

# Prioritization of Organic Compounds for Suspect Screening in (Drinking) Water and Biota

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## Abstract

Suspect and non-target screening are powerful tools for identification of organic compounds using high resolution mass spectrometry (HRMS). However, there is a lack of smart suspect screening lists for the identification of organic compounds which are persistent, have high mobility in the environment and are potentially bioaccumulative and toxic. In this study, we created a prioritization strategy for top candidates among ~32,000 compounds including pesticides, pharmaceuticals, flame retardants and many other chemicals based on their physiochemical characteristics (e.g.  $\log K_{ow}$ ,  $\log K_{oc}$ ), predicted environmental fate characteristics (e.g. biodegradability, bioconcentration factor), human toxicity, emission sources and emission quantity for (drinking) water and biota. For charged compounds (18% of the list), a  $\log D$  value was estimated based on the  $\log K_{ow}$  at  $pH 7$  to account for different behavior of charged compounds in the environment. Three lines of specialization were developed, one for water and two for biota (LC- and GC-HRMS, respectively). For biota, the driving factor for achieving a high score was high mobility towards biota and bioaccumulation potential, whereas for water, the mobility in the aquatic environment and human toxicity was of most significant importance. Typically, halogenated compounds ended up in the “(drinking) water-” (26,4%) and “biota-GC-HRMS” (19,4%) suspect lists due to their high bioaccumulation factor. In summary, we present three suspect screening lists developed for anthropogenic compounds that may end up in water and biota providing new opportunities to screen for environmentally relevant compounds by HRMS.

**Keywords:** Suspect screening; prioritization of compounds; physiochemical properties; environmental fate; emerging pollutants

## 1. Introduction

The introduction of conventions and directives has successfully phased out high risk organic pollutants.

However, the chemicals and product markets constantly introduce new compounds for replacement. The CAS registry<sup>SM</sup> contains more than 124,000,000 organic and inorganic substances with CAS numbers, of which 347,020 substances are regulated worldwide<sup>1</sup>, and approximately 145,000 substances are pre-registered in REACH<sup>2</sup>. The Swedish Chemicals Agency (KemI) has registered over 130,000 substances with CAS numbers. On a daily basis, ~50 new regulated substances and 60-75 patent citations are added to the CAS database<sup>3</sup>. Only a small fraction of these compounds is regulated; for example, the Stockholm Convention comprises 30 compounds/groups<sup>4</sup> and the Water Framework Directive (WFD) comprises 33 priority compounds/groups<sup>5</sup>. However, the fate, bioaccumulation potential and toxicity to wildlife and humans of emerging compounds are largely unknown. Moreover, it is challenging to assess all these compounds, due to limitations of analytical methods and capacity.

Recent suspect and non-target screening studies<sup>6-10</sup> successfully identified some emerging compounds and developed identification techniques. A well implemented approach to identify a broad range of compounds in environmental monitoring and assessment is suspect screening<sup>11-14</sup>. Nowadays, suspect lists are mainly dominated by known, highly produced and/or trend compounds. In general, these pre-set suspect screening lists focus on certain compound groups or are based on previous identified compounds<sup>15-18</sup>. Moreover, the environmentally relevant charge of compounds is rarely considered. A systematic approach to develop suspect lists for monitoring and screening purposes in different environments, like water and biota, is desired.

In this study, a flexible tool was developed for the development of suspect screening lists, which can be used for screening of environmental samples for organic compounds using high resolution mass spectrometry (HRMS). The organic compounds are ranked based on their physiochemical properties, environmental fate characteristics, emission source, emission quantity range, and toxicity, while the criteria of the ranking can be easily adjusted and applied for different matrices. Three suspect screening lists were created, one focusing on water and two on biota (LC- and GC-HRMS, respectively).

## 2. Materials and Methods

### 2.1. Databases

The main database of organic compounds was created by merging three databases including chemical structures (SMILES) information (US EPA<sup>19</sup>, Farmaceutiska specialiteter i Sverige (FASS), and the Noman list of emerging substances). Merging these databases and excluding duplicates resulted in a list of about 32,000 compounds including pesticides, pharmaceuticals, flame retardants, industrial additives and many more chemicals and compound groups. The above-mentioned databases were chosen due to their completeness of information (number of compounds, SMILES notation, and CAS numbers) and relevance for the Swedish environment. Depending on the final purpose of the suspect list, other databases may be selected as a starting point.

