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Guidance for identification of RBSPs and list of Danube RBSPs including quantification of their ecological impact and modelling-based exposure and risk predictions validated with case-study data

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PP	Restricted to other programme participants (including EC)	
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1 Summary

In this deliverable a methodology/guidance is described for selection of River Basin Specific Pollutants (RBSPs) at the river basin scale. Using this guidance, a draft list of the Danube RBSPs was identified and their provisional Predicted No-Effect Concentrations were proposed to be considered for the derivation of the basin-wide Environmental Quality Standards. From among various prioritisation approaches explored in the SOLUTIONS sub-project (SP) Concepts & Solutions (see Deliverable 2.1) the most mature NORMAN approach, modified to handle data from a single survey, has been applied to a dataset of ca. 47,000 measurements of 719 substances obtained within the Joint Danube Survey 3 (JDS3). A preliminary list of RBSPs was drafted in 2015 and adopted by the ICPDR in the second Danube River Basin Management Plan. An extended dataset of ca. 410,000 monitoring data on occurrence of 846 substances compiled from all Danube countries was then processed with the NORMAN approach modified for large international river basins. A final list of 20 Danube RBSPs with their respective PNECs was proposed.

It became obvious that there is a lack of environmental occurrence data on thousands of additional substances which might be considered for prioritisation. Therefore, a basin-wide study, focused on input of chemical pollutants from waste water treatment plants (WWTP) effluents, was designed and carried out in autumn of 2017. A state-of-the-art target and non-target screening methodology developed in SOLUTIONS SP Tools was applied on samples from 12 WWTPs.

The modified NORMAN prioritisation methodology was also used for a ranking of 1835 substances in datasets generated by modelling tools developed in SP Models where occurrence of environmental pollutants in the Danube River Basin was predicted from other than monitoring data. Among the data sources also emission inventories and input from the Pressures and Measures Expert Group of the ICPDR were considered. A first provisional ranking is presented in Deliverable 1.5.

In addition to a basin-wide identification of pollutants, an approach for identifying site specific toxicants was proposed employing effect-based tools. A tiered approach for identifying site relevant toxicity drivers is presented, including a mass balance approach, virtual effect-directed analysis (EDA), and higher tier EDA. For the data collection, a battery of bioassays (Deliverable 11.1) was systematically applied together with the chemical target and non-target screening. The methodology had been tested at the case study site Novi Sad in Serbia and re-

sulted in the identification of 14 drivers of effects seen in *in vitro* screens for endocrine disrupting modes of action.

All data obtained within the project are stored in the on-line SOLUTIONS Knowledge Base which is interlinked with the Information Platform of Chemical Monitoring (IPCHEM) of the European Commission.

1.1 Graphical abstract

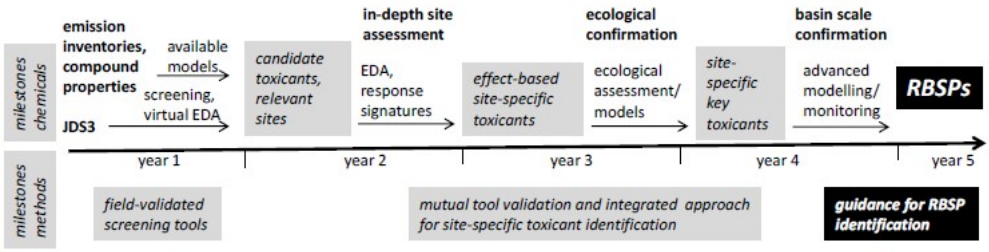


Fig. 1: Sequence of activities for the RBSPs identification in the Danube case study

2 List of abbreviations

EC	Effect concentration for the selected effect level
EU	European Union
DRB	Danube River Basin
DRBMP	International river basin management plan for the Danube River Basin District
EBTs	Effect-based tools
EQS	Environmental quality standard
GC-MS	Gas chromatography mass spectrometry
HC5	Hazardous Concentration for 5% of species
ICPDR	International Commission for the Protection of the Danube River
LC-(HR)MS/MS	liquid chromatography (high resolution) tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
LVSPE	Large volume solid-phase extraction

MoA	Mode of Action
MEC	Measured Environmental Concentration
MS	Member States
PE	People equivalent
PEC	Predicted Environmental Concentration
PNEC	Predicted No-Effect Concentration
PoM	Programme of Measures
RBD	River Basin District
RBMP	River Basin Management Plan
RBSPs	River Basin Specific Pollutants
REF	Relative enrichment factor for the selected effect level
REP	Relative potency compared to reference compound x
SPE	Solid phase extraction
SPM	Suspended particulate matter
SSD	Species sensitivity distribution
TNMN	ICPDR Transnational Monitoring Network
TU	Toxic Units
QSAR	Quantitative Structure-Activity Relationship
WFD	EU Water Framework Directive 2000/60/EC
WWTP	Wastewater treatment plant

3 Guidance for identification of RBSPs

3.1 Scope for the guidance document for identification of RBSPs

To achieve the environmental objectives of the EU Water Framework Directive 2000/60/EC (WFD; [1]) the Member States (MS) should adopt measures to eliminate pollution of surface waters by the priority substances and progressively to reduce pollution by other substances, which would otherwise prevent Member States from achieving the good chemical and ecological status. Besides the set of Priority Substances laid down in Annex X of the WFD, which are regulated and monitored at EU level, the EU MS need to identify pollutants of regional or local importance (in particular substances listed in WFD, Annex VIII) and provide environmental quality standards (EQS), monitoring schemes, and regulatory measures to mitigate their effects. This means that MS need to decide which substances should be declared as River

Basin Specific Pollutants (RBSPs). This requires assessments of impacts as well as prioritisation efforts and strategic screening for substances possibly causing concern. The WFD (Annex V, section 1.2.6) establishes the principles to be applied by the MS to develop EQSs for RBSPs. Compliance with EQSs for RBSPs forms part of the assessment of ecological status. EQSs are therefore key tools in assessing and classifying ecological status and can therefore affect the overall ecological status classification of a water body under the WFD. In addition, EQSs will be used to set permits for discharge to waterbodies, so that chemical emissions do not lead to EQS exceedance within the receiving water (see Deliverable 20.1).

Article 8.1 of the WFD requires MS to establish monitoring programmes for the assessment of the status of surface waters in order to provide a coherent and comprehensive overview of water status within each river basin district (RBD). The results of monitoring play a key role in determining whether water bodies are in good status and what measures need to be included in the River Basin Management Plans (RBMPs) in order to reach good status. Precise and reliable monitoring results are therefore a prerequisite for sound planning of investments in the Programme of Measures (PoM). The selection of the quality elements and parameters to be monitored should enable the detection of all significant pressures on water bodies. This is particularly important where the pressures and impact assessment may not have been adequate to identify all potential pressures and impacts in the River Basin District (RBD) perhaps because of lack of information or methods or because of unexpected, anthropogenic activities within the RBD.

The obligation to identify RBSPs and set EQS for them was not equally observed in the first RBMP cycle, with some MS identifying many more than others, and some standards being much less stringent than others for the same substances. This had implications for the comparability of conclusions drawn regarding ecological status.

This guidance document for the identification of RBSPs should contribute to an overall understanding of the process of identification of RBSPs. Under the process of the Common Implementation Strategy for the WFD, a Guidance Document No. 27 “Technical Guidance for Deriving Environmental Quality Standards” has been developed to support the derivation of EQSs for priority substances and for RBSPs that need to be regulated by MS according to the provisions of the WFD. The document focuses on the steps required to derive EQSs that comply with the requirements of Annex V of the WFD. It assumes that the chemicals for which EQSs are required have been identified, *i.e.* that EU guidance does not cover the prioritisation

of chemicals. In this respect, the guidance document developed by SOLUTIONS provides an extended advice for selecting pollutants of basin-wide importance for which a Programme of Measures has to be established.

SOLUTIONS developed this guidance on the basis of a number of experimental and computational modules, such as the NORMAN prioritisation framework, a methodology for the identification of candidate toxicants, toxicological endpoints and possible hot spots using chemical and effect-based screening, basin wide wastewater effluents screening, effect-based identification of site specific toxicants, identification of mixture toxicity drivers, and advanced integrated exposure, effect and risk models for assessment and toxicant prioritisation on a basin scale. Methodological details of all these components is provided in the corresponding deliverables from the sub-projects *TOOLS* and *MODELS*, in particular D9.1 on effect-based tools, D13.1 on ecological effect assessment, D14.1 on integrated exposure and risk modelling, and D18.1 on component-based mixture risk modelling and driver identification.

A conceptual outline of a future advanced methodological framework which integrates mixture risk assessments into prioritisation procedures under the WFD is provided in Deliverable D2.1 from the sub-project CONCEPTS & SOLUTIONS. Full implementation of the proposed advanced framework, however, would require changes in the legal text of the WFD and corresponding amendments to the existing guidance for EQS setting. In contrast, the guidance for identification of RBSPs described in this deliverable report stays with procedures for single substance prioritisation that are applicable under the existing legal framework, in particular the NORMAN approach.

A practical testing of this guidance was carried out through the process of developing a proposal of RBSPs for the DRB (Fig. 1). The DRB case study carried out in the frame of SOLUTIONS, focused on in-depth testing, demonstration and harmonisation of the state-of-the-art chemical and biological diagnostic tools, prioritisation approaches and multivariate statistical methodologies for the selection of RBSPs required for the assessment of ecological status of DRB waters according to the WFD.

3.2 Universe of candidate toxicants – Joint Danube Survey 3

The ICPDR provided all officially available data used for the development of an international RBMP for the Danube River Basin District (DRBMP) in a format allowing for their use in the prioritisation schemes and models developed within the SOLUTIONS project. This included

data from the ICPDR Transnational Monitoring Network (TNMN), and from the previous large-scale surveys organized by the ICPDR (JDS1 2001, AQUATERRA 2004, JDS2 2007; http://www.icpdr.org/main/activities-projects/joint-danube-survey).

The interim results concerning the identification of the Danube RBSPs have been regularly presented at the regional stakeholder meetings organised by the ICPDR (meetings of the ICPDR Monitoring and Assessment Expert Group and of the Pressures and Measures Expert Group) increasing awareness in the project activities and getting a critical feedback to optimise further studies.

SOLUTIONS had a unique opportunity to test the developed tools against the results of the Third Joint Danube Survey (JDS3; [2]), organized by the ICPDR from 12 August till 26 September 2013, covering the largest and most international river basin in Europe. The JDS3 was carried out on a 2,600 km stretch of the Danube River on which samples of surface water, suspended particulate matter (SPM), sediments and biota were collected from 68 sites assigned by the DRB countries (Fig. 2). All data are stored and publicly accessible in the on-line SOLUTIONS Knowledge Base.

3.3 Universe of candidate toxicants - basin wide screening of waste water effluents

It became obvious during the assessment of the JDS3 outcomes that there is still a lack of environmental occurrence data on thousands of substances which might be considered for prioritisation. Therefore, a basin-wide study focused on one of the most important inputs of chemical pollutants – WWTP effluents – was designed and carried out in autumn of 2017. A state-of-the-art target and non-target screening methodology developed in SOLUTIONS SP Tools was applied. The main goals were to:

- Get representative chemical patterns from WWTP effluents with different treatment and from different European countries;
- Get representative effect-based patterns for the same WWTP effluents;
- Support RBSPs selection for the Danube basin;
- Provide data to modelers for advanced exposure and risk modeling in the Danube river and comparison with JDS3 data;
- Provide a starting point for the planning and implementation of the JDS4;
- Support ICPDR and local stakeholders with valuable data for the DRBMP;

- Gather all data available in the open access SOLUTIONS Knowledge Base/ NORMAN/ ICPDR databases and used for the goals defined above resulting in common publications.

3.3.1 Concept for sampling WWTP effluents for hazardous substances analyses

The ICPDR aims to strengthen its efforts for pollution control of hazardous substances and expressed its interest to deepen the knowledge on sources and pathways of hazardous substances in the DRB as a basis for efficient management strategies. In line with this, the SOLUTIONS consortium offered the ICPDR the possibility to analyse samples from ca. 15 wastewater treatment plant (WWTP) effluents in the Danube Basin for a wide range of organic emerging chemicals in highly advanced laboratories, in case the ICDPR can organize the sampling and can provide the samples. This concept provides a selection of WWTPs to be sampled, parameters to be analysed and serves as guidance for the sampling procedures.

3.3.2 Selection of WWTPs for sampling

A selection of possible WWTPs to be monitored in the campaign has been made based on 2012 data of the ICPDR Urban Wastewater Inventory. The selection process considered the following criteria:

- Only those Danube countries were considered, which expressed their interest to support the monitoring campaign;
- The number of WWTPs to be monitored in each country was determined according to the expected data availability and the capacity of the countries to organize WWTP sampling;
- The selected WWTPs should represent the countries` predominant technology;
- The WWTPs were chosen to be as big as possible (in terms of population equivalents, PE) to ensure the best technical equipment and the best “know how” to perform the monitoring.

In total eight countries gave positive feedback to the WWTP effluents monitoring campaign supported by the SOLUTIONS project, representing almost 90% of PE treated in the Danube Basin in 2012. The list of WWTPs sampled and analysed is shown in Table 1.

Table 1: List of WWTPs in the Danube River Basin selected for effluent monitoring

Country	Town	PE*	Treatment type
Romania	Bucharest	1327995	Tertiary
Romania	Cluj-Napoca	382031	Tertiary
Serbia	Šabac	84000	Tertiary
Croatia	Varaždin	97450	Secondary
Croatia	Zagreb	842425	Secondary
Slovenia	Ljubljana	462872	Secondary
Slovenia	Vipap	152487	Tertiary
Hungary	Budapest	1174643	Tertiary
Slovak Republic	Žilina	139934	Tertiary
Czech Republic	Brno-Modřice	397945	Tertiary
Austria	Amstetten	150000	Tertiary
Germany	Augsburg	659387	Tertiary

* PE; People Equivalent as capacity

For the monitoring campaign, flow proportional automatic samplers at the WWTP effluent were preferred. Alternatively, if flow proportional samplers were not available, time proportional sampling was considered also suitable. In case automatic device was not in place, the composite samples were taken manually (minimum requirement). Additionally, a fridge and a freezer for storing the collection vessels were required either at the WWTP or in a suitable laboratory. WWTPs should be able to analyse general parameters in their labs or in a suitable laboratory.

3.3.3 Sampling procedure

The effluent sampling for the analysis of the considered substances followed the established routine at the WWTP as far as possible. Sampling was performed over seven days preferably with automatic samplers to get seven daily composite samples for the organic target parameters, one seven-days composite sample for heavy metals and several daily composite samples for the general parameters. In order to undertake additional analyses on target parameters (organic emerging chemicals/hazardous substances), heavy metals and general parameters, three sub-samples were collected in parallel. Sampling was performed during late summer/early autumn according to the specific arrangements with the SOLUTIONS team responsible for col-

lecting the samples. The SOLUTIONS team directly collected the samples at the WWTPs. The stepwise sampling procedure is shown in Fig. 3.

