ-PCR)

ACH PATHWAY

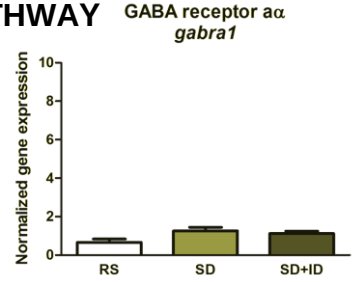




SEROTONIN AND DOPAMINE PATHWAY



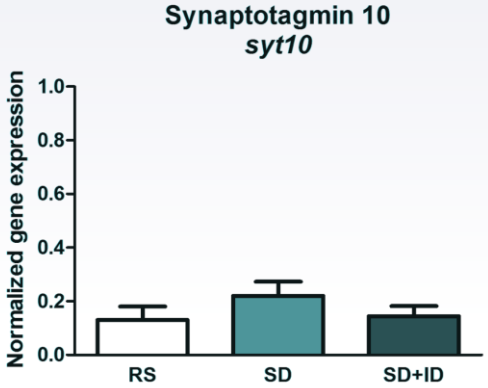
GABA PATHWAY



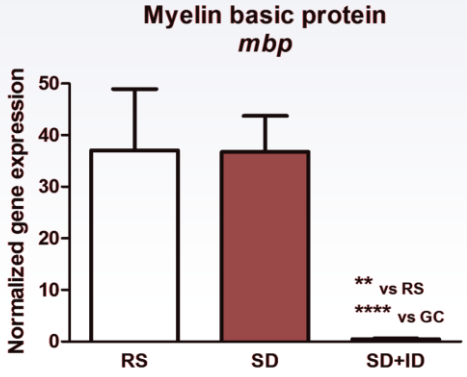
- ***AChE:*** **tendency of inhibition of gene expression and stimulation of enzyme activity**
- ***htr1aa*** **distinguishes as the most sensitive parameter and promising candidate for biomarker of effect of NCs**

Biomarkers of neurotoxicity in fish brain tissue
(gene expression analyses – RQ-PCR)

EXOCYTOSIS OF NT

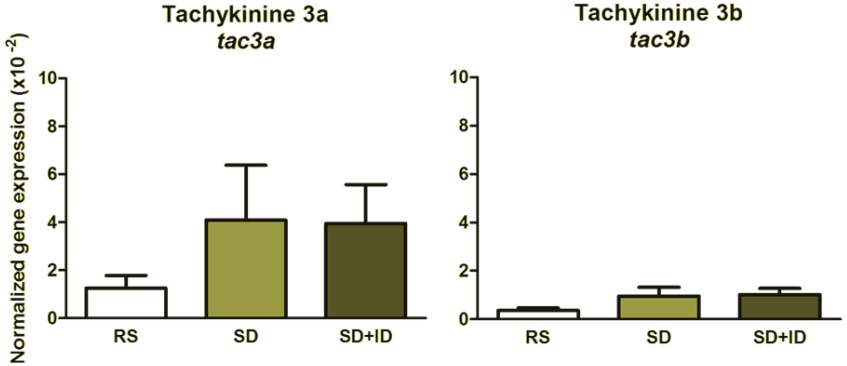


MYELINATION AND NEUROPROTECTION



- ***mbp*** **strong inhibition at SD+ID site – possible biomarker of effect for industrial compounds?**

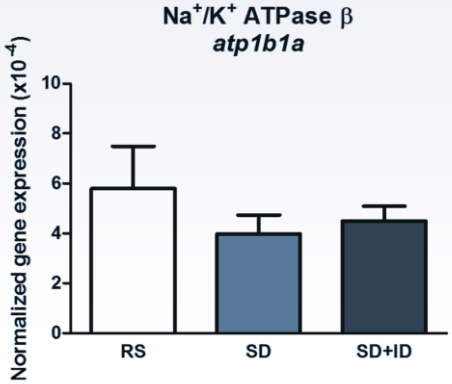
NEUROENDOCRINE REGULATION OF REPRODUCTION



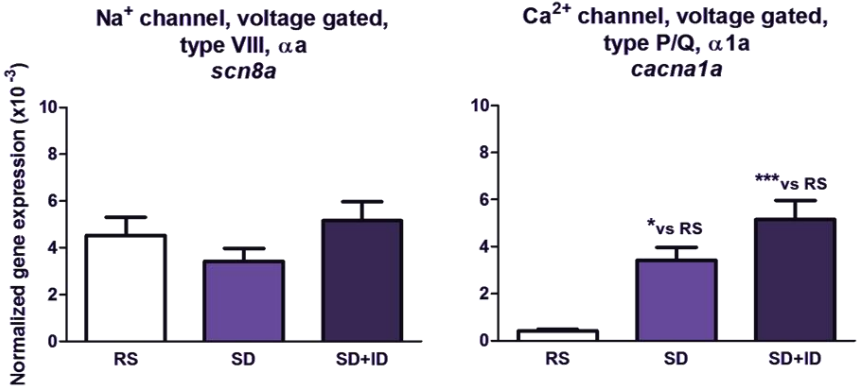
- ***tac3a*** **stimulation**

Biomarkers of neurotoxicity in fish brain tissue
(gene expression analyses – RQ-PCR)

DISTURBANCE OF MEMBRANE RESTING POTENTIAL



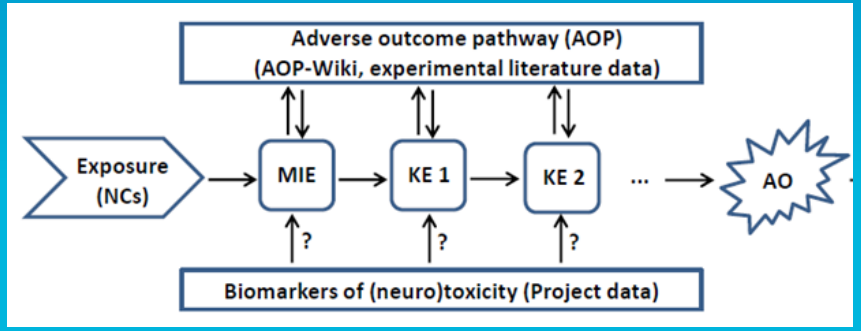
NERVE IMPULSE CONDUCTION



- ***cacna1a*** **strong stimulation – possible relation also to effect on exocytosis of NTs?**

Integration of the responsive biomarkers into AOP database

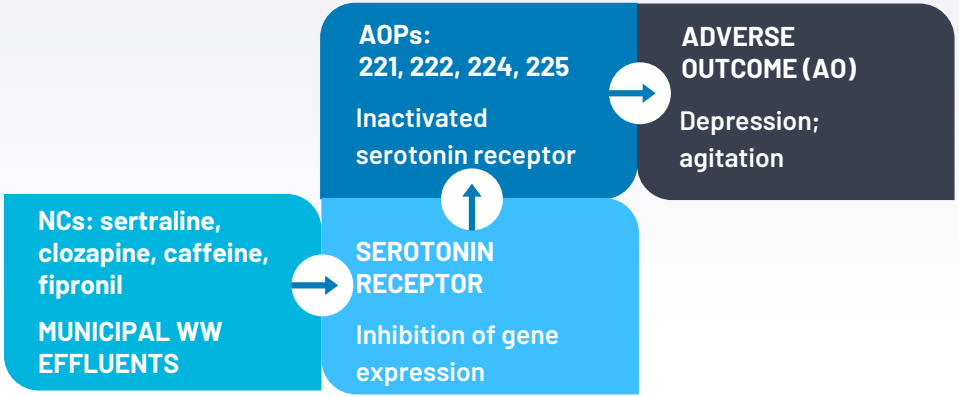
Prediction of AO of exposure to NCs

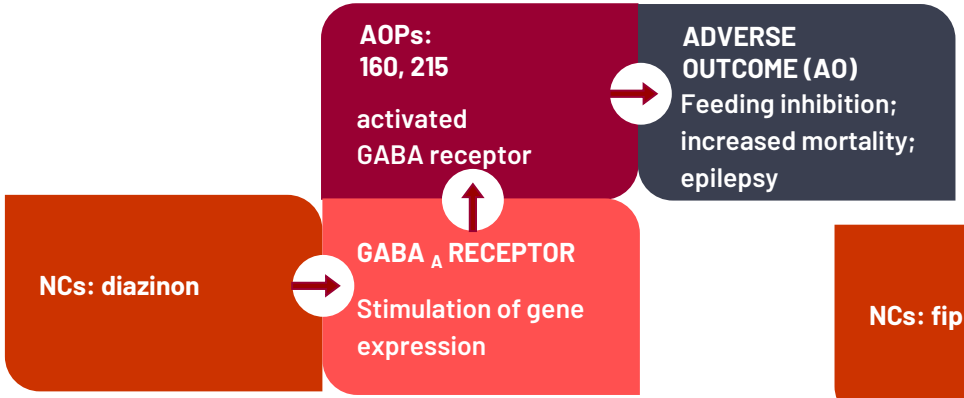


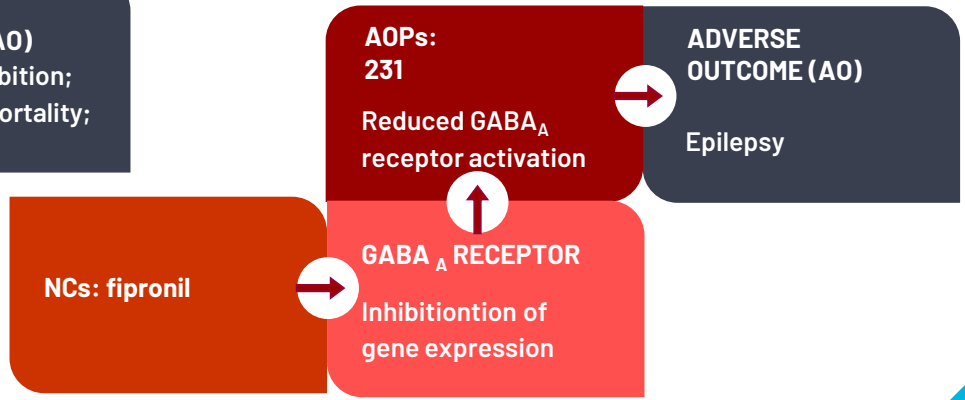
Linking responsive biomarkers with KEs in AOPs and resulting AOs

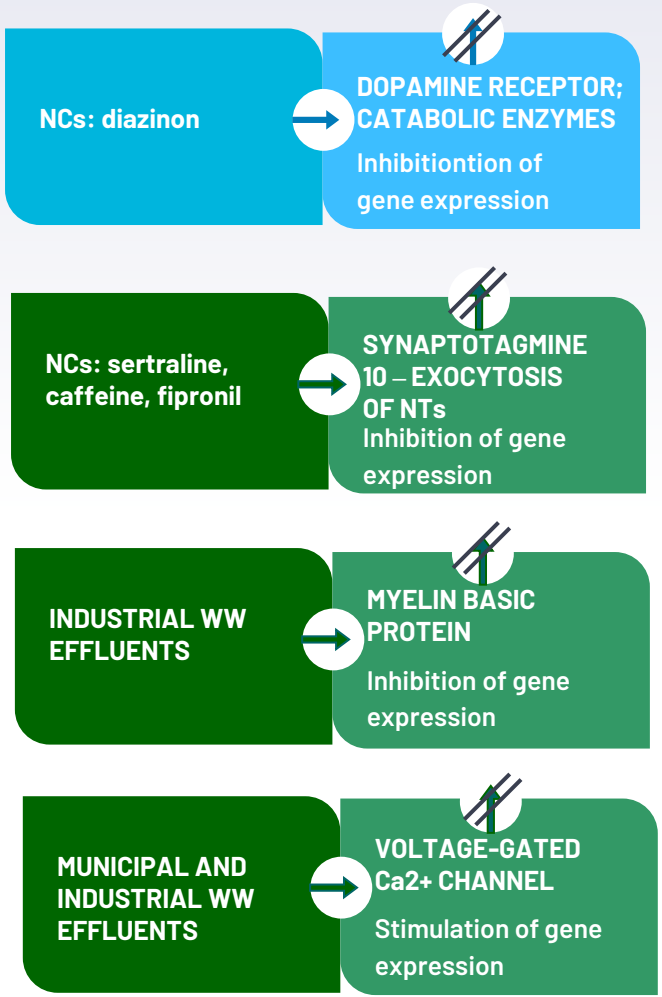
- ***HTR3A and htr1aa (serotonin receptor)*** **inhibition/inactivation results in depression and agitation**
- ***GABRB3 (GABA$_A$ receptor)*** **stimulation/activation result in feeding inhibition, mortality, epilepsy**

inhibition/reduced activation result in epilepsy









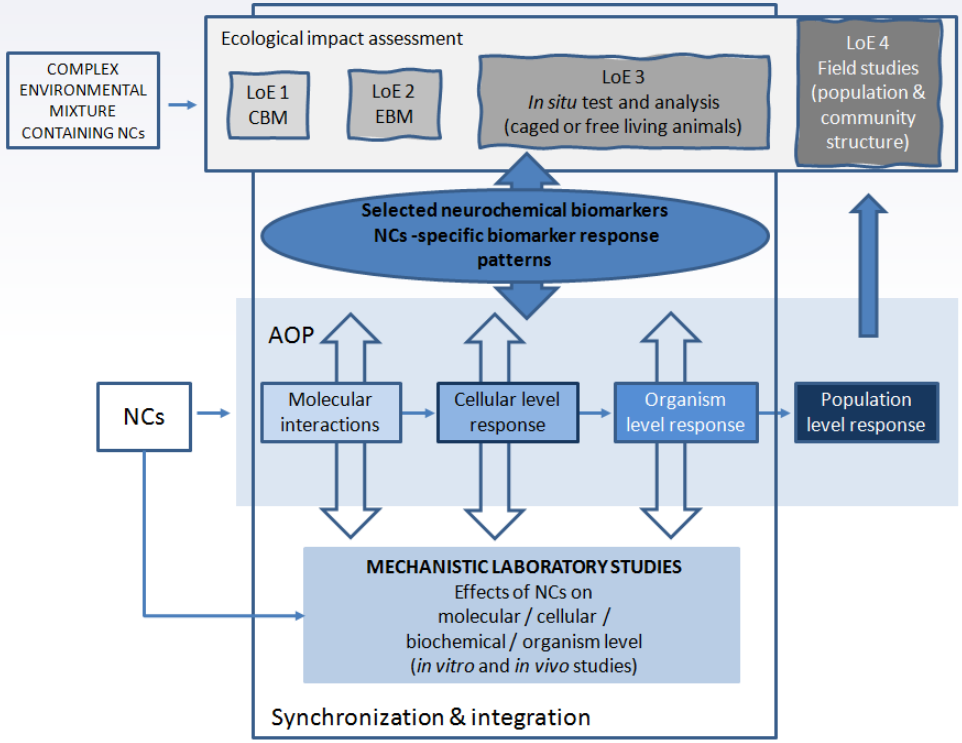
- **Other responsive and potentially relevant biomarkers** – still no available AOPs with corresponding MIEs and/or KEs; the question of AO remains open

Conclusions and further work

✓**Validation of suggested biomarkers and further search for novel biomarkers clearly related to adverse effects in the exposed organisms**

✓ **Testing additional NCs with various primary MoA (single and in mixture)**

✓**The holistic approach : synchronization of mechanistic laboratory studies and biomarker research with further development of AOP database in development of biomarker-base strategy for impact assessment of NCs in the aquatic environment.**



Kaisarevic et al. (2021) Environ Sci Eur 33:115

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Science Fund
of the Republic of Serbia

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