### 2.2. Chemical Descriptors

All compounds in the final database were characterized by empirical data or estimated chemical descriptor data calculated in EPI Suite<sup>TM</sup> 20, MarvinView 15.10.12.0<sup>21</sup> and the On-line CHEMical database and Modeling environment<sup>22</sup> from their SMILE structures. The descriptors comprised physicochemical properties ( $\log K_{ow}$ ,  $\log K_{oc}$ ,  $\log K_{oa}$  and  $\log S_w$ ), predicted environmental fate characteristics (biodegradation and  $\log BCF$ ), predicted numerical *in vitro* response toxicity data for several receptors (ER= estrogen receptor, AR= androgen receptor and TR= thyroid hormone receptor), and index values (0-5) from the SPIN database<sup>23</sup> characterizing emission sources and emission quantity ranges ( $EI_{air}$ ,  $EI_{water}$ ,  $EI_{soil}$ ,  $EI_{sewage\ treatment}$ ,  $EI_{consumer}$ ,  $EI_{occupational}$  and  $QI$ = quantity index). These descriptors were selected for their relevance in describing mobility and bioaccumulation potential of the compounds in water and biota. Biodegradation was estimated via BIOWIN 3 (EPI Suite<sup>TM</sup>), and the toxicity data was predicted via OCHEM. Physicochemical properties (empirical and estimated) and predicted environmental fate characteristics were retrieved via EPI Suite<sup>TM</sup>. For some parameters ( $\log K_{ow}$ ,  $\log K_{oc}$ ,  $\log S_w$  and  $\log BCF$ ), empirical data were available. All parameters, showed high correlation between experimental and estimated data. This indicates that the empirical data are reliable and can be given preference over the estimated values.

### 2.3. Charged compounds

At given environmental conditions, compounds exist in positively charged, negatively charged, as zwitterions, or neutral form. According to Franco *et al.* (2010)<sup>24</sup>, 41% of the REACH compounds are positively or negatively charged, 8% are zwitterions, and 51% occur in their natural form at typical environmental *pH* conditions. Charged ions may obviously behave different in the environment as compared to their uncharged counter compounds<sup>25, 26</sup>. However, it has been shown that positively charged compounds behave relatively similar to their neutral

counter compounds<sup>27</sup>. Therefore, in this work, positively charged ions were treated as neutral. If a compound is most likely negatively charged at typical environmental conditions, this compound should be assigned a  $\log D$  value (estimated  $\log K_{ow}$  at *pH* 7) rather than a  $\log K_{ow}$  value. The  $pK_a$  values for all compounds were estimated in MarvinView. Since the typical environmental *pH* is around 7, we considered a compound with a  $pK_a < 5$  negatively charged and  $pK_a > 9$  uncharged. If a compound has a  $pK_a$  between 5 and 9, it was attributed an average value of  $\log D$  and  $\log K_{ow}$ . (Figure 1).

## 3. Results and Discussion

### 3.1. Prioritization strategy

**Scoring range.** Before prioritization of the compounds, a scoring range for each descriptor was defined to create suspect screening lists for biota using LC-HRMS, biota using GC-HRMS and (drinking) water. The descriptors  $\log K_{ow}/D$ ,  $\log K_{oc}$ ,  $\log S_w$ ,  $\log BCF$ , biodegradation, and quantity index were used for scoring of the final lists (Figure 1). For the biota suspect lists, the exposure indices air, water, and soil were included, whereas the water, sewage treatment and occupational exposure indices were scored for the (drinking) water suspect list. The toxicity data used here are relevant for human endpoints, and therefore only included in the prioritization for the (drinking) water suspect list. The suspect screening list for biota using GC-HRMS contained only chemicals with  $\log K_{ow} > 3$ <sup>28</sup> and got an additional score for  $\log K_{oa}$ .

**Scoring.** For the development of the suspect screening lists for water and biota, the compounds were prioritized by assigning a scoring within a range of 0 to 1 for all descriptors. A high score is associated with a high rank in the suspect lists. The mobility and bioaccumulation potential were two important factors for ranking of the compounds<sup>29</sup>. For biota, a centered scoring system was applied for most descriptors ( $\log K_{ow} / \log D$ ,  $\log K_{oc}$  and  $\log S_w$ ) in the biotic environment due to the fact that they describe both high mobility and bioaccumulation potential<sup>29</sup>. In addition, the descriptors  $\log BCF$ , biodegradation,  $\log K_{oa}$ , exposure indices and emission quantity index are scored linear. For water, the mobility in the aquatic environment was the driving factor resulting in linear scoring of the descriptors  $\log K_{ow} / \log D$ ,  $\log K_{oc}$ ,  $\log S_w$ ,  $\log BCF$ , biodegradation, toxicity, exposure indices and emission quantity.

**Weighing.** Before summing up the scores, a weighing factor was added to each descriptor to put more focus on certain descriptors which describe bioaccumulation (e.g.  $\log BCF$ , biodegradation) and mobility (e.g.  $\log K_{ow} / \log D$ ,  $\log S_w$ ) in the desired environment best. A weighing of the descriptors was necessary to get a sufficient spread in the end scores. This resulted in three suspect lists (two for biota and one for (drinking) water), each containing the top-1000 ranked compounds which can be used for suspect screening.

### 3.2. Sensitivity analysis

Some of the chosen physiochemical properties and predicted environmental fate characteristics have been shown to be significantly correlated. The parameters  $\log K_{ow}$ ,  $\log K_{oc}$  and  $\log BCF$  correlate, and should therefore not be weighted equally in the prioritization step.

The toxicity data used in our approach were predicted using *in vitro* response toxicity data for several receptors (ER= estrogen receptor, AR= androgen receptor and TR= thyroid hormone receptor) because there is a lack of (available) species-specific measurement data for such a great number of compounds. For each predicted *in vitro* response, an estimation of the accuracy was made within the OCHEM prediction environment (high percentage = high accuracy). If the accuracy estimation was lower than 60%, the result was considered unreliable and discarded.

The descriptor biodegradation comprises semi-quantitative data, rather than half-lives. The software BIOWIN 3 generates data for compound classes rather than individual compounds. Certain compounds may get the same score for this parameter, even though they might differ in biodegradation potential. However, the BIOWIN 3 software is suitable for biodegradation in water and has been used successfully to search for drugs of potential environmental concern<sup>30</sup>.

### 3.3. Suspect lists for biota and (drinking) water

The prioritization strategy as described in section 3.1 resulted in three lists of top-1000 highest scored

compounds for biota LC-HRMS, biota GC-HRMS and (drinking) water.

About 18% of all compounds in the used databases are negatively charged in the environment ( $pK_a < 5$ ) with 8.9%, 4.2% and 13% in the generated suspects list biota LC-HRMS, biota GC-HRMS and (drinking) water, respectively (Table 1). Charged compounds are mainly present in the LC-HRMS and (drinking) water suspect lists, because ionizable compounds are highly mobile in aquatic solutions. Parameters estimated for these compounds should be well considered, because EPI Suite<sup>TM</sup> estimated most descriptors based on the chemical's neutral form. In this work, we tackled this issue with replacing  $\log K_{ow}$  with  $\log D$  for negatively charged compounds.

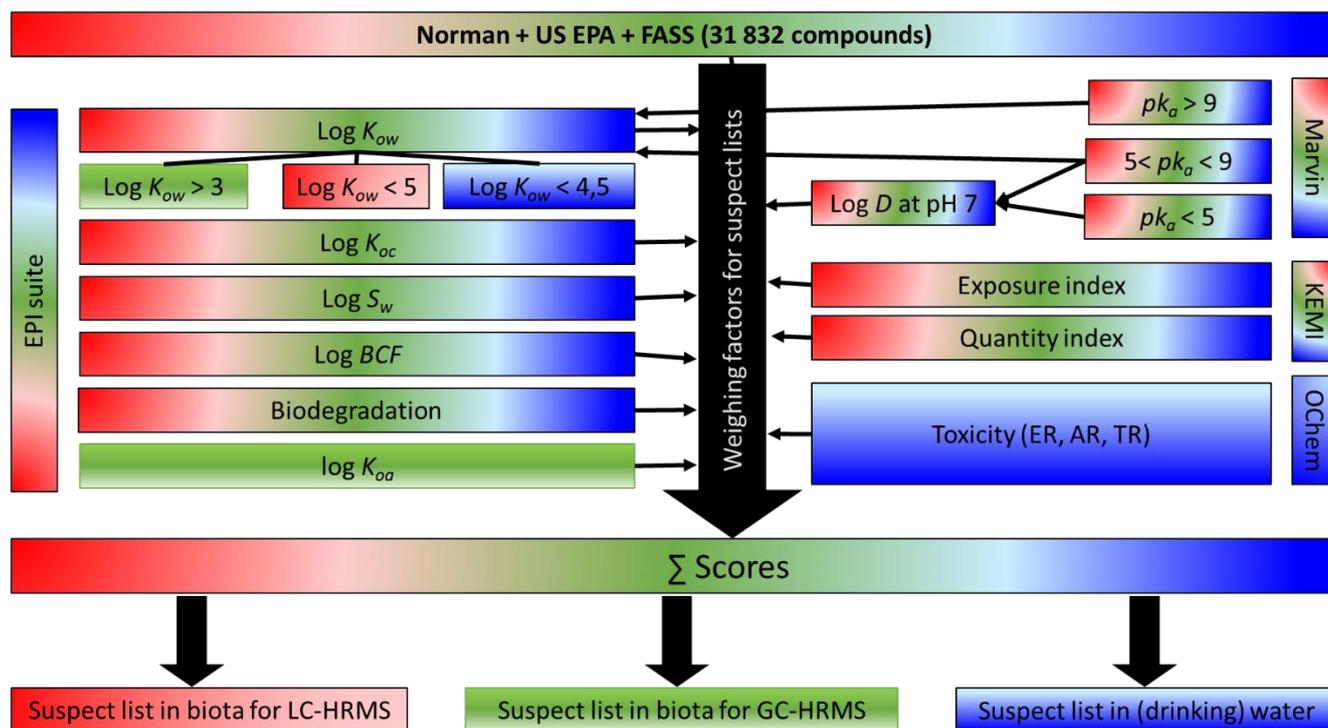
Typically, halogenated compounds end up in the (drinking) water (26,4%) and Biota GC-HRMS (19,4%) suspect lists due to their high bioaccumulation factor.

## 4. Conclusion

The identification of emerging contaminants by non-target screening is time-consuming and an accepted common approach is lacking<sup>31, 32</sup>. Suspect screening reduces the work tremendously by screening for certain group of compounds<sup>12</sup>. Ultimately, we developed a tool to create suspect screening lists for various matrices (e.g. biota, water) for the identification of anthropogenic compounds providing new opportunities to screen for environmentally relevant compounds using HRMS.

**Table 1.** The percentage of halogenated and negatively charged compounds in the three suspect lists for biota using GC-HRMS ( $n = 1000$ ), biota using LC-HRMS ( $n = 1000$ ), and (drinking) water ( $n = 1000$ )

	Halogenated			Negatively charged			
	Biota LC-HRMS	Biota GC-HRMS	(Drinking) Water	Biota LC-HRMS	Biota GC-HRMS	(Drinking) Water	
<b>F</b>	2,6%	6,3%	4,7%	$pK_a < 5$	8,9%	4,2%	13,3%
<b>Br</b>	1,3%	1,7%	2,6%	$5 < pK_a < 9$	3,1%	1,4%	6,9%
<b>Cl</b>	10,9%	11,1%	18,7%				
<b>I</b>	0,2%	0,3%	0,4%				
<b>F + Br</b>	0,2%	0,8%	0,1%				
<b>Cl + F</b>	1,7%	1,8%	0,6%				
<b>Cl + Br</b>	0,1%	0%	0,7%				
<b>Cl + I</b>	0%	0,1%	0%				
<b>F + I</b>	0,2%	0,9%	0%				
<b>Br+ I</b>	0%	0%	0%				
<b>Total</b>	15,0%	19,4%	26,4%		12,0%	5,6%	20,2%



**Figure 1.** Overview of the prioritization strategy of organic compounds for the development of suspect screening lists of environmentally relevant compounds in (drinking) water and biota. Blue = (drinking) water, Red = biota LC- HRMS, green= biota GC-HRM.  $K_{ow}$  = octanol-water partitioning coefficient;  $D$  = adjusted  $K_{ow}$  at pH 7;  $K_{oc}$  = organic carbon-water partitioning coefficient;  $S_w$  = water solubility;  $BCF$  = bioconcentration factor;  $K_{oa}$  = octanol-air partitioning coefficient; ER= estrogen receptor; AR= androgen receptor; TR= thyroid hormone receptor. Exposure index and quantity index according to the SPIN database<sup>23</sup>.

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