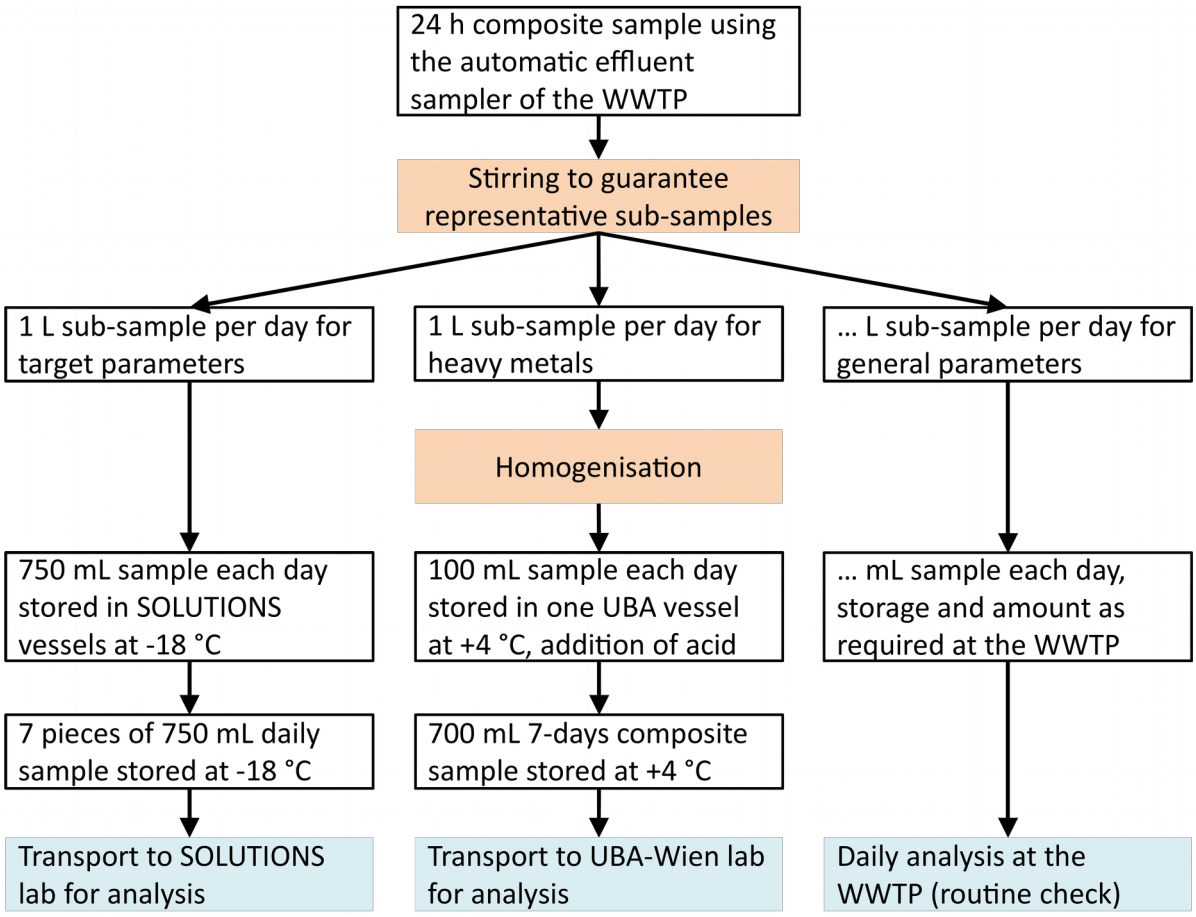


Fig. 3: Scheme of the sampling procedure. Parameters to be analysed were general parameters (by WWTPs) – pH, conductivity, COD and/or TOC, BOD5, NH4-N, NO3-N, TP, PO4; metals (UBA Vienna) – Cd, Cu, Cr, Pb, Ni, Hg, Zn, As and emerging substances (SOLUTIONS) in seven-days composite samples and LVSPE spot 20 l samples.

The described sampling methodology is well suited to considerably reduce fluctuations in the analysis results generated. This is of an advantage if the number of samples to be analysed is limited. General parameters (see Annex 2) were analysed to assess whether significantly varying target parameter concentrations can be attributed to specific situations in the WWTP. Results of the analyses are expected to be available in June 2018.

Fig. 2: Joint Danube Survey 3 - overview map

3.4 NORMAN Prioritisation framework

Prioritisation of chemical contaminants remains a task of primary importance for environmental managers and for the scientific community as regards the definition of priority actions for pollution prevention & control and for the allocation of resources to address current knowledge gaps. It is widely recognised that the lack of data is the primary cause of the lack of regulation of contaminants of emerging concern, as a result of the often discussed vicious circle where: "*no monitoring means no data, and no data means no regulations*". Currently, the NORMAN prioritisation approach is widely accepted in the EU as a reliable tool to remove the huge knowledge gaps about emerging substances. However, it is confined to single substance assessments and the procedure builds on evidence from chemical monitoring as the crucial starting point.

As a step further SOLUTIONS developed a concept which integrates mixture risk assessments into prioritisation procedures. The aim was to derive a proposal for an '*Advanced methodological framework for the identification and prioritisation of pollutants and pollutant mixtures in the aquatic environment*' as one of the final SOLUTIONS deliverables. The advanced framework shall not replace existing approaches for single substance prioritisation, but aims to integrate existing procedures with novel methodologies into a multiple-lines of evidence approach which is able to identify both individual priority pollutants and priority mixtures of pollutants. The novel elements of the proposed prioritisation framework include evidence from:

- Ecological monitoring;
- Effect-based tools (EBTs);
- State-of-the-art (co-)exposure modelling; and
- Component-based approaches (CBA) to mixture risk assessment (MRA).

These elements were separately developed in different branches of the SOLUTIONS project and coordinated to fit into a consistent framework (for more details, see Deliverable D2.1). The development proceeded in parallel to the Danube RBSPs identification reported here. The methodological developments included feasibility tests of individual elements in separate case studies. These have not yet all been completed and they have not all been performed in the Danube or with Danube data. Partly they are smaller scale studies on selected sample sites in the Danube, the Rhine, or smaller European creeks and rivers. It was not possible to apply all novel methodological elements together and retrospectively on a very large scale such as the

entire Danube river basin. Hence, full application of the proposal for a future advanced framework to the task of current Danube RBSPs identification would have been premature. Exceptions apply to selected elements and sites, namely results from an effect-based identification of site-specific toxicants in the area of Novi Sad, Serbia, and results from an application of the SOLUTIONS integrated exposure and risk modelling methodology to the JDS3 sampling sites.

As a consequence, the existing NORMAN approach was applied to the RBSPs identification in this report (cf. also text below and 4.1). The NORMAN Prioritisation framework [3, 4] provides a powerful integrated strategy to take knowledge gaps into account in the prioritisation of chemical contaminants. The concept combines the traditional risk-based ranking process with the preliminary application of a decision tree, which allows the allocation of substances into six action categories, based on the knowledge gaps and actions needed to fill them, e.g. development of more powerful analytical methods, launch of monitoring campaigns, performing additional ecotoxicity tests, etc.

The overall prioritisation procedure is therefore carried out in two successive stages. In the first stage (see Fig. 4) a decision tree classifies chemicals into the above-mentioned six categories. The second stage entails the ranking of the substances within each category on the basis of their occurrence, hazard and risk indicators. This is a transparent and rational approach to deal with the knowledge gaps which still prevent, for most emerging substances, proper risk assessment and risk ranking to justify their classification as RBSPs.

The overall process is an iterative one that involves a periodic revision of the priority substances in each category whenever e.g. new information / more reliable data are generated or a feedback from applied pollution reduction measures is available.

3.4.1 Categorisation process

As illustrated in Fig. 4, the first step in the decision tree consists of grouping the compounds by degree of investigation and evidence of exposure. The exposure indicators used in the categorisation phase are aimed at assessing whether the quantity and quality of the available mon-

itoring data are sufficient to allow exposure assessments for the identified emerging substances.

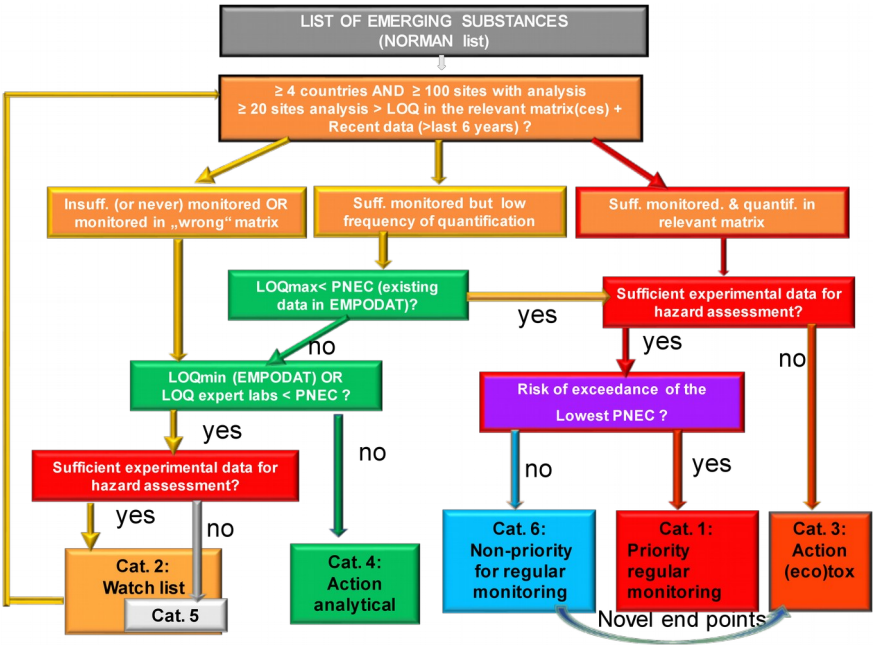


Fig. 4: NORMAN decision tree for the categorisation of substances

The indicators used for this assessment are as follows:

a) *Consistency between the monitored matrix and the relevant matrix for a given substance*

This indicator describes the distribution of the substance among the different media as a result of the application of fugacity models, plus assessment of the octanol–water partition coefficient (K_{ow}), the organic carbon–water partition coefficient (K_{oc}) and water solubility (S_w).

b) *Number of countries and number of sites with analyses*

The number of countries and the number of sites in which the substance was looked for is used as an indicator of the level of investigation of the given substance (well monitored substances *vs* insufficiently monitored substances).

c) *Number of sites with quantified data (above the Limit of Quantification, LOQ)*

The number of sites at which the substance was detected above the LOQ indicates whether the exposure is widespread or only a "local problem", knowing that the actions of NORMAN might address both compounds that are of concern at a river basin or local level and compounds that are of concern at the European level.

d) Compatibility of the analytical performance with the target environmental threshold

If the substance is not quantified (*i.e.* occurrence levels are reported to be below the LOQ) but the LOQ is above the effect threshold (i.e. "PNEC used", for detailed explanation see Section 3.4.4) the available monitoring data will not be sufficient to exclude a potential risk. For these chemicals, further monitoring is needed and analytical methods should be improved to assess the real risk of the substance.

On the basis of these criteria (see Table 2), the candidate substances are divided into distinct groups:

- Substances that are sufficiently monitored and sufficiently quantified in the relevant matrix;
- Substances that are sufficiently monitored in the relevant matrix, but with a low frequency of quantification;
- Substances for which we have no or insufficient data in the NORMAN Database System or other existing datasets (labelled as "never monitored").

The definition of the terms "sufficiently / insufficiently monitored", "sufficiently / insufficiently quantified" has to be adjusted to the geographical scale of the prioritisation study. At present, for Europe-wide scale are used 100 sites, whereas 50 sites were considered sufficient for the large international river basins (see also text below).

3.4.2 Application at the EU level

The criteria and cut-off values associated with the different indicators for exposure assessment at European level are summarised in Table 2 below.

Table 2: Cut-off values associated with the different indicators used for exposure assessment in the categorisation process at the EU level

Indicators / Substances sub-groups	Analyses available in the relevant matrix(ces)	Number of countries with analyses	Number of sites with analyses	Number of sites with analyses > LOQ
Subst. suff. monitored and sufficiently quantif. in relevant matrix	Yes	≥4 countries	≥100 sites	≥50 sites
Subst. suff. monitored but with low frequency of quantification	Yes	≥4 countries	≥100sites	<50 sites
Subst. insufficiently monitored	Yes	<4 countries AND / OR <100 sites with analyses		Not relevant
Subst. never monitored (i.e. data not available in EMPODAT or other existing datasets)	Not relevant	No data	No data	No data
Subst. monitored in a "not relevant" matrix	No	Not relevant	Not relevant	Not relevant

3.4.3 Application at the national / river basin level

The "sufficiently monitored" criterion should be defined at the level of a country based on the requirements for "sufficient" level of monitoring in the national regulation. In the studies conducted in France, compounds were considered as "sufficiently monitored" when monitored at 20% of the stations of the regular monitoring network (total ca. 1500 stations). This was then the monitoring level applied for the substances on the French Watch List [5]. Based on these principles, "sufficiently monitored" substances at the national level are substances that are monitored in:

- At least 1/3 of the river basins;
- And at least 20% of the stations of the regular monitoring network.

"Sufficiently quantified" substances at the national level are substances that are quantified at:

- At least 20 – 50 stations with a risk evaluation calculated with MEC_{95} for countries / basins with a large number of stations;
- At least 10 stations with a risk evaluation calculated with MEC_{90} for countries / basins with lower monitoring coverage.

3.4.4 Risk indicator

The indicator used for the identification of potential risks in the categorisation process is based on the 95th percentile of all MEC_{max} values per site devided by the *PNEC (i.e. Exceedance of the lowest environmental threshold).* This indicator is based on the PEC/PNEC ratio concept, where PEC (Predicted Environmental Concentration) and PNEC (Predicted No-Effect Concentration) correspond in this study to MEC_{95} and Lowest PNEC, respectively. The definitions of the parameters Lowest PNEC and MEC_{95} can be found in the NORMAN Prioritisation reference document [3]. The main principles are recalled in the sections below.

Lowest PNEC

In the NORMAN methodology the Lowest PNEC for a substance refers to the lowest available PNEC value that might be derived on the basis of acute, chronic or non-standard tests and is intended as a non-legally binding threshold value for the protection of the receptors at risk in, or via, the aquatic environment. In order to be consistent with the scope of the WFD and its definition of Priority (Hazardous) Substances, both environmental risks to aquatic ecosystems and human health via the aquatic environment can be considered in the derivation of the Lowest PNEC, provided that the data are available. The Lowest PNECs are derived for the water matrix and then converted to the corresponding PNECs, for sediment and biota, depending on the relevance of the substances in those matrices / compartments.

MEC_{95}

The maximum concentration observed at a given site is referred to as measured Maximum Environmental Concentration (MEC). More specifically:

- MEC_{site} refers to the measured Maximum Environmental Concentration at one site.
- MEC_{95} refers to the 95th percentile of all MEC_{site} values, taking into account that data with real concentrations for at least 20 sites are needed for calculation of a MEC_{95} with acceptable confidence.

MEC_{site_max} refers to the measured Maximum Environmental Concentration among all sites with recent measurements (*i.e.* last 6 years). For substances that are sufficiently monitored (*i.e.* more than 4 countries and more than 100 sites) with satisfactory analytical performance (*i.e.* all LOQ values are below the Lowest PNEC), but for which there are less than 20 sites with measurements above LOQ (*i.e.* for most sites the concentration levels are below the LOQ), the MEC_{site_max} value can be used to replace MEC_{95} in the calculation of the risk ratio.

This is done in order to identify whether there is still a possible risk of exceedance of the Lowest PNEC at local level.

The justification for considering the maximum concentrations for exposure assessment at each site is to avoid underestimating the risks associated with substances released intermittently (e.g. pesticides), which have rather short-term peaks, as compared to average concentration values. As the general sampling procedure consists of monthly grab samples, an annual or quarterly average of these measurements cannot be seen as an appropriate representation of the real exposure situation. Concentrations are known to fluctuate much more, which means that even the maximum annual grab sample is highly unlikely to represent the maximum exposure situation, which is expected to have effects on the aquatic communities as shown in numerous publications [6 – 11].

The maximum concentration can also be used for substances with continuous exposure patterns, as a conservative approach. The maximum is often between 2- and 10-fold higher than the annual average in surface water. For emerging substances there are usually not enough data available to calculate a reliable annual average.

Moreover, the use of the maximum concentration values avoids the uncertainty associated with the integration of "less than" values (*i.e.* non-quantified monitoring data <LOQ) in the calculation of the PEC and allows the identification of a potential risk at each site in a worst-case scenario.

Finally, the 95th percentile of the maximum concentrations at each site (MEC_{95}) is preferred here, instead of the 90th percentile of the average concentrations (used in the DG ENV prioritisation exercises published by Fraunhofer Institute (1999) and INERIS, IOW (2009) for revision of the list of Priority Substances), because the 95th percentile allows for a more conservative approach to the identification of a potential risk.

3.4.5 Categorisation process: details by category

As explained above the substances are first divided into three main groups:

- Substances that are <u>sufficiently monitored</u> and <u>sufficiently quantified</u> in the relevant matrix;

- Substances that are sufficiently monitored in the relevant matrix, but with a low level of quantification;
- Substances that are insufficiently monitored OR "never monitored" (i.e. insufficient or no data are available in the EMPODAT database) OR the only monitoring data available correspond to a "non-relevant matrix".

The further steps to allocate the substances to six different categories can be followed directly on the decision tree (Tables 3-6). An explanation of the different categories is given below and more details can be found in the NORMAN Prioritisation reference document [3].

Category 1

Table 3: NORMAN scheme – Category 1

Category		Current scheme with target monitoring data
1	1A	Sufficiently monitored and sufficiently quantified substances for which a risk is identified
	1B	Sufficiently monitored substances, with a low level of quantification, but for which a risk is identified at the local level (i.e. MEC_{site_max} > Lowest PNEC)

Table 4: NORMAN scheme – Category 2

Category		Current scheme with target monitoring data
2	2A	Insufficiently monitored substances for which further monitoring data are needed
	2B	Sufficiently monitored substances, with a low level of quantification and poor quality data (target monitoring data), further monitoring data are needed
	2F	No occurrence data are available in EMPODAT (or other datasets) but the literature data show that the LOQs associated with existing analytical methods are lower than the Lowest PNEC

Category 3

Compounds in Category 3 are compounds which are sufficiently monitored and sufficiently quantified substances for which there are insufficient experimental ecotoxicity data for hazard assessment.

Category 4

Monitoring data show that the analytical performance need to be improved (LOQ_{min} associated with current analytical methods are above the Lowest PNEC).

Table 5: NORMAN scheme – Category 4

Category		Current scheme with target monitoring data
4	4A	Insufficiently monitored substances for which analytical methods need to be improved (LOQs associated with current analytical methods are above the Lowest PNEC)
	4B	Sufficiently monitored substances, with low level of quantification, for which analytical methods need to be improved (LOQs associated with current analytical methods are above the Lowest PNEC)
	4F	No monitoring data are available in EMPODAT (or other datasets) and no LOQ data retrieved from the literature to define whether existing analytical methods are compatible or not with the Lowest PNEC, OR Monitoring data available in EMPODAT show that the LOQs associated with the available data are above the Lowest PNEC BUT no LOQ data have been retrieved from the literature to define whether the LOQs associated with current analytical methods are above or below the Lowest PNEC

Category 5

The features of the substances in Category 5 are the same as the substances in Category 2. The only difference between Category 2 and Category 5 is that the Lowest PNEC values will be here P-PNEC values (data predicted from QSAR models).

Category 6

Table 6: NORMAN scheme – Category 6

Category		Current scheme with target monitoring data
6	6A	Sufficiently monitored and sufficiently quantified substances, with experimental ecotoxicity data, but no risk is identified
	6B	Sufficiently monitored substances, with low level of quantification, AND LOQs < Lowest PNEC AND no risk is identified (either at wide or at local level i.e. MEC_{site_max} < Lowest PNEC)

Categorisation allows water managers to focus on the next steps to be taken, e.g. (not exhaustive): (1) derivation of EQS for substances already well investigated with sufficient amount of data on their occurrence and toxicity; (2) improvement of analytical methods for substances monitored whose limits of quantification (LOQs) are higher than PNEC values; (3) additional screening when more occurrence data are needed to confirm a basin wide thread; and, (4) discontinue with monitoring of substances that are already well investigated and proved not to represent a threat to the environment.

3.4.6 Prioritisation process

Once the substances have been allocated to the various action categories, a subsequent ranking of the substances within each action category takes place. In the NORMAN framework, the prioritisation of the substances within the different categories is done by applying various exposure, hazard and risk indicators that are subsequently aggregated to a total score. For further detailed explanation on the application of these indicators we refer to the NORMAN methodology document [3].

NOTE: Since the objectives differ from one category to another, due to the data gaps that are addressed by each Category (e.g. Category 4 aims to improve the analytical performance; Category 3 requires the compilation or derivation of additional toxicity data), the prioritisation indicators may differ from one category to another as well.

3.4.7 Risk indicators used in the Danube case study

Two main indicators were applied to decide which compounds have the highest priority in terms of potential risk according to the data available:

- ***Extent of exceedance (EoE) of the Lowest PNEC*** = MEC_{95} / *Lowest PNEC*, to address the intensity of impacts.

This indicator ranks compounds with regard to the extent of the expected local effects. For the calculation of this indicator all raw data is used. All concentration data above the LOQ are pooled and used to calculate a MEC_{95}. The MEC_{95} is the 95th percentile of the measured concentrations, separately for each compound. It is recommended to have at least 20 monitoring sites to get a reliable statistical result. For the calculation, the Excel formula "QUANTIL" can be used. The MEC_{95} is then divided by the lowest PNEC to derive the "*Extent of Exceedance*". This value can consist of values below 1 and up to several thousands. Risk score (RS) is assigned as follows:

- EoE <1 → RS = 0
- 10≥ EoE ≥1 → RS = 0.1
- 100≥ EoE >10 → RS = 0.2
- 1000≥ EoE >100 → RS = 0.5
- EoE >1000 → RS = 1

For the example above, we assume that the MEC_{95} of compound A is 2 µg/l, while the MEC_{95} of compound B is 25 µg/l, due to generally higher concentrations. If the lowest PNEC in this example is 1 µg/L for both substances, the "*Extent of Exceedance*" calculates as follows:

Compound A: EoE = MEC_{95} of 2 µg/l / lowest PNEC of 1 µg/l = 2

Compound B: EoE = MEC_{95} of 25 µg/l / lowest PNEC of 1 µg/l = 25

The risk score (RS1) is then 0.1 for compound A and 0.2 for compound B.

- ***Spatial Frequency of exceedance of the Lowest PNEC***, to address the spatial aspect of exposure

  Spatial Frequency of Exceedance of the Lowest PNEC = *n / N* where:

  - *n* is the number of sites with MEC_{site}/Lowest PNEC ratios above 1
  - *N* is the total number of sites with analytical measurements for the respective compound.

This indicator considers the spatial distribution of potential effects of a certain compound, *i.e.* the frequency of sites with observations above a certain effect threshold. For the calculation of this indicator, the compound's maximum observed concentration at each site (MEC_{site}) is compared to the Lowest PNEC. Subsequently, the number of sites where the threshold was exceeded is divided by the total number of sites where the respective compound was monitored.

To give an example of the calculation, a hypothetical dataset consists of 20 sites with one sample each. In total, compound A was found 18 times, while compound B was found 12 times. The maximum concentrations of compound A exceeded the lowest PNEC at ten sites, while the maximum concentrations of compound B exceed the lowest PNEC only at 5 sites. The risk score for the indicator "Frequency of Exceedance" (RS2) calculates as follows:

Compound A: FoE = 10 sites exceeding lowest PNEC / 20 sites = 0.50

Compound B: FoE = 5 sites exceeding lowest PNEC / 20 sites = 0.25

Hence, compound B has a lower risk as compared to compound A.

This index can be applied irrespective of the number of sites with concentration above the LOQ. The resulting value indicates the share of sites where potential effects are expected and lies between 0 and 1. These values can therefore be used directly for the overall prioritisation.

Final Ranking Score

The final ranking score is then calculated by simply adding both risk scores (RSs). Please note that the maximum score is therefore a value of 2. In our example (see also text above), the ranking score calculates as follows:

Compound A: RS1 0.1 + RS2 0.50 = 0.60

Compound B: RS1 0.2 + RS2 0.25 = 0.45

In this example, compound A has a higher priority than compound B, although both compounds had the highest score in one of the two indicators. However, the relatively large distribution of compound A (50% of sites exceeded the lowest PNEC) lead to the overall higher priority.

3.5 Identification of effect-based site-specific toxicants

3.5.1 Effect-based tools (EBTs)

Target screening of emerging chemicals and the increasing availability of analytical properties and toxicity data for these chemicals from data repositories as created by large projects such as ToxCast [12] provide an enormous progress in chemical monitoring, assessment and prioritisation and thus in the identification of RBSPs. However, they are still based on known and suspected chemicals, while the majority of the tens of thousands of compounds that occur in typical environmental samples remain unconsidered. This holds also for known chemicals such as steroids, which are active at concentrations which are below typical screening analysis and are thus easily overlooked.

Effect-based tools (EBTs) are a very promising complementary approach for monitoring complex environmental contamination that detect toxic chemicals independent from their occurrence on target lists and independent from their analytical detection limits. Since the chemicals present in the environment cover a large number of modes of action (MoA) [13] and many orders of magnitudes in effect concentrations on different organisms [14] a battery of bioassays is required and has been recommended in Deliverable D12.1 and published [15]. After enrichment using for example large volume solid phase extraction [16, 17] EBTs together with trigger values [18] may be used to identify hot spots of toxic contamination and to prioritise toxic endpoints.

3.5.2 Tiered approach to identify toxicity drivers

Since EBTs as such are not able to identify the chemicals causing effects and typical environmental mixtures are extremely complex, these tools need to be combined with methods to reduce complexity and with chemical analytical tools to identify the compounds that may be causing the effect. This may be achieved with a tiered approach as provided in Deliverable D12.1 and published as an in-depth overview on effect-directed analysis (EDA) [19]. This approach may involve three different methods that are applicable in tiers respective under specific circumstances (Fig. 5).

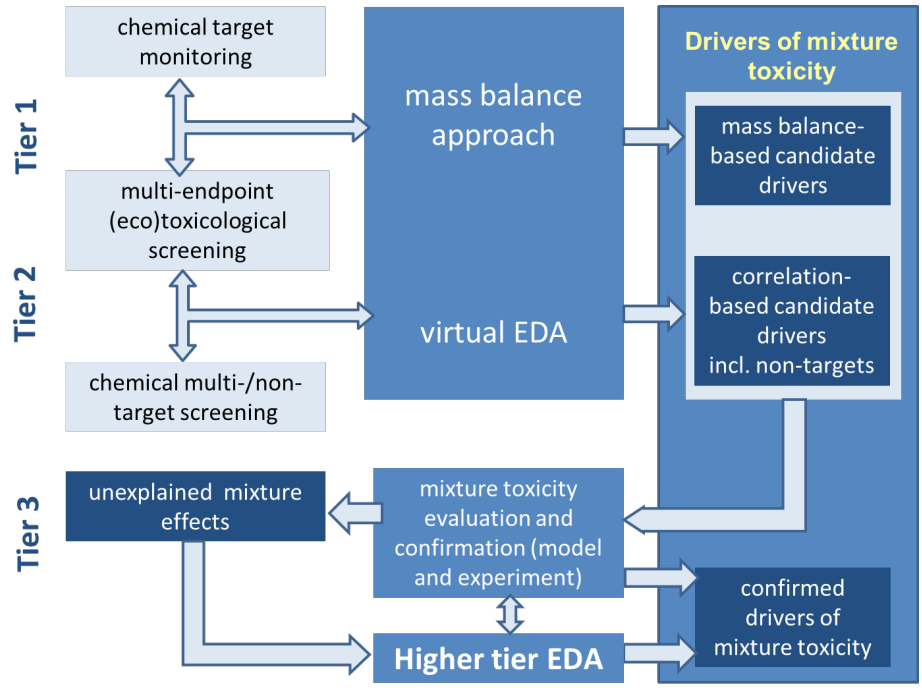


Fig. 5: Tiered approach for the effect-based identification of toxicants

3.5.3 Mass balance approach

As a first tier it is highly recommended to apply a mass balance approach based on chemical target monitoring including known toxicants relevant for the toxicological endpoints under consideration together with multi-endpoint (eco)toxicological screening using EBTs. This approach is particularly promising for endpoints for which an extensive knowledge base on candidate compounds exists. Examples may be apical endpoints such as toxicity to *Daphnia magna* as a frequently used model organism for macroinvertebrates or to algae. Chemical target analysis of known insecticides respective herbicides and biocides may provide a promis-

ing basis to explain toxicity towards these organisms. This also holds for some *in vitro* assays addressing specific MoAs such as estrogenicity and androgenicity. Mass balance approaches are based on the mixture effect model of concentration addition (CA) using Toxic Units (TUs) derived from chemical analysis (TU_{chem}) as the sum of the ratios of individual chemicals' measured concentrations and their effect concentrations. In the case of *in vitro* effects often reference compounds as positive controls are used allowing the application of Biological Equivalent Quantities (BEQs) using relative potencies (REPs) compared to the reference compounds. $\sum TU_{chem}$ and $\sum BEQ_{chem}$ are compared with TU_{Bio} and BEQ_{Bio}, respectively, derived from biotesting as the reciprocal of the relative enrichment factor (REF; Fig. 6).

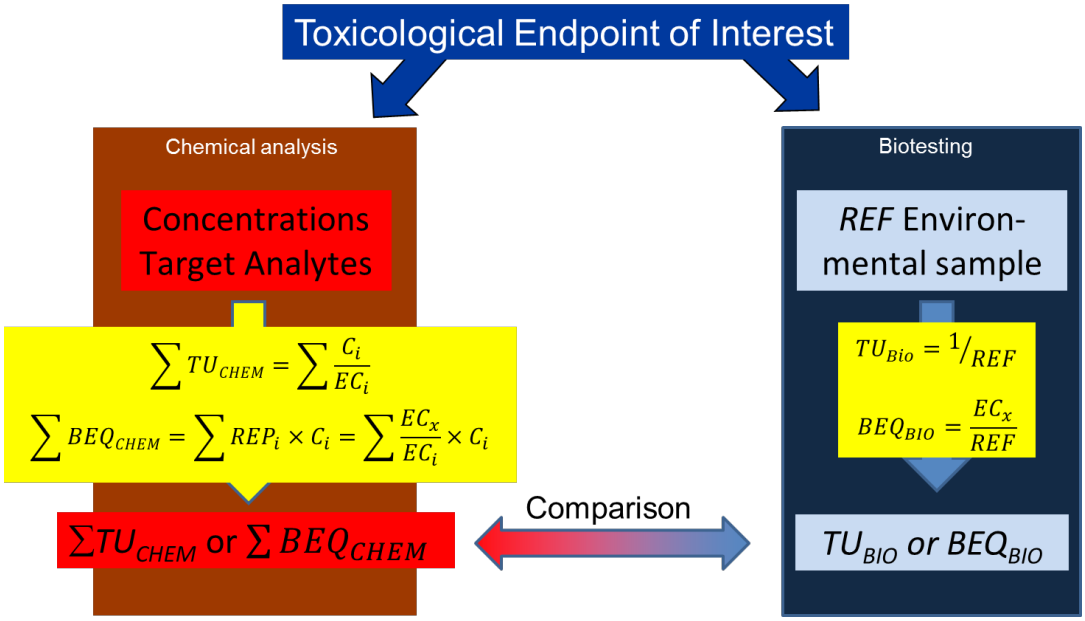


Fig. 6: Mass balance approach to link chemical target monitoring and (eco)toxicological screening/biotesting. Abbreviations: TU: Toxic Units, EC: Effect concentration for the selected effect level, REP: Relative potency compared to reference compound x, REF: Relative enrichment factor for the selected effect level.

This approach provides a quantitative measure for the contribution of individual chemicals to the overall toxicity as well as a measure for the total fraction of toxicity explained by target chemicals. An agreement of $\sum TU_{chem}$ or $\sum BEQ_{chem}$ and TU_{Bio} or BEQ_{Bio}, respectively, within the uncertainty of the method indicates that major contributors to the observed effect have been identified. In case of larger deviations, there is an indication for missing important drivers and higher tiers of identification based on non-target analysis can be applied to identify them.

In the Danube River Basin case study the mass balance approach has been applied on 22 sites sampled during the JDS3 along the whole river [20] as well as downstream of the inlet of untreated wastewater from the city of Novi Sad as one specific source of pollution in the River Danube.

In the study based on the JDS3 the focus was on six different endpoints including the activation of the arylhydrocarbon (AhR) and pregnan-X-receptor (PXR) relevant for the metabolism of xenobiotics, the activation of the estrogen receptor (ER), oxidative stress response, p 53 response as an indicator for genotoxicity, NF-κB response as an endpoint related to inflammation and fish embryo toxicity (FET). Significant fractions of biological activity could be explained with target compounds only for AhR, namely the phyto-hormone daidzein and the herbicide terbutylazine, and for ER activation with the hormone estrone and the phyto-estrogen genistein as major contributors to the measured effects. However, it should be considered that other estrogenic candidates such as estradiol and ethinylestradiol were below the detection limits but still may have contributed major fractions of estrogenicity. For all other endpoints less than 1% of the activity could be related to measured chemicals suggesting either an effect of the complex mixture or of chemicals that have not been targeted. Due to the rather unspecific nature of these endpoints a mixture effect has been considered as the most probable.

In a second attempt to apply mass balances to identify drivers of endocrine effects in extracts of water from the River Danube downstream of the wastewater inlet from Novi Sad we applied optimized target analysis for steroidal hormones and other known endocrine disruptors and could explain major fractions of the effects with our target chemicals (Fig. 7) [21]. Estrogenicity was largely explained by estrone, estriol and 17β estradiol, androgenicity was caused by testosterone, progesterone and the pharmaceutical medroxy-progesterone with some contribution of 4-androstene-3,17-dione. Anti-androgenicity could be linked to genistein, daidzein, bisphenol A, 2,4-dinitrophenol and estrone and antagonistic effects on the glucocorticoid receptor were driven by progesterone, estriol, estrone, bisphenol A and 1,2-benzisothiazolinone. In agreement with the JDS3 study oxidative stress could be explained to a minor extent with high caffeine concentrations dominating.

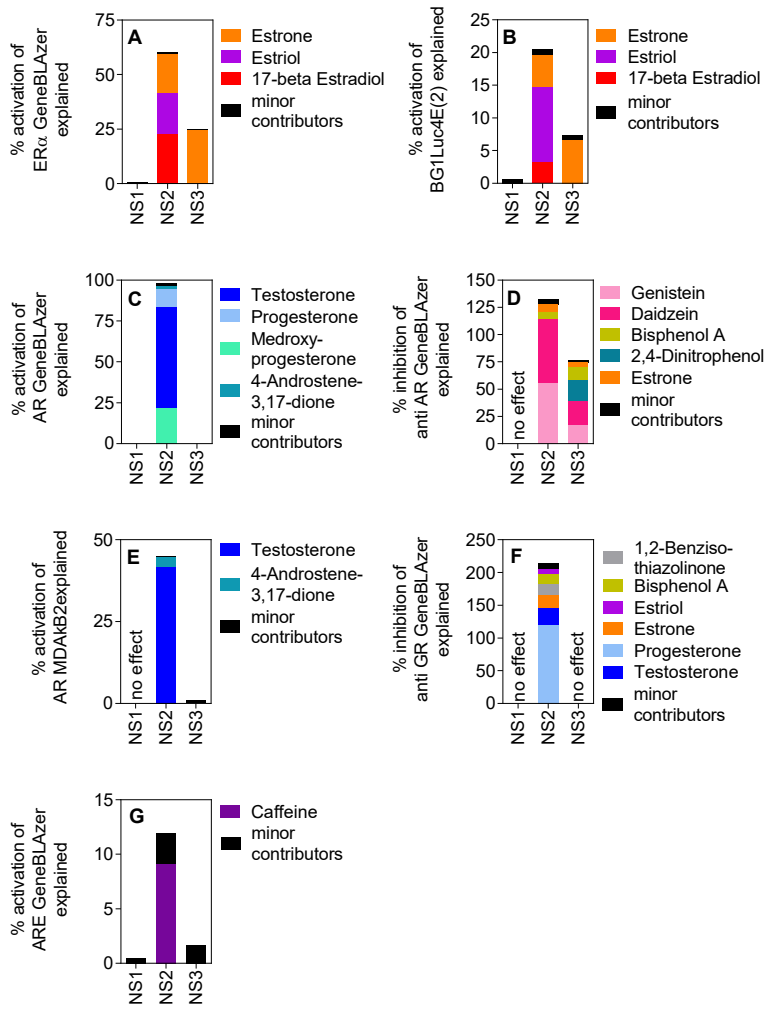


Fig. 7: Percent effect explained by individual detected chemicals for A) activation of ER (GeneBLAzer), B) activation of ERa (BG1Luc4E2)), C) activation of AR (GeneBLAzer), D)inhibition of AR (GeneBLAzer), E) activation of AR (MDA-kB2), F) inhibition of GR (GeneBLAzer) and G) oxidative stress response.

3.5.4 Virtual EDA

Virtual EDA is an approach that can involve also non-target analysis together with (eco)toxicological screening and is based on statistical correlations between chemical signals and measurable effects. It is described in detail in Deliverable D11.1. The basic concept involves a stepwise exclusion of chemicals signals that do not contribute to the explanation of effects using Partial Least Square (PLS) analysis. This approach is designed for the analysis of a larger number of samples and thus could help to provide a basis for the identification of RBSPs for

the whole river basin rather than for individual sites. We successfully applied this tool for a time series of industrial wastewater effluents [22, 23].

Virtual EDA has been also applied to the JDS3 dataset that has been used for the mass balance approach [20]. This approach confirmed the relevance of wastewater for most toxic endpoints by identifying typical marker compounds such as carbamazepine transformation products, artificial sweeteners, cotinine and others. The list of candidate chemicals identified in this study may be a basis for further confirmation. However, there are several circumstances that make virtual EDA in this case less promising:

1. For successful virtual EDA samples with sufficient variance in contamination and effects are required. Deviations by a factor of 10 and more are helpful to outweigh uncertainties of biotests and non-target screening. At the same time, causative chemicals should occur rather independent instead of just correlating with wastewater input in general and thus with all the wastewater markers that enter the river from the same sources. In the Danube River these conditions are not met for any of the investigated endpoints.
2. The toxicological endpoints that have been applied in the JDS3 study were quite unspecific (except estrogenicity). Thus, effects are probably caused by the complex mixture rather than by individual risk drivers. A link of effects to individual chemicals is not possible.

3.5.5 Higher Tier EDA

Higher tier EDA for river water is a powerful method for the identification of risk drivers that has been significantly advanced[8] and successfully demonstrated in different case studies in SOLUTIONS [24 - 26]. It is a site-specific approach typically starting with large volume solid phase extraction (LV SPE) in order to yield sufficient material for biotesting and chemical analysis. Extracts are tested with bioassays for the toxicological endpoints of concern, for example in the frame of effect-based monitoring (Fig. 8). If substantial effects are detected, the extracts are subjected to chromatographic fractionation in order to reduce the complexity of the mixture and derive fractions for further testing. Toxic fractions are either sub-fractionated or subjected to chemical analysis. This might involve target screening of known chemicals that should elute in the respective retention time window. The major focus typically is on non-target analysis in order to identify the chemicals in the toxic fractions. The procedure is com-

pleted by a confirmation step involving the analytical confirmation, effect confirmation in the respective biotest and where possible also a confirmation of effects on a higher level of biological organization [27].

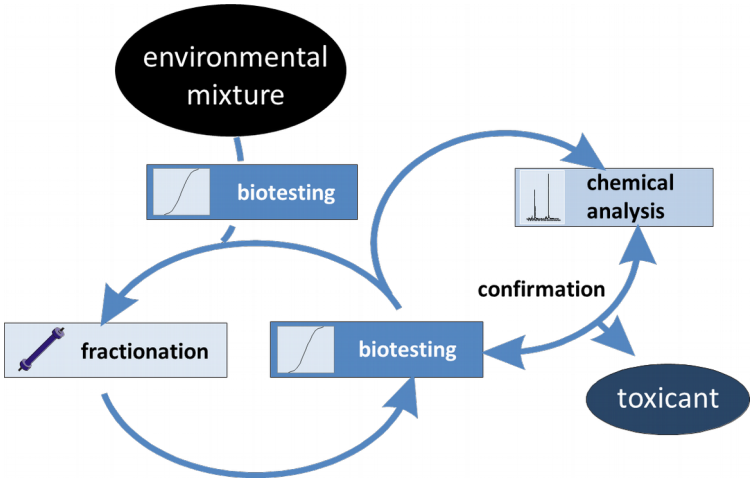


Fig. 8: Scheme of higher tier EDA [19, 28]

In the Danube River case study, higher tier EDA has been successfully applied for the confirmation of the results from the mass balance approach focusing on drivers of endocrine disruption and to further investigate drivers of oxidative stress [26]. Estriol, estrone and estradiol were confirmed as drivers of estrogenicity, while additional contributions by the contraceptive 17α-ethinylestradiol and the phytohormone genistein have been found (Fig. 9). EDA of drivers of androgenicity could confirm the contributions of testosterone, medroxyprogesterone and progesterone but additionally highlighted the contribution of dihydrotestosterone. In both cases the good agreement between the potency of the parent extract, the recombination of the fraction and the calculated sum of fractions' potencies indicate neither significant losses during fractionation nor substantial deviations from the CA mixture effect model have occurred.

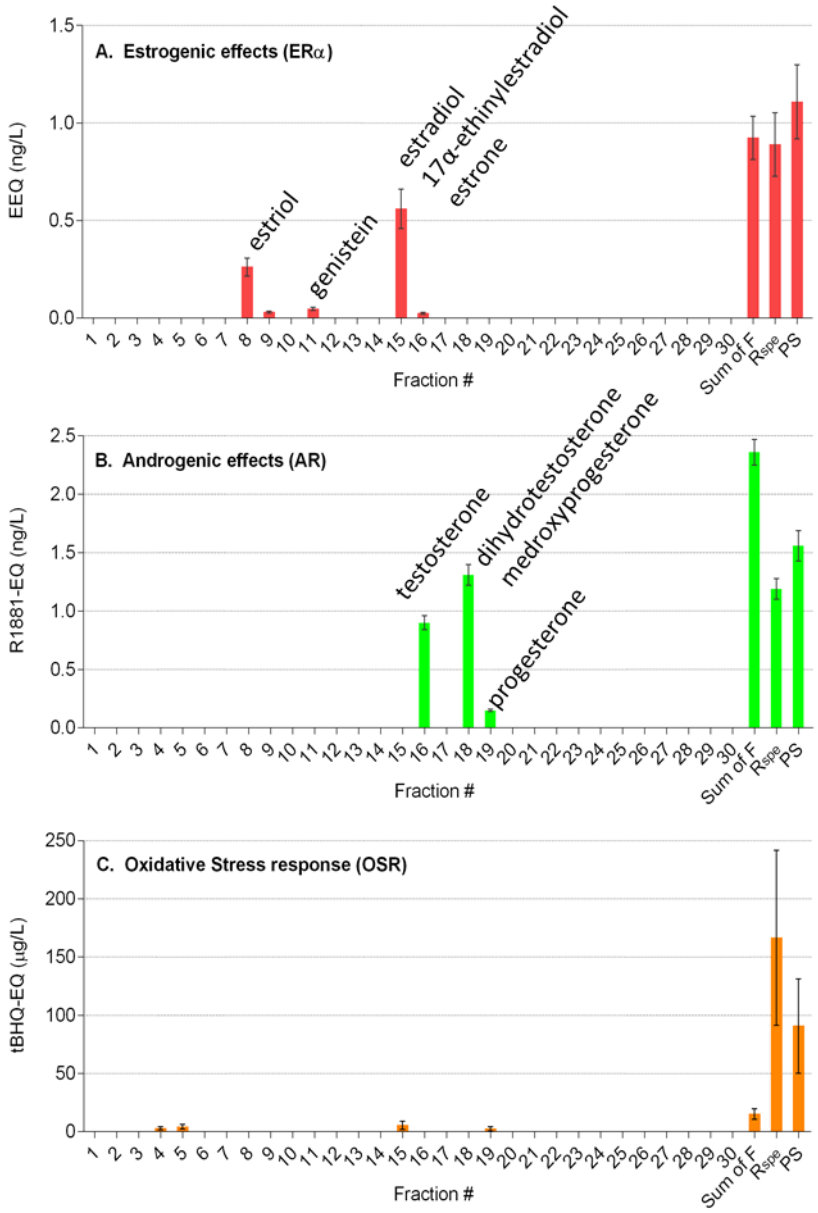


Fig. 9: Endocrine potency of fractions from Danube water extracts downstream of Novi Sad

3.6 Modeling-based exposure and risk predictions validated with case-study data on contamination and impact

3.6.1 Background

The guidance is described in more detail in the Internal Deliverable C1.5 'Modelling –based exposure and risk predictions validated with case-study data on contamination and impact'

and it is based on the model train & validation efforts described in detail in the Deliverable D14.1 ‘Modelling framework and model-based assessment for substance screening’.

3.6.2 Objectives and methodology

The main objective is to feed the Danube Case Study with a model-based prioritisation of chemicals that can serve as one of the Lines of Evidence in an overall prioritisation exercise.

Modelling offers the advantage of having more complete Predicted Environmental Concentrations (PEC) as compared to Measured Environmental Concentrations (MEC). In particular, PECs can be produced for more substances, for all rivers with full temporal coverage, while avoiding issues with analytical Limits of Detection (LOD) and Quantification (LOQ) and natural patchiness. The price we pay for that is reduced accuracy. The methodology used to deal with the reduced accuracy of PECs in a prioritisation context is the following:

- Establish appropriate accuracy limits for the PECs;
- Those substances which still cause no problems even if we increase the PEC with this error margin are “true negatives” and could be safely omitted from any further analysis;
- Those substances which still cause problems even if we decrease the PEC with this error margin are “true positives” and need to be prioritised for further analysis;
- The remaining substances cannot be conclusively classified in view of the inaccuracy of the PECs. However, they can still be ranked from *more likely to be a problem* to *less likely to be a problem*.

3.6.2 Substances considered

The prioritisation exercise has been conducted for 1835 chemicals, which satisfy the following conditions:

- Emission data and substances properties data are available to allow a simulation with the combined emission and fate and transport models;
- The substance is non-volatile (defined as having a boiling point higher than 430K);

- Ecotox data are available to allow the derivation of a PNEC.

Volatile substances have been omitted because the model validation demonstrated that the accuracy of the PECs is significantly lower for volatile substances.

3.6.3 Sites considered

The selection of sites for the simulation and assessment of PECs is theoretically unlimited. In practice however, for the results to be tangible enough, a choice needs to be made. In this case were selected the same sampling sites as those used for the MEC-based substances prioritisation taking into account 68 JDS3 sampling sites.

3.6.4 Derivation of PNECs

For the current exercise, we checked the availability of harmonised "Lowest PNEC" values from the NORMAN framework. These were available for 333 out of 1,835 chemicals. Therefore, it was needed to fill a data gap.

Here we used the widely accepted Species Sensitivity Distribution (SSD) modelling approach to derive a PNEC. This method has recently been operationalised for a much wider range of substances then what was possible before (De Zwart et al., in prep). In particular, the SSDs were used to calculate a median HC5 value for the PNEC-definition (HC5 = Hazardous Concentration for 5% of the species, based on an SSD-NOEC, an SSD model derived from No Observed Effect Concentrations of a chemical). If chronic NOEC ecotoxicology data are available for 3 or more species, the PNEC is calculated by fitting a lognormal SSD through these three data points and deriving the concentration that would affect 5% of species. We note that this approach is more conservative than taking the lowest PNEC, because for low numbers of data, the 5%tile of the fitted distribution is on average lower than the lowest data point.

If chronic NOEC ecotoxicology data for three or more species are not available, the chronic NOEC values are extrapolated from other test endpoints and an extrapolation safety factor is added to compensate for this. In particular, if there is an endpoint with three or more species

tested, we use that endpoint for extrapolation. If that is not the case, we combine all available ecotoxicity data after extrapolation to the chronic NOEC. Extrapolation factors used are listed in Table 7.

Table 7: Safety factors used for extrapolation of ecotox data to other endpoint

To / From	Order of extrapolation attempts to Chronic NOEC	Chronic NOEC Extrapolation factor
Acute EC50	3	Multiply by 1/10
Acute NOEC	2	Multiply by 1/3
Chronic EC50	1	Multiply by 1/3
Chronic NOEC	0	Multiply by 1

The procedure that was followed resulted in an estimate of the PNEC of each of the chemicals incorporated in the study.

On the basis of the above, we still expect a difference between lowest PNECs from the NORMAN framework and present PNECs set equal to HC5 levels of NOEC based SSD's. Since the NORMAN framework follows the relevant EU Guidance, it applies a safety factor of 10 on PNECs derived from chronic NOEC ecotoxicology data for 3 or more species. This safety factor is omitted in the presently used PNECs. We note that such a safety factor has a clear purpose while deriving protective EQSs, but has no apparent advantage for the ranking of chemicals in a prioritisation context. A comparison between the lowest PNECs from the NORMAN framework and present PNECs, and an analysis of differences further analysis is provided in the Results section below.

3.6.5 Risk indicators

To enhance transparency towards stakeholders and comparability between the PEC-based and the MEC-based approaches, the same risk indicators as in the MEC-based prioritisation were used (see Section 3.4.7). These are:

1. The "*Extent of Exceedance*" (EoE) to address the intensity of impact:

   the 95%tile of the PEC at all sites and all days, divided by the PNEC;

2. The "*Frequency of Exceedance*" (FoE) to address the spatial exposure aspects:

   the fraction of sites where the 95%tile of the PEC exceeds the PNEC.

These two indicators are then transformed into a risk score:

score = function(EoE) + FoE

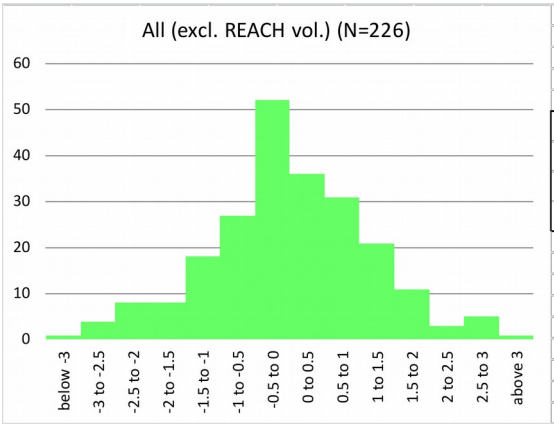
where the function of EoE is defined as in Section 3.4.7:

- EoE <1 → score = 0
- 10≥ EoE ≥1 → score = 0.1
- 100≥ EoE >10 → score = 0.2
- 1000≥ EoE >100 → score = 0.5
- EoE >1000 → score = 1

Thus, the overall score is a number in the range between 0 and 2, with higher values indicating a higher potential of an exposure situation to cause harm to ecosystems.

3.6.6 Accuracy of the PECs

Fig. 10 illustrates the accuracy of the PECs as it was established in D14.1. The bias is defined as the logarithm of the PEC/MEC ratio. This implies that a bias of 1 is equivalent to an overestimation by one order of magnitude, and a bias of -1 is equivalent to an underestimation by one order of magnitude.



N	226	100%
Abs(Bias) < 1	146	65%
Abs(Bias) < 2	204	90%
Abs(Bias) > 2	22	10%

Fig. 10: Distribution of bias obtained for individual chemicals for all validation datasets (pesticides, pharmaceuticals and REACH registered chemicals)

The results indicate that the bias is on average close to zero, but shows a quite wide distribution, with 65% of results being accurate within one order of magnitude and 90% of results ac-

curate within two orders of magnitude. For the prioritisation exercise we consider an accuracy range of two orders of magnitude.

4 List of Danube RBSPs

4.1 Preparation of an interim list of the Danube River Basin Specific Pollutants

Given the vast number of chemicals which may be released into the environment and existing time and budget constraints of all involved parties to deal with thousands of potential pollutants, there is a need to prioritise chemicals for their regulatory risk assessment and monitoring. Article 16 of the WFD sets out the strategy to reduce the chemical pollution of European waters [1]. Thereby, the chemical status assessment is used alongside the ecological status assessment to determine the overall status of a water body and to define management measures. The Directive 2013/39/EU [29] establishes EQSs, expressed as both annual average (AA) concentrations and maximum allowable concentrations (MACs) for 45 priority substances. Compliance with AA-EQSs and MAC-EQSs sets the chemical status of the water body as ‘‘good’’. Under the WFD, Member States must set quality standards (according to Annex V, 1.2.6) for ‘‘river basin specific pollutants’’ (RBSPs; listed in Annex VIII, 1–9) that are ‘‘discharged in significant quantities’’ and take action to meet those quality standards by 2015 as part of ecological status (Article 4, 11, and Annex V, 1.3 [1]. EQSs are therefore key tools in assessing and classifying both chemical and ecological status. Whether a compound is ‘‘discharged in significant quantities’’ is commonly decided based on the substance’s exposure level, referred to as PEC. This, in turn is compared to an ecological safety threshold expressed as PNEC. PEC/PNEC risk ratios above 1 would trigger the substance's consideration as RBSP and its inclusion in the routine monitoring and the derivation of a legally-binding EQS.

Despite the fact that the majority of the Danube countries have already defined their national RBSPs and related EQSs, there is no recent update of the Danube river basin-wide list of specific pollutants. The currently valid list includes only arsenic, chromium, copper and zinc without specifying their EQSs. A prioritisation methodology to select RBSPs in a wider European context, including the data from the Danube river basin, was introduced by von der Ohe et al. [30]. It was based on the methodology developed by the prioritisation working group of the NORMAN network [3]. The approach has then been applied for the prioritisation of the monitoring data from the Slovak Republic [31]. All of the prioritisation efforts run so far either at the EU, river basin or national level concluded that there is a need for more occurrence and ecotoxicity data of high quality. This has been understood also at the design of the JDS3 and one of the specific goals of the survey was to provide a complex dataset allowing

for selection of the Danube RBSPs. The aim of this activity was to prioritise among the large number of substances detected in the surface water samples during the JDS3, using the simplified NORMAN prioritisation approach [30, 31].

4.1.1 Prioritisation methodology

The NORMAN prioritisation methodology as described in Section 3.4 uses a decision tree that first classifies chemicals into six categories depending on the information available. The priority within each category is then evaluated based on several indicators, including exposure (e.g. frequency of observations above LOQs of used methods, annual usage, use pattern, etc.), hazard (e.g. Persistence, Bioaccumulation, Toxicity (PBT), Endocrine Disruption (ED) and Carcinogenicity, Mutagenicity and Reprotoxicity (CMR) properties) and risk.

Considering the specifics of the JDS3 dataset, no categorisation was run and only two risk indicators were proposed for the prioritisation of target analytes detected in surface water samples, namely the *Frequency of Exceedance (FoE)* and the *Extent of Exceedance (EoE)*, that are subsequently added to a final ranking score (values between 0 and 2; see Section 3.4.6). The surface water samples from the 68 monitoring sites have been analysed by different laboratories, using various analytical methods. Hence, multiple entries for the same site/compound combination exist. In order to aggregate them to a single measure of exposure for each sampling site, the maximum concentration from all measurements was used. The reason for this was not to bias towards substances, which have been analysed only by one laboratory.

For the calculation of the *Frequency of Exceedance (FoE)*, the maximum observed concentration at each site (MEC_{site}) is compared to the lowest PNEC. In the JDS3 case, quite often several measurements of a single compound were performed by different laboratories at the same sample using different methodologies. The maximum concentrations per compound per site were directly used to compare them with the lowest PNEC. Subsequently, the number of sites where the threshold was exceeded was divided by the total number of sites, where the respective compound was measured. Please note that the total number of 68 sites was used for all prioritised substances despite some of the substances were not determined in all samples for some analytical methods (e.g. LVSPE samples for special organic pollutants analysis taken only from 22 sites).

4.1.2 Data for prioritisation

Data on 719 target organic substances in water, sediment, SPM and biota were measured by 13 JDS3 laboratories. Out of these, 654 substances were analysed in surface water samples. All data (more than 47,000 data entries) were collected in the JDS3 specific Data Collection Templates and first stored in a JDS3 Access database developed by Environmental Institute to be later uploaded into the ICPDR Water Quality Database and SOLUTIONS Knowledge Base. The prioritisation dataset also included semi-quantitative results from target suspect screening by one of the laboratories. The prioritisation at this stage did not consider substances determined in sediments, SPM and biota matrices. It also did not take into account findings from passive sampling.

The ecotoxicity threshold (PNEC) values were either taken from the NORMAN Working Group on Prioritisation or newly derived for 189 out of 277 JDS3 substances actually determined in the samples above their respective LOQs. Substances not provided with PNEC and thus not included into the prioritisation were for the time being not considered of prior importance based on the expert judgement, which had to be applied due to the lack of time to collect all needed information. It was planned to continue with deriving PNECs for all JDS3 target substances and re-run the prioritisation when completed.

4.1.3 Prioritisation results

First results of the prioritisation of the Danube RBSPs are presented in Table 8. Altogether 20 substances exceeded the PNEC values at more than 1% of the investigated (68) sites. Considering that benzo(a)pyrene together with other polyaromatic hydrocarbons (benzo(g,h,i)perylene and indeno(1,2,3-c,d)pyrene), fluoranthene and PFOS are already regulated (and thus will have to be monitored by all Danube countries) the list is showing additional 17 pollutants of potential basin-wide concern. 2,4-Dinitrophenol, chloroxuron, bromacil, dimefuron, diazinon, linuron, metazachlor and bentazon represent a general class of pesticides causing exceedances of ecotoxicological limit values across the basin. A quality check of the database for 2,4-dinitrophenol and respective safety factors revealed that the revised PNEC value is significantly higher and therefore this substance was no longer ranked as a potential Danube RBSP in the follow up studies. Transformation products of pesticides atrazine

(2-hydroxy atrazine) and terbutylazine (desethylterbutylazine) exceeded the lowest PNEC value at 76 and 79% of the investigated sites, respectively. Amoxicillin, 17beta-estradiol and diclofenac were among the pharmaceuticals to be considered of importance. The latter two substances were already included in the proposal for update of the EQS Directive [32] and finally not considered for inclusion among the WFD priority substances with the justification that more evidence on their occurrence in European surface waters is needed. Both substances are on the EU Watch List of substances included in the national monitoring programmes [33, 34]. The widely discussed plasticiser bisphenol A was found in surface water samples from 30 sites of which the newly proposed lowest PNEC of 0.1 µg/l was exceeded at ten sites (e.g. 1.94 µg/l downstream Olt; JDS52). A new class of biocides represents fipronil, which exceeded the PNEC value at the JDS58 (Arges).

4.1.4 Conclusions regarding the interim list of the Danube RBSPs

The analysis of a large amount of organic substances during the JDS3 enabled us to provide suggestions for the update of the Danube RBSPs. The applied prioritisation methodology using a modified NORMAN approach produced a list of 20 substances suggested as relevant for the DRB based on the results of the JDS3 target screening of 654 substances in the Danube water samples by 13 laboratories. PNEC values were available for 189 out of 277 JDS3 substances actually determined in the samples. The cut off criteria to include a compound in the list was its exceedance of the ecotoxicological threshold value (PNEC or EQS) at minimum of one JDS3 site. The list contains five WFD priority substances (three PAHs, fluoranthene and PFOS) and two EU Watch List candidate compounds (17-beta-estradiol, diclofenac). The ‘top ten’ substances were (i) the pesticides 2,4-dinitrophenol (removed later on from the list due to unreliable PNEC), chloroxuron, bromacil, dimefuron, diazinon and transformation products of widely used atrazine and terbuthylazine, (ii) polyfluorinated substance PFOS, (iii) the plasticiser bisphenol A and polyaromatic hydrocarbon benzo(g,h,i)perylene.

The update of the Danube RBSPs has been presented in the 2015 Update of the Danube RBMP (https://www.icpdr.org/main/activities-projects/river-basin-management-plan-update-2015) not only as a fulfilled requirement of the provisions of the WFD but also as an excellent practical example of a science-to-policy interface.

Table 8: Results of the prioritisation of pollutants determined in the JDS3 surface water samples

No.	Substance	CAS No.	No. of sites substance detected	C_{max}[1]	MEC_{95}[2]	Lowest PNEC/EQS [µg/L]	Key study	Type	EoE[3]	EoE score	FoE[4]	Final score
1	2,4-Dinitrophenol (DNP)*	51-28-5	68	0.06	0.04	0.001	RIVM 2014	EQS chronic water[5]	40	0.2	1.00	1.20
2	PFOS (Perfluorooctansulfonate)	1763-23-1	63	0.026	0.02	0.00065	EU 2013	EQS chronic water[5]	31	0.2	0.93	1.13
3	Chloroxuron	1982-47-4	65	0.04	0.02	0.0024	James et al. 2009	PNEC acute	8.3	0.1	0.93	1.03
4	Desethylterbutylazine	30125-63-4	54	0.028	0.01	0.0024	RIVM 2014	EQS chronic water[5]	4.2	0.1	0.79	0.89
5	2-hydroxy atrazine	2163-68-0	53	0.06	0.02	0.002	Ecostat 2013	EQS chronic water[5]	10	0.1	0.76	0.86
6	Bromacil	314-40-9	31	0.19	0.14	0.01	INERIS 2013	EQS chronic water[5]	14	0.2	0.46	0.66
7	Dimefuron	34205-21-5	58	0.041	0.04	0.008	Oekotoxzentrum 2014	EQS chronic water[5]	5.0	0.1	0.56	0.66
8	Bisphenol A	80-05-7	30	1.94	1.03	0.1	Nendza 2003	EQS chronic water[5]	10	0.2	0.16	0.36
9	Benzo(g,h,i)perylene	191-24-2	65	0.029	0.003	0.002	CEC 2008	EQS chronic water[5]	1.5	0.1	0.26	0.36
10	Diazinon	333-41-5	21	0.009	0.01	0.001	Management Team PPDB 2009	PNEC acute	10	0.1	0.12	0.22
11	Indeno(1,2,3-c,d)pyrene	193-39-5	15	0.005		0.002	CEC 2008	EQS chronic water[5]			0.19	0.19
12	Linuron	330-55-2	32	1.42	1.12	0.26	Oekotoxzentrum 2014	EQS chronic water[5]	4.3	0.1	0.07	0.17
13	Amoxicillin	26787-78-0	33	0.28	0.08	0.078	van der Aa et al. 2011	PNEC chronic	1.0	0.1	0.03	0.13
14	Metazachlor	67129-08-2	30	0.03	0.02	0.019	INERIS 2014	EQS chronic water[5]	1.1	0.1	0.03	0.13
15	17 beta-estradiol	50-28-2	8	0.029		0.0004	CEC 2011	EQS chronic water[5]			0.12	0.12
16	Benzo(a)pyrene	50-32-8	3	0.002		0.00017	EU 2013	EQS chronic water[5]			0.04	0.04
17	Diclofenac	15307-79-6	51	0.318	0.036	0.05	Oekotoxzentrum 2014	EQS chronic water[5]			0.04	0.04
18	Bentazon	25057-89-0	61	0.1	0.02	0.06	USEPA 2008	PNEC acute			0.01	0.01
19	Fipronil	120068-37-3	1	0.02		0.012	EU 2011	EQS chronic water[5]			0.01	0.01
20	Fluoranthene	206-44-0	58	0.02	0.006	0.0063	EU 2013	EQS chronic water[5]			0.01	0.01

1 C_{max} – Maximum concentration in µg/L reported in case the substance has been measured by several JDS3 laboratories

2 MEC_{95} – 95th percentile of the Maximum Environmental Concentration in µg/L; calculated only if the substance has been found above LOQ at minimum 20 sites

3 EoE – Extent of Exceedance

4 FoE – Frequency of Exceedance

5 Equal to Annual Average EQS (AA-EQS)

*The PNEC revised in a follow up study, the substance removed from the list of potential RBSPs.

4.2 Preparation of the Final list of the Danube River Basin Specific Pollutants

At the SOLUTIONS General Assembly in September 2016 an agreement was made that as a follow up to the Danube RBSPs list presented in the 2015 Update of the Danube RBMP a revised list of Danube RBSPs will be provided to the ICPDR after incorporating (i) additional monitoring data from the DRB, (ii) evidence from the effect-based identification of site-specific toxicants, and (iii) evidence from an application of the integrated SOLUTIONS modelling approach to the JDS3 sampling sites. This final list of the Danube RBSPs has been developed using the following approach:

1. The JDS3 dataset extended for additional monitoring data from countries in the Danube basin (>410,000 data entries) was used as a basis. Extensive curation of the monitoring data has been carried out to exclude outliers. However, more effort should be put into this, possibly together with the data providers from the Danube countries. Unfortunately, many of the statistically outlying values were related to PAHs and therefore none of the PAHs are in the final list of RBSPs (Table 9). An agreement of the data providers would be needed to remove/correct the outlying values.

2. A distribution of all substances with monitoring data in the DRB collected in the SOLUTIONS Knowledge Base into respective categories and their prioritisation within the categories is given in Annex 1. The final list of the Danube RBSPs (Category 1) is presented in Table 9. This list has been approved by the Monitoring and Assessment Expert Group of the ICPDR to be used for the preparation of the Fourth Joint Danube Survey in 2019 and for preparation of the 2021 Update of the Danube RBMP. Additional evidence from the application of novel effect-based and modelling approaches will be taken into consideration for defining the final list of substances to be screened in the DRB.

After a first analysis of the available data, the initial list of 195 candidate substances was reduced to address only the substances for which at least one exceedance was identified from the available data. Substances for which no exceedance of the PNEC was identified and for which the LOQ_{max} was below the Lowest PNEC were stored separately and not considered further in this study. Looking in more detail into the list of candidate compounds which were selected for further assessment, an overview of the range of the min – max values for the various metadata used in this study, is reported in Table 10.

Table 10: Minimum and maximum values for metadata used in the prioritisation process

Metadata	Min value	Max value
Years	2011	2017
No. of Countries	1	17
No. of Stations	1	424
No. of Analyses	1	410000
No. of Stations with data > LOQ	1	169
No. of Analyses with data > LOQ	0	8451
LOQ_{min}	0,000001 µg/l	0,2 µg/l
LOQ_{max}	0,001 µg/l	2 µg/l
Lowest PNEC	0,0001 µg/l	7,8 µg/l
MEC_{95}	0,0057 µg/l	15,5 µg/l
$MEC_{site\ max}$	0,0091 µg/l	283 µg/l
Risk ratio	0,1	42,3

4.2.1 Discussion

The NORMAN methodology was applied with the main aim of identifying the priority candidate RBSPs for the DRB, *i.e.* selecting those substances for which there is already sufficient evidence from current monitoring data that they are present in many sites of the river basin (widespread presence) at concentration levels which may lead to a risk for the aquatic ecosystems. In line with the terminology applied in the NORMAN Prioritisation framework these substances will be referred to as “Category 1” substances in the text. Based on the result of this prioritisation study, it is possible to provide proposals as regards substances that need further monitoring (Category 2 and Category 5), substances that need improvement of the performance of analytical methods (Category 4), substances that need improved ecotoxicity testing for assessment of their hazardous character (Category 3). Finally, some substances for which current monitoring data do not provide sufficient evidence of a potential risk for the ecosystems are also highlighted (Category 6). For these chemicals, monitoring efforts could be reduced, unless new ecotoxicity studies (including studies on non-standard endpoints) would show evidence of effects, in which case these conclusions should be revised and the compounds would go back to Category 3 for further assessment.

Table 9: Results of the prioritisation of pollutants (Category 1) in the Danube River Basin based on available data in the SOLUTIONS Knowledge Base. WFD priority substances are depicted in grey colour.

No.	Substance	CAS No.	No. of countries Measuring	Position prioritisation 2014[1]	No. of sites with measurements	No. of sites with concentrations >LoQ	MEC_{site} [2]	MEC_{95} [3]	Lowest PNEC [µg/L]	Reference key study	EoE[4]	Score EoE	Score FoE[5]	Final score
1	Arsenic - dissolved	7440-38-2	7	DRBSP	68	68	5.3	3.57	0.83	INERIS SPAS	4.3	0.1	1.00	1.10
2	PFOS	1763-23-1	10	2	77	70	0.026	0.021	0.00065	2013/39/EU	32.3	0.2	0.90	1.10
3	Chloroxuron	1982-47-4	8	3	71	65	0.04	0.022	0.0024	INERIS (COMPPS II)	9.2	0.1	0.89	0.99
4	Caffeine	58-08-2	10	-	77	76	4	0.7	0.1	SOLUTIONS 2017	7.0	0.1	0.84	0.94
5	Bromacil	314-40-9	7	6	68	31	0.19	0.15	0.01	INERIS 2013	15.0	0.2	0.46	0.66
6	Copper - dissolved	7440-50-8	7	DRBSP	68	68	283	5.6	1.6	INERIS SPAS	3.5	0.1	0.51	0.61
7	Diazinon	333-41-5	10	10	79	29	0.012	0.011	0.001	Footprint	11.0	0.2	0.35	0.55
8	Carbamazepine	298-46-4	10	-	77	77	0.2	0.1	0.05	SOLUTIONS 2017	2.0	0.1	0.27	0.37
9	Metolachlor	51218-45-2	9	-	87	87	2.2	1.04	0.07	INERIS SPAS	14.9	0.2	0.17	0.37
10	Zinc - dissolved	7440-66-6	7	DRBSP	68	68	61	11.65	7.8	INERIS SPAS	1.5	0.1	0.19	0.29
11	Metazachlor	67129-08-2	9	14	76	38	0.29	0.23	0.02	OZ 2016	11.5	0.2	0.08	0.28
12	Nickel (Ni) - dissolved	7440-02-0	7	PS	68	68	230	15.5	4	2013/39/EU	3.9	0.1	0.15	0.25
13	Lead - dissolved	7439-92-1	7	PS	68	68	8.1	1.73	1.2	2013/39/EU	1.4	0.1	0.12	0.22
14	Desethylterbutylazine	30125-63-4	11	4	170	140	0.27	0.15	0.03	INERIS SPAS	5.0	0.1	0.10	0.20
15	Linuron	330-55-2	8	12	79	36	1.4	1.05	0.26	OZ 2015	4.0	0.1	0.08	0.18
16	Diclofenac	15307-86-5	11	17	77	61	0.32	0.069	0.05	OZ 2014	1.4	0.1	0.06	0.16
17	Tebuconazole	107534-96-3	9	-	82	54	1.3	0.32	0.24	OZ 2016	1.3	0.1	0.06	0.16
18	Isoproturon	34123-59-6	9	PS	100	99	17	0.35	0.3	2013/39/EU	1.2	0.1	0.06	0.16
19	Bisphenol A	80-05-7	10	8	160	74	1.9	0.75	0.2	JRC PS dossier	3.8	0.1	0.04	0.14
20	Amoxicillin	26787-78-0	7	13	68	33	0.28	0.081	0.078	van der Aa et al., 2011	1.0	0.1	0.03	0.13

[1] See Table 8

[2] MEC_{site} – Maximum concentration in µg/L

[3] MEC_{95} – 95th percentile of the Maximum Environmental Concentration in µg/L; calculated only if the substance has been found above LOQ at minimum 20 sites

[4] EoE – Extent of Exceedance

[5] FoE – Frequency of Exceedance

Substances in Category 1 and 6

As explained above, the compounds reported in Table 9 refer to Category 1 and correspond to compounds for which we have sufficient evidence of exposure (i.e. > 4 countries and > 50 sites with monitoring data and quantified data in > 20 sites) and sufficient evidence of a potential risk, based on the calculation of the risk ratio MEC_{95} / Lowest PNEC (where the Lowest PNEC is supported by experimental ecotoxicity data of sufficient quality). They are proposed as Danube RBSPs.

The substances reported in Category 6 correspond to substances for which the risk ratio MEC_{95} / Lowest PNEC is below 1, which indicate that the concentration levels in which they occur in the river do not represent a widespread risk for the ecosystem. However, it is important to notice that some of these compounds, such as PFOA, appear as frequently quantified in the monitored sites. Although the risk ratio calculated as a result of a MEC_{95} concentration can be considered as sufficiently conservative, yet the fact that the substance is frequently measured above the LOQ should be considered and the additional hazardous properties should be taken into account for possible re-evaluation of the necessary actions for this compound. Please note that for some compounds the PNEC is still exceeded, indicating at least local risks.

Chromium (VI) was discussed as a candidate compound for the update of the EU Watch List [34]. Further to the most recent conclusions, chromium (VI) is no longer proposed for inclusion in the 2nd Watch List. As reported in the Watch List Report [33], the JRC's assessment of the new monitoring data (received before January 2018) together with the data from the 2014 prioritisation does not support the idea that chromium (VI) would be posing a risk in freshwaters. However, chromium (VI) could be considered for inclusion in the 3rd Watch List in transitional and coastal waters, after confirmation of the PNEC via consultation with the WG Chemicals and after collection and analysis of any additional existing monitoring data for these categories of water. Furthermore, the JRC reviewed the ecotoxicological data available not only for chromium (VI) but also for chromium (III). This led to an update for the PNEC in freshwaters of 2.06 µg/l and 1.8 µg/l for chromium (VI) and chromium (III) respectively. The PNEC value reported in Annex 1 should therefore be updated. To be noted that if the PNEC value is updated the risk ratio for chromium in the Danube will be > 1. The data for the calculation of the risk ratio should be verified before confirmation of the final category for this metal.

Substances in Category 2

Category 2 consist of compounds for which either there were less than 4 countries and / or less than 50 sites with analysis available (insufficiently monitored compounds). Allocated to this category were also compounds that are sufficiently monitored but for which the quality of the non-quantified data is not sufficient (*i.e.* not all LOQs associated to non-quantified data are below the Lowest PNEC values). This is the case for 17-beta-estradiol which was part of the first Watch List and it is confirmed to remain on the 2nd EU watch List because of insufficient quality of the data (LOQ > Lowest PNEC).

Azithromycine was part of the first Watch List and it is confirmed to remain on the 2nd EU Watch List because of reduced quality of the non-quantified samples for some countries[1]. In the assessment made in the DRB it appears that azithromycine was sufficiently monitored (8 countries and 73 sites) and with good quality data (LOQ always below the PNEC values), but it was quantified only at 2 sites in 1 country. This would lead to the conclusion that azithromycine is not a priority compound for further monitoring actions in the DRB. It has been allocated to Category 2 but it might also be considered to suggest this substance for further assessment only in the country in which the substance was quantified (1 site with exceedance was observed) and shift it to Category 6.

Substances in Category 3

All seven listed substances were frequently present in samples across the basin in more than four countries. In case their proposed PNECs would be confirmed, all compounds would be candidates for Category 1 (RBSPs). However, MECsite was exceeded only for flupentixol and trimipramine at 50 and 27 sites, respectively.

Substances in Category 4

A clear need for improvement of analytical methodologies for certain substances of this category was identified. For example, even for the priority substance dichlorovos with an EQS 0.6 ng/l, no laboratory had a suitable method established.

[1] Please note also that the JRC has updated the PNECs for azithromycine on the basis of the most recent ecotoxicological data, which led to a change in the maximum acceptable limit of detection for this substance.

When comparing results of prioritisation based only on JDS3 data with those coming from an extended dataset, 10 out of the top 20 candidate RBSPs were included in both lists. In addition, 4 substances from the JDS3 prioritisation set were categorized in Category 2 (17-beta-estradiol, fipronil), Category 3 (2-hydroxy atrazine) and Category 6 (fluoranthene). Three PAHs (benzo(g,h,i)perylene, indeno(1,2,3-c,d)pyrene and benzo(a)pyrene), ranked among the top 20 in JDS3 prioritisation, were not considered since the obtained datasets contained a high amount of outlying values. The compounds are on the list of the WFD priority substances and therefore should be monitored in the DRB anyway. Updated PNECs for dimefuron, bentazone and 2,4 – dinitrophenol caused that these substances are not in the final list of candidate RBSPs.

4.3 Site specific toxicants identified by EDA in the area of Novi Sad, Serbia

As described in Section 3.5, effect-based identification of site-specific toxicants focused on the Danube River downstream of Novi Sad. The area is on the one hand sufficiently highly contaminated in order to be able to detect chemicals such as steroids, which are active at concentrations that often fall below the analytical detection limits and, on the other hand, are believed to be representative for many sites in the lower Danube where untreated wastewater enters the river. Endocrine disruptors are believed to provide a particular risk under these circumstances since most steroidal hormones retained to a significant degree in WWTPs but can display their full endocrine disrupting potency if treatment is missing. The compounds that have been identified as drivers of endocrine disruption *in vitro* downstream of Novi Sad are summarized in Table 11.

Table 11: Drivers of endocrine disruption identified in the River Danube downstream of Novi Sad

No.	Compound	Effect
1	Estrone	estrogenicity, antiestrogenicity, antiglucocorticoid [21, 26]
2	Estriol	estrogenicity [21,26]
3	17β-estradiol	estrogenicity [21,26]
4	17α-ethinylestradiol	estrogenicity [21,26]
5	Daidzein	estrogenicity, anti-androgenicity [21,26]
6	Genistein	estrogenicity, anti-androgenicity [21,26]
7	Bisphenol A	anti-androgenicity, anti-glucocorticoid [26]
8	2,4-Dinitrophenol	anti-androgenicity [26]
9	Testosterone	androgenicity, anti-glucocorticoid [21,26]
10	Dihydrotestosterone	androgenicity [21]
11	Progesterone	androgenicity, anti-glucocorticoid [21,26]
12	Medroxy-progesterone	androgenicity [21,26]
13	4-Androstene-3,17-dione	androgenicity [26]
14	1,2-Benzisothiazolinone	anti-glucocorticoid [26]

4.4 Proposal of RBSPs identified by modelling-based approaches

Simulations of the combined emission and fate and transport models were carried out for 1835 chemicals, the success rate is compiled in Table 12. This table shows that >97% of simulations provided technically correct results. We note that “Incorrect” in this respect means that the simulation was showing numerical problems, which means that the output does not correctly relate to the input.

Table 12: Statistics of PEC simulation success rate in the DRB

	Total	Crashes	Incorrect	Reliable
Danube	1835	8	39	1788

4.4.1 Results for the Danube Basin District

The results (top 100 ranking substances after prioritisation) are summarised in Annex 3. A full list of 1835 prioritised substances is provided in Deliverable 1.5. The results give a clear guidance on:

1. Which chemicals can anyhow be excluded from further assessment? (“true negatives”)

- Order all chemicals on the Risk Score assuming that the model underestimates the PEC by 2 orders of magnitude (“Score (PEC*100)”).
- Filter out all simulations which are not correct.

- This provides 1220 chemicals with a risk score of 0.
- These chemicals are not expected to cause any problem, regardless of the model accuracy.

2. Which chemicals can anyhow be classified as “high priority”? (“true positives”)

- Order all chemicals on the Risk Score assuming that the model overestimates the PEC by 2 orders of magnitude (“Score (PEC/100)”).
- Filter out all simulations which are not correct.
- This provides only 1 chemical with a risk score higher than 0 **(CAS 541-02-6, decamethylcyclopentasiloxane).**
- By inspection of the FoE, we conclude that this chemical exceeds the PNEC at at least 12% of sites, regardless of the model accuracy, and can be prioritised anyhow.

3. How to rank the remaining chemicals?

- Order all chemicals:
  - first on the Risk Score assuming that the model correctly simulates the PEC (“Score (PEC)”);
  - next on the Risk Score assuming that the model underestimates the PEC by a factor of 100 (“Score (PEC*100)”).
- Filter out all simulations which are not correct.
- The “Score (PEC)” now provides the ranking, while the numbers in “Score (PEC/100)” and “Score (PEC*100)” show the expected range of the score if the model inaccuracy is considered.

There is obviously not a clear overlap between the ‘PEC-based’ (modelling) and ‘MEC-based’ (monitoring) lists of substances. This is a matter of concern and the substances prioritised by the modelling tools should be included systematically in the suspect screening (part of the non-target screening workflow) of the samples from the DRB.

5 Conclusions

One of the key tasks of SOLUTIONS was to support an implementation of the WFD in an international river basin district through coming up with a proposal of candidate Danube RBSPs. The project provided a substantial contribution to the ICPDR by analysing the samples collected in the frame of the JDS3 for a wide range of hazardous substances. Analysing this large amount of organic substances and the follow-up data processing enabled SOLUTIONS experts to provide first suggestions for the update of the Danube RBSPs. This list was presented in the 2015 Update of the Danube RBMP (https://www.icpdr.org/main/activities-projects/river-basin-management-plan-update-2015) and was contributed significantly to addressing the Danube Significant Water Management Issue “Hazardous substances” in river

basin management planning. Further activities within SOLUTIONS using the extended Danube dataset led to the refinement of the list of the Danube RBSPs which will be monitored during the fourth Joint Danube Survey in 2019/20. This finalised and verified list of RBSPs will then be published in the 2021 Update of the Danube RBMP. Several pollutants were identified as candidates to extend the list of 20 Danube RBSPs using modelling tools and approaches of identifying site-specific toxicity drivers.

The results of SOLUTIONS in the field of identifying the Danube RBSPs can be considered not only as a support to fulfilling requirements of the provisions of the WFD but also as an excellent tangible example of a science-to-policy interface driven by the European Commission. Appreciating the significant inputs by SOLUTIONS into the river basin management activities in the Danube River Basin the ICPDR at its 20th Ordinary Meeting in December 2017 adopted the following resolution:

The ICPDR appreciates the substantial support the SOLUTIONS project continuously provides to the ICPDR, in particular with the development of the list of Danube River Basin Specific Pollutants during and after the JDS3 and welcomes the ongoing cooperation on the preparation of the JDS4.

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Annex 1: Results of the final prioritisation of the Danube River Basin Specific Pollutants

Substance	CAS No.	No. of countries with measurements	Position prioritisation 2014	No. of countries with concentrations >LoQ	No. of sites	No. of sites with concentrations >LoQ	Category	LoQ min	LoQ max	No. of sites where MEC-site>PNEC	MECsite Max	95th MECsite	Lowest PNEC	Reference key study	PNEC type	Species	AF	Extent of Exceedence	Score EoE	Score FoE	Final score
Arsenic - dissolved	7440-38-2	7	DRBSP	7	68	68	1	0.03	0.03	68	5.3	3.57	0.83	INERIS SPAS	QSeco	Scondary poisoning	300	4.3	0.1	1.00	1.10
PFOS	1763-23-1	10	2	9	77	70	1	0.001	0.01	69	0.026	0.021	0.00065	2013/39/EU	EQS chronic water (=AA-EQS)	-	-	32.3	0.2	0.90	1.10
Chloroxuron	1982-47-4	8	3	7	71	65	1	0.0017	0.0027	63	0.04	0.022	0.0024	INERIS (COMPPS II)	PNEC acute	*Selenastrum capricornutum*	1000	9.2	0.1	0.89	0.99
Caffeine	58-08-2	10	-	10	77	76	1	0.001	0.08	65	4	0.7	0.1	SOLUTIONS 2017	PNEC chronic	*fish*	10	7.0	0.1	0.84	0.94
Bromacil	314-40-9	7	6	6	68	31	1	0.0015	0.0015	31	0.19	0.15	0.01	INERIS 2013	V-PNEC	*Selenastrum capricornutum*	1000	15.0	0.2	0.46	0.66
Copper - dissolved	7440-50-8	7	-	7	68	68	1	0.027	0.027	35	283	5.6	1.6	INERIS SPAS	Qseco (before 2009)	-	-	3.5	0.1	0.51	0.61
Diazinon	333-41-5	10	10	8	79	29	1	0,.01	0.023	28	0.012	0.011	0.001	Footprint	PNEC acute	-	-	11.0	0.2	0.35	0.55
Carbamazepine	298-46-4	10	-	10	77	77	1	0.00015	0.02	21	0.2	0.1	0.05	SOLUTIONS 2017	PNEC chronic	-	10	2.0	0.1	0.27	0.37
Metolachlor	51218-45-2	9	-	9	87	87	1	0.0017	0.05	15	2.2	1.04	0.07	INERIS SPAS	V-PNEC	*Selenastrum capricornutum*	10	14.9	0.2	0.17	0.37
Zinc - dissolved	7440-66-6	7	DRBSP	7	68	68	1	0.04	0.04	13	61	11.65	7.8	INERIS SPAS	V-PNEC	BLM PNEC (EU RAR-based)	not applicable	1.5	0.1	0.19	0.29
Metazachlor	67129-08-2	9	14	6	76	38	1	0.0027	0.05	6	0.29	0.23	0.02	OZ 2016	EQS chronic water (=AA-EQS)	-	-	11.5	0.2	0.08	0.28
Nickel - dissolved	7440-02-0	7	PS	7	68	68	1	0.04	0.04	10	230	15.5	4	2013/39/EU	EQS chronic water (=AA-EQS)	-	-	3.9	0.1	0.15	0.25
Lead - dissolved	7439-92-1	7	PS	7	68	68	1	0.009	0.009	8	8.1	1.73	1.2	2013/39/EU	EQS chronic water (=AA-EQS)	-	-	1.4	0.1	0.12	0.22
Desethylterbutylazine	30125-63-4	11	4	10	170	140	1	0.001	0.05	9	0.27	0.15	0.03	INERIS SPAS	P-PNEC exp.	*Pseudokirchneriella subcapitata*	1000	5.0	0.1	0.10	0.20
Linuron	330-55-2	8	12	6	79	36	1	0.0016	0.024	6	1.4	1.05	0.26	OZ 2015	EQS chronic water (=AA-EQS)	-	-	4.0	0.1	0.08	0.18
Diclofenac	15307-86-5	11	17	10	77	61	1	0.00086	0.05	5	0.32	0.069	0.05	OZ 2014	EQS chronic water (=AA-EQS)	-	-	1.4	0.1	0.06	0.16
Tebuconazole	107534-96-3	9	-	8	82	54	1	0.001	0.05	5	1.3	0.32	0.24	OZ 2016	EQS chronic water (=AA-EQS)	-	-	1.3	0.1	0.06	0.16
Isoproturon	34123-59-6	9	PS	9	100	99	1	0.0003	0.05	6	17	0.35	0.3	2013/39/EU	EQS chronic water (=AA-EQS)	-	-	1.2	0.1	0.06	0.16
Bisphenol A	80-05-7	10	8	10	160	74	1	0.001	0.1	7	1.9	0.75	0.2	JRC PS dossier	PNEC chronic	*Salmo trutta*	10	3.8	0.1	0.04	0.14
Amoxicillin	26787-78-0	7	13	5	68	33	1	0.003	0.003	2	0.28	0.081	0.078	van der Aa et al., 2011	PNEC chronic	Crustacae	10	1.0	0.1	0.03	0.13
Chlorothalonil	1897-45-6	1	-	1	1	1	2	0.045	0.045	1	0.81	0	0.06	Aquire 156339	PNEC chronic	Thalassiosira pseudonana	10	0.0	0	1.00	1.00
Dicamba	1918-00-9	1	-	1	16	16	2	0.29	0.64	13	2.4	0	0.13	INERIS SPAS	V-PNEC	-	-	0.0	0	0.81	0.81
Dimethenamid	87674-68-8	4	-	2	19	14	2	0.001	0.001	7	0.57	0	0.2	INERIS SPAS	V-PNEC	-	-	0.0	0	0.37	0.37
2-Phenylphenol	90-43-7	1	-	1	3	3	2	0.003	0.003	1	0.51	0	0.36	INERIS 2013	P-PNEC exp.	*Pimephales promelas*	100	0.0	0	0.33	0.33
17-beta-Estradiol	50-28-2	9	15	9	76	9	2	0.0003	0.003	8	0.029	0	0.0004	OZ 2011	EQS chronic water (=AA-EQS)	-	-	0.0	0	0.11	0.11
4-terc-Octylphenol	140-66-9	3	PS	3	291	21	3	0.003	0.05	5	0.5	0.5	0.10	2013/39/EU	EQS chronic water (=AA-EQS)			5.0	0.1	0.02	0.12
Fipronil	120068-37-3	8	19	8	34	9	2	0.0001	0.018	3	0.02	0	0.00077	UBA 2017	RAC	-	-	0.0	0	0.09	0.09
Azithromycin	83905-01-5	8	-	1	73	2	2	0.00047	0.001	1	0.37	0	0.019	OZ 2015	EQS chronic water (=AA-EQS)	-	-	0.0	0	0.01	0.01
Ibuprofen	15687-27-1	10	-	8	32	2	2	0.01	0.15	1	0.013	0	0.011	OZ 2016	EQS chronic water (=AA-EQS)	-	-	0.0	0	0.03	0.03
Flupentixol	2709-56-0	7	-	7	68	68	3	0.003	0.003	50	0.63	0.5	0.082	ToxTram QSAR	P-PNEC	*Selenastrum capricornutum*	1000	6.1	0.1	0.74	0.84
Trimipramine	739-71-9	7	-	7	66	63	3	0.003	0.003	27	0.69	0.36	0.17	ToxTram QSAR	P-PNEC	*Selenastrum capricornutum*	1000	2.1	0.1	0.41	0.51
Iopromide	73334-07-3	5	-	5	21	21	3	-	-	3	0.7	0.3	0.14	ToxTram QSAR	P-PNEC	*Selenastrum capricornutum*	1000	2.1	0.1	0.14	0.24
Clozapine	5786-21-0	9	-	7	76	47	3	0.00025	0.0036	8	0.33	0.072	0.037	ToxTram QSAR	P-PNEC	*Selenastrum capricornutum*	1000	1.9	0.1	0.11	0.21
CP 47,497	70434-82-1	7	-	7	68	38	3	0.003	0.003	6	0.51	0.46	0.15	ToxTram QSAR	P-PNEC	*Selenastrum capricornutum*	1000	3.1	0.1	0.09	0.19
6-Deisopropylatrazine	1007-28-9	9	-	6	419	42	3	0.001	1	1	4.2	1.76	0.39	ToxTram QSAR	P-PNEC	*Selenastrum capricornutum*	1000	4.5	0.1	0.00	0.10
Atrazine-2-hydroxy	2163-68-0	7	5	7	53	53	3	-	-	6	0.06	0.02	0.01	JRC PS dossier	PNEC	-	-	2.0	0.1	0.11	0.21
Nicosulfuron	111991-09-4	1	-	1	19	13	4	0.052	0.052	13	0.87	0	0.0087	INERIS SPAS	V-PNEC	-	-	0.0	0	0.68	0.68
Deltamethrin	52918-63-5	1	-	1	5	1	4	0.012	0.024	1	0.0091	0	0.0001	INERIS SPAS	V-PNEC	-	-	0.0	0	0.20	0.20
Dichlorvos	62-73-7	9	PS	2	76	2	4	0.001	0.05	2	0.011	0	0.0006	2013/39/EU	EQS chronic water (=AA-EQS)	-	-	0.0	0	0.03	0.03
Diatrizoate	117-96-4	1	-	1	1	1	5	-	-	1	0.5	0	0.073	ToxTram QSAR	P-PNEC	*Selenastrum capricornutum*	1000	0.0	0	1.00	1.00
Iohexol	66108-95-0	4	-	4	7	7	5	-	-	5	1.1	0	0.18	ToxTram QSAR	P-PNEC	*Selenastrum capricornutum*	1000	0.0	0	0.71	0.71
Terbutylazine CGA 324007	309923-18-0	2	-	2	2	2	5	-	-	1	0.1	0	0.065	ToxTram QSAR	P-PNEC	Selenastrum capricornutum	1000	0.0	0	0.50	0.50
Lauryl diethanolamide	120-40-1	6	-	2	26	3	5	0.028	0.028	1	2	0	0.95	ToxTram QSAR	P-PNEC	*Selenastrum capricornutum*	1000	0.0	0	0.04	0.04
CP 47,497-C8 homologue	70434-92-3	6	-	6	45	45	5	0.003	0.003	1	0.12	0.024	0.08	ToxTram QSAR	P-PNEC	Selenastrum capricornutum	1000	0.3	0	0.02	0.02
alpha-Hydroxyalprazolam	37115-43-8	7	-	7	68	68	5	0.003	0.003	1	0.16	0.15	0.16	ToxTram QSAR	P-PNEC	Selenastrum capricornutum	1000	0.9	0	0.01	0.01
Nitrazepam	146-22-5	7	-	7	68	44	5	0.003	0.003	1	0.92	0.058	0.49	ToxTram QSAR	P-PNEC	Selenastrum capricornutum	1000	0.1	0	0.01	0.01
Olanzapine	132539-06-1	7	-	7	68	57	5	0.003	0.003	1	0.19	0.02	0.054	ToxTram QSAR	P-PNEC	Selenastrum capricornutum	1000	0.4	0	0.01	0.01
Perfluorooctanoic acid	335-67-1	11		11	161	150	6	0.000001	0.006	0	0.053	0.025	0.3	UBA 2009	PNEC drinking water	-	-	0.1	0	0.00	0.00
Chromium - dissolved	7440-47-3	7	DRBSP	7	68	68	6	0.1	0.1	3	67	2.46	3.4	INERIS SPAS	QSeco	-	-	0.7	0	0.04	0.04
Cadmium - dissolved	7440-43-9	7	PS	7	68	64	6	0.01	0.01	2	1.1	0.11	0.25	2013/39/EU	EQS chronic water (=AA-EQS)	-	-	0.4	0	0.03	0.03
Fluoranthene	206-44-0	8	20	8	73	60	6	0.001	0.002	1	0.02	0.0057	0.0063	2013/39/EU	EQS chronic water (=AA-EQS)	-	-	0.9	0	0.01	0.01
Propiconazole	60207-90-1	9	-	7	78	22	6	0.001	0.05	1	1.2	0.11	1	UBA 2017	EQS chronic water (=AA-EQS)	-	-	0.1	0	0.01	0.01
Desethylatrazine	6190-65-4	11	-	11	161	145	6	0.001	0.006	1	0.036	0.025	0.03	INERIS SPAS	P-PNEC exp.	-	-	0.8	0	0.01	0.01
Methyl-1H-benzotriazole (mix of isomers 4- and 5-)	29385-43-1	5	-	5	22	21	6	0.011	0.011	0	0.093	0.075	0.1	SOLUTIONS 2017	PNEC chronic	*Daphnia magna*	100	0.8	0	0.00	0.00
Di(2-ethylhexyl)phthalate (DEHP)	117-81-7	7	PS	7	68	31	6	0.2	0.2	0	0.84	0.52	1.3	2013/39/EU	EQS chronic water (=AA-EQS)	-	-	0.4	0	0.00	0.00

Annex 2: Sampling protocol for sampling WWTPs effluents used in the Danube case study

The required sampling protocol required the following:

General information	
Name of the WWTP	
Country	
Town	
Postal code	
Street address	
Name of the compiler	
Position of the compiler	

Basic WWTP data	
Connection rate	Population: Population equivalents:
Specific connections besides population (industry, hospital, commercial buildings, etc., please describe)	
Collection system	☐ separated sewer system ☐ combined sewer system ☐ trucks
Treatment type	☐ mechanical ☐ carbon removal ☐ nitrification ☐ denitrification ☐ biological P-removal ☐ P-removal by precipitation ☐ other specific technology (please specify):
Annual average daily wastewater discharge	m^3/day
Annual average BOD_5 concentration	Influent: g/m^3 Effluent: g/m^3

Annual average COD or TOC concentration	Influent:	g/m^3	Effluent:	g/m^3
Annual average NH4-N concentration	Influent:	g/m^3	Effluent:	g/m^3
Annual average NO3-N concentration	Influent:	g/m^3	Effluent:	g/m^3
Annual average TN concentration	Influent:	g/m^3	Effluent:	g/m^3
Annual average PO4-P concentration	Influent:	g/m^3	Effluent:	g/m^3
Annual average TP concentration	Influent:	g/m^3	Effluent:	g/m^3
Annual average flow rate of the recipient	m^3/s			
Extreme flow rates of the recipient	Minimum:	m^3/s	Maximum:	m^3/s
Observed performance problems in WWTP operation (please describe)				

Sampling conditions		
Sampling period	Starting day:	Ending day:
Number of daily samples	Organic parameters: Heavy metals: General parameters:	
Sampling method	☐ flow proportional automatic sampling ☐ time proportional automatic sampling ☐ time proportional manual sampling ☐ random manual sampling	
Duration of freezing (organic parameter samples)	days	
Freezing temperature	°C	
Duration of cooling (heavy metal samples)	days	
Cooling temperature	°C	

General sampling results (during the seven days campaign)		
Indication of wet weather conditions *0: no rainfall, 1: little*	Day 1: Day 3:	Day 2: Day 4:

rainfall (<10 mm/day), 2: significant rainfall (10-40 mm/day), 3: extreme rainfall (>40 mm/day)	Day 5:		Day 6:		
	Day 7:				
Daily wastewater inflow rates	Day 1:	m^3/day	Day 2:	m^3/day	
	Day 3:	m^3/day	Day 4:	m^3/day	
	Day 5:	m^3/day	Day 6:	m^3/day	
	Day 7:	m^3/day			
Estimated share of groundwater infiltration in daily wastewater inflow rates	Day 1:	%	Day 2:	%	
	Day 3:	%	Day 4:	%	
	Day 5:	%	Day 6:	%	
	Day 7:	%			
Daily average effluent wastewater temperature	Day 1:	°C	Day 2:	°C	
	Day 3:	°C	Day 4:	°C	
	Day 5:	°C	Day 6:	°C	
	Day 7:	°C			
Daily average effluent wastewater pH value	Day 1:		Day 2:		
	Day 3:		Day 4:		
	Day 5:		Day 6:		
	Day 7:				
BOD_5 concentration of the daily sample*	Day 1:	g/m^3	Day 2:	g/m^3	
	Day 3:	g/m^3	Day 4:	g/m^3	
	Day 5:	g/m^3	Day 6:	g/m^3	
	Day 7:	g/m^3			
TOC concentration of the daily sample*	Day 1:	g/m^3	Day 2:	g/m^3	
	Day 3:	g/m^3	Day 4:	g/m^3	
	Day 5:	g/m^3	Day 6:	g/m^3	
	Day 7:	g/m^3			
NH4-N concentration of the daily sample*	Day 1:	g/m^3	Day 2:	g/m^3	
	Day 3:	g/m^3	Day 4:	g/m^3	
	Day 5:	g/m^3	Day 6:	g/m^3	
	Day 7:	g/m^3			
NO3-N concentration of the daily sample*	Day 1:	g/m^3	Day 2:	g/m^3	
	Day 3:	g/m^3	Day 4:	g/m^3	
	Day 5:	g/m^3	Day 6:	g/m^3	
	Day 7:	g/m^3			
TN concentration of the daily sample*	Day 1:	g/m^3	Day 2:	g/m^3	
	Day 3:	g/m^3	Day 4:	g/m^3	
	Day 5:	g/m^3	Day 6:	g/m^3	
	Day 7:	g/m^3			

PO4-P concentration of the daily sample*	Day 1:	g/m^3	Day 2:	g/m^3
	Day 3:	g/m^3	Day 4:	g/m^3
	Day 5:	g/m^3	Day 6:	g/m^3
	Day 7:	g/m^3		
TP concentration of the daily sample*	Day 1:	g/m^3	Day 2:	g/m^3
	Day 3:	g/m^3	Day 4:	g/m^3
	Day 5:	g/m^3	Day 6:	g/m^3
	Day 7:	g/m^3		
pH value of the daily sample*	Day 1:		Day 2:	
	Day 3:		Day 4:	
	Day 5:		Day 6:	
	Day 7:			
Conductivity of the daily sample*	Day 1:	µS/cm	Day 2:	µS/cm
	Day 3:	µS/cm	Day 4:	µS/cm
	Day 5:	µS/cm	Day 6:	µS/cm
	Day 7:	µS/cm		
Methods/standards for analysing the routine parameters and for analytical quality data assurance (please describe)				
Any specific performance conditions in WWTP operation (please describe)				

* data for 7 days are preferred but at least two days are the minimum requirement

Annex 3: Top hundred ranking substances from the modelling-based prioritisation of 1835 substances in the DRB

No.	CAS Number	Name	Emissions?	Simulation correct?	Score (PEC/100)*	Score (PEC/10)*	Score (PEC)*	Score (PEC*10)*
1	541-02-6	Decamethylcyclopentasiloxane	3_REACH	TRUE	0.22	1.20	1.50	2.00
2	106-47-8	4-Chloroaniline	3_REACH	TRUE	0.00	1.04	1.20	1.50
3	80-05-7	4,4'-Isopropylidenediphenol	3_REACH	TRUE	0.00	0.23	1.20	1.50
4	120-12-7	Anthracene	3_REACH	TRUE	0.00	0.19	1.20	1.50
5	70356-09-1	1-[4-(1,1-Dimethylethyl)phenyl]-3-(4-methoxyphenyl)propane-1,3-dione	3_REACH	TRUE	0.00	0.17	1.20	1.50
6	62-53-3	Aniline	3_REACH	TRUE	0.00	0.04	1.10	1.20
7	2243-62-1	1,5-Naphthylenediamine	3_REACH	TRUE	0.00	0.00	1.10	1.20
8	80-15-9	A,α Dimethylbenzyl hydroperoxide	3_REACH	TRUE	0.00	0.00	1.10	1.20
9	98-82-8	Cumene	3_REACH	TRUE	0.00	0.00	1.09	1.20
10	106-50-3	P-Phenylenediamine	3_REACH	TRUE	0.00	0.00	1.04	1.20
11	122-39-4	Diphenylamine	3_REACH	TRUE	0.00	0.00	1.04	1.20
12	5598-13-0	Chlorpyrifos-methyl	2_Pest	TRUE	0.00	0.04	1.03	1.20
13	7287-19-6	Prometryn	2_Pest	TRUE	0.00	0.32	0.88	1.34
14	1897-45-6	Chlorothalonil	2_Pest	TRUE	0.00	0.00	0.85	1.13
15	950-37-8	Methidathion	2_Pest	TRUE	0.00	0.00	0.84	1.20
16	86479-06-3	Hexaflumuron	2_Pest	TRUE	0.00	0.00	0.79	1.11
17	52-68-6	Trichlorfon	2_Pest	TRUE	0.00	0.00	0.75	1.20
18	34256-82-1	Acetochlor	2_Pest	TRUE	0.00	0.00	0.73	1.04
19	142-90-5	Dodecyl methacrylate	3_REACH	TRUE	0.00	0.01	0.60	1.20
20	87392-12-9	S-metolachlor	2_Pest	TRUE	0.00	0.00	0.60	1.20
21	872-05-9	Dec-1-ene	3_REACH	TRUE	0.00	0.00	0.44	1.20
22	112-53-8	Dodecan-1-ol	3_REACH	TRUE	0.00	0.00	0.29	1.20
23	2921-88-2	Chlorpyrifos	2_Pest	TRUE	0.00	0.01	0.28	1.14
24	101-54-2	N-(4-Aminophenyl) aniline	3_REACH	TRUE	0.00	0.00	0.28	0.95
25	41859-67-0	Bezafibrate	1_Pharma	TRUE	0.00	0.00	0.26	0.82
26	79538-32-2	Tefluthrin	2_Pest	TRUE	0.00	0.00	0.25	1.19
27	2642-71-9	Azinphos-ethyl	2_Pest	TRUE	0.00	0.03	0.23	1.20
28	111-88-6	Octane-1-thiol	3_REACH	TