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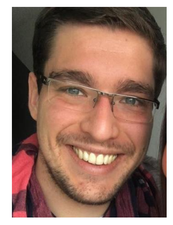

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Effect-directed Monitoring (EDM) Tools as a Toxicological Fingerprint for Ecological and Human Risk Assessment of Water Bodies

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Introduction

Because of widespread human activities, water pollution is a global issue, potentially threatening human and environmental health due to exposure to complex mixtures of chemicals. Nowadays, regulatory risk assessment in the EU is mainly based on analytical methods, which measure the concentrations of known individual chemicals in water samples. This approach has several shortcomings. Preferably, novel, next generation risk assessment tools should be using effect-based human health, 3R models. These bioassays will allow measuring the effects of unknown chemical mixtures in water samples. Within the EMERCHE project, one of the objectives is to select a battery of bioassays covering a broad panel of endpoints.

List of chemicals

Table 1 shows an overview of 12 literature sources that were used to compile a total of 317 concerning and prioritized chemicals occurring in different water bodies (waste, surface and drinking water).

Figure 1 shows the percentages of the different categories of the 317 chemicals. The majority of chemicals are pesticides (40 %, including several metabolites), followed by functional industrial compounds (26%) and pharmaceuticals (22%). This distribution also reflects the causative anthropic activities (agriculture, chemical industry, pharmaceutical industry). Interestingly, 37% of the chemicals appears not to be approved by the EU of the pesticides, 49% is not approved by the EU.

Table 1: Classification of the 12 references used to select 317 chemicals detected in waste water, water cycle, surface water and drinking water.

	12 references*			
	Waste water	Water cycle	Surface water	Drinking water
Number of references	1	2	4	5
Number of chemicals	20	36	170	153

* Schriks et al, Water Research, 2010. Roos et al, Sc of Tot Env, 2012. de Voogt et al, Water Sc and Tech 2009. Sjerps et al, Water Research 2016. Busch et al, ET&C 2016, Van den Ohe et al, Sc of Tot Env 2011. Decision 2015/495/EU. Decision 2013/39/EU. Gros et al J haz mat 2017. Baken et al Env Inter 2018. Vewin list problematic pesticides. WHO guidelines for drinking-water quality 2017.

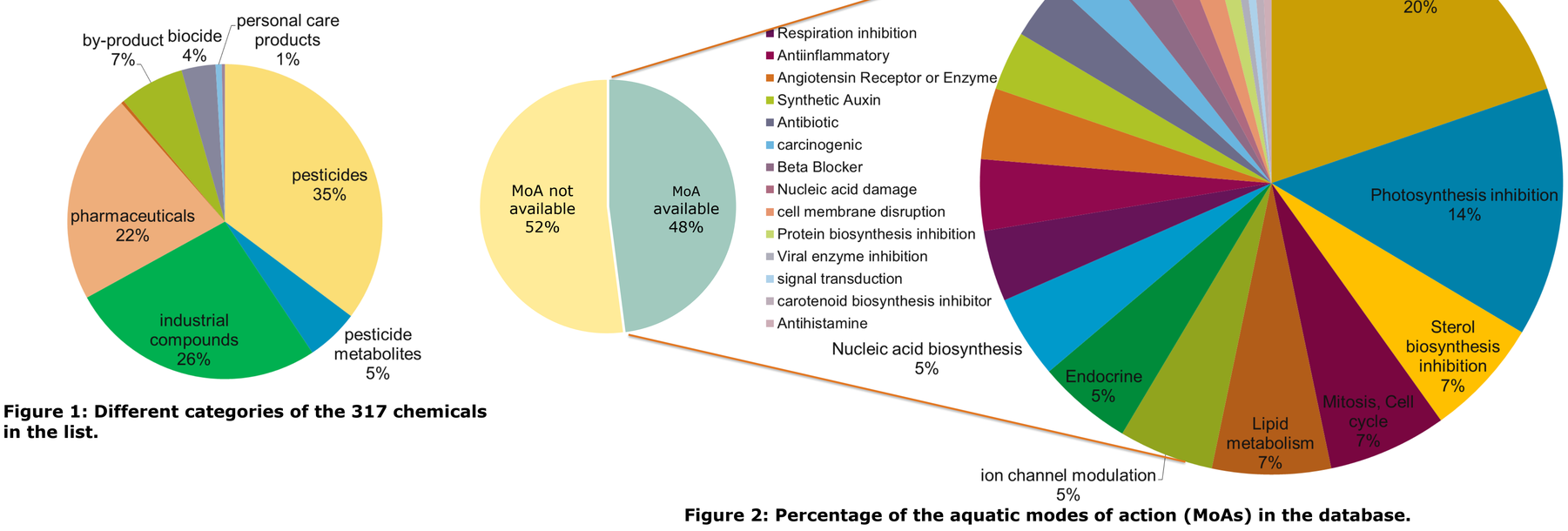

Figure 1: Different categories of the 317 chemicals in the list.

Figure 2: Percentage of the aquatic modes of action (MoAs) in the database.

Modes of Action (MoA)

Available aquatic acute MoAs for the chemicals in Table 1 were identified based on the database presented in Busch et al. (2016).

MoAs were available for 152 chemicals (48%). Figure 2 presents the percentages of the different available MoAs, distributed over 22 different aquatic MoAs. Neuroactive chemicals constitute the main portion (20%), followed by photosynthesis inhibitors (14%, which are herbicides), sterol biosynthesis inhibitors (7%), and chemicals impacting mitosis or cell cycle (7%), lipid metabolism (7%), ion channel modulation (5%), nucleic acid biosynthesis (5%) and the endocrine system (5%). These MoAs cover 70% of the chemicals with an available MoA. The rest of the list of MoAs concerns less than 4% of the chemicals with an available MoA.

Table 2:List of different endpoints which should be assessed for human health risk and associated with different bioassays currently used in water quality assessment. From this list, 14 bioassays has been pre-selected for each endpoints.

Bioassay endpoint	Number of bioassays applied in water quality assessment	Examples of pre-selected bioassays	Reference
cytotoxicity	21	microtox	[1]
		MTT/XTT	[1]
Ah receptor (anta)agonist	10	AhR-CALUX	[2]
(anti)estrogenic activity	33	ER-CALUX	[1, 3]
(anti)androgenic activity	22	AR-CALUX	[1, 3]
(anti)glucocorticoid activity	5	GR-CALUX	[2]
(anti)progestagenic activity	8	PR-CALUX	[3]
(anti)thyroid activity	11	TR-CALUX	[3]
lipid metabolism	1	PPARγ-CALUX	[2]
genotoxicity	22	Comet assay	[1, 4]
		micronucleus	[1, 4]
mutagenicity	10	Ames test	[2]
oxidative stress response	8	AREc32	[4]
neurotoxicity	2	AChE inhibition	[1, 4]
develomental toxicity	2	N/A	
hepatotoxicity	4	N/A	
Reprotoxicity	1	N/A	
Immunotoxicity	4	N/A	
heamatoxicity	0	N/A	
Epigenetic/ carcinogenicity	0	N/A	
Nephrotoxicity	0	N/A	

References : [1] Connon R., Geist J. and Werner I. Effect-based tools for monitoring and predicting the ecotoxicological effect of chemicals in the aquatic environment, *Sensors* (2012), 12, 12741-12771.[2] Neale P. et al. Development of a bioanalytical test battery for water quality monitoring: fingerprinting identified micropollutants and their contribution to effects in surface water, *Water Research* (2017), 123, 734-750; [3] Leusch F., Neale P., Hebert A., Scheurer M., Schriks M. Analysis of the sensitivity of *in vitro* bioassays for androgenic, progestagenic, glucocorticoid, thyroid and estrogenic activity: Suitability for drinking and environmental waters, *Environmental International* (2017), 99, 120-130. [4] Escher B. and Leusch F., Bioanalytical tools in water quality assessment. *IWA Publishing* (2012).

Bioassays

A literature review was performed in order to produce an overview of different bioassays, which have already been used in the context of water quality assessment.
For each endpoint, bioassays were selected based on criteria such as sensitivity, availability of standard protocols, and specificity.
Here we propose a preliminary set of 14 bioassays covering the different relevant endpoints for human health. The list is shown in Table 2.
For 7 endpoints, none or very few bioassays are available and no pre-selected bioassays were proposed. Work in progress will attempt to define specific bioassays to cover these relevant endpoints in the context of water quality assessment as well.

Table 3: List of different mechanisms of action of the 30 neurotoxicants in the list of chemicals.

Mode of action	Number of neurotoxicants
GABA receptors agonists	4
GABA receptors antagonists	2
nAChR agonists	6
AChE inhibitors	12
Sodium channel modulators	2
alteration of neuronal excitability	4
	30

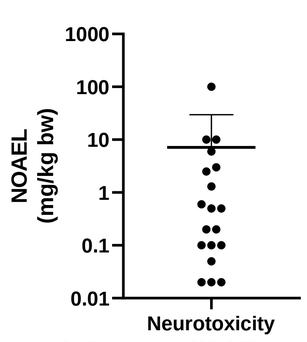

Figure 4: Range of NOAELs of the 30 neurotoxicants from the list of chemicals

Neurotoxicity endpoint

Because the majority of the chemicals is neuroactive (Figure 2), special focus is put on the neurotoxicity endpoint. The pre-selected bioassay in Table 2 allows to detect only one neurotoxic effect - Acetylcholine esterase (AChE) inhibition.
Valdivia et al. (2017) suggested to apply a multielectrode array (MEA) to detect neurotoxicity, based on a set of chemicals. These chemicals were screened by different specific neurotoxic MIE in the ToxCast program. Figure 3 shows the link between those MIEs and MEA measurements.
The list of chemicals presented here contains 30 neurotoxicants for which MoAs are available. These MoAs are shown in Table 3. Interestingly, except for AChE inhibition, all MoAs are part of the MIE of the Valdivia's study (indicated in red in Figure 3). Therefore, we suggest to apply MEA on rat cortical neurons to assess the neurotoxicity of water samples , in addition to the AChE inhibition assay. To be able to determine the potential neurotoxic risk for human health in the context of water quality assessment, it is recommended to define trigger values. To obtain these, we will apply toxicokinetics models on *in vivo* data, using NOAELs from neurotoxicological studies of the chemicals listed in Table 3. These neurotoxicological NOAELs cover a concentration range of a factor of 1000 (Figure 4) and a trigger value is suggested to be calculated and validated with *in vitro* data from the lower NOAEL of 0,002 mg/kg bw in rat.

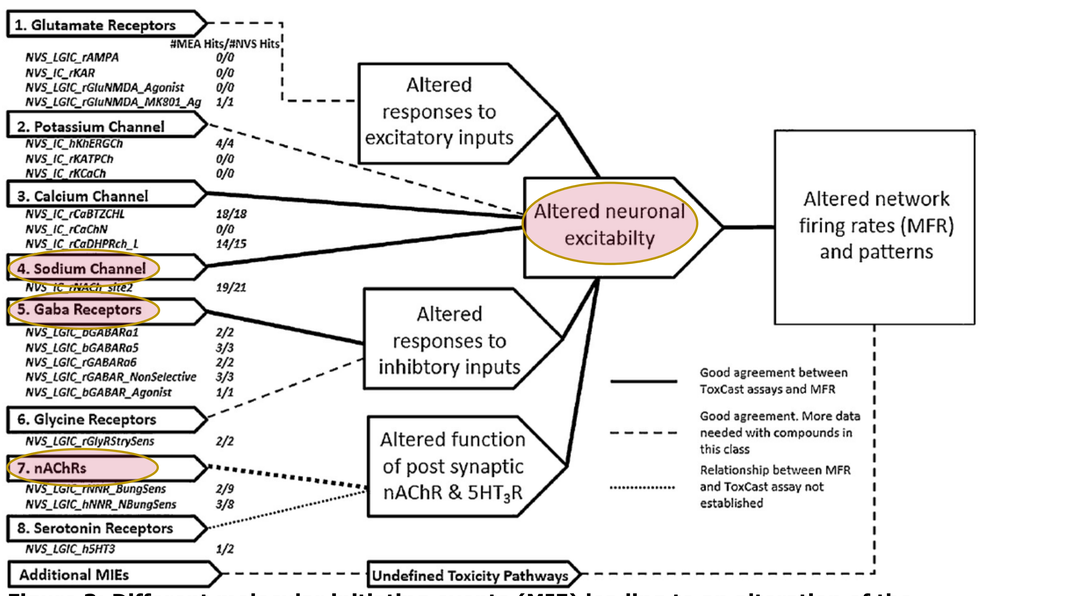

Figure 3: Different molecular initiating events (MIE) leading to an alteration of the neuronal excitability which could be assessed by multielectrode array (MEA).
Ref: P. Valdivia, M. Martin, W. R. LeFew, J. Ross, K. A. Houck, and T. J. Shafer, "Multi-well microelectrode array recordings detect neuroactivity of ToxCast compounds," *Neurotoxicology*, vol. 44, pp. 204–217, 2014.

Conclusion

The main aim of this study was to provide EDM tools – a battery of bioassays – in order to assess risks to human health of chemicals in water. Firstly, a representative list of chemicals in drinking and surface water was established to highlight specific adverse outcomes, or modes of action, which need to be assessed by the set of bioassays. This list contains mainly pesticides (40%) and neurotoxic compounds (10%). Secondly, the most sensitive and specific bioassays were selected for each endpoint (i.e., when a choice was relevant and possible). This resulted in a battery of 14 bioassays, covering 12 toxicological endpoints. Thirdly, for some endpoints, such as neurotoxicity, there was no appropriate bioassay available. Therefore, we suggest to apply MEA techniques, which have been demonstrated to be potentially usable for detecting different neurotoxic modes of actions of environmental chemicals.
In the context of water quality assessment, the battery of bioassays will be applied in a concrete practical way, i.e., in mesocosm studies and the water monitoring program in the Netherlands , after defining accurate trigger values extracted from the literature or as determined by modelling.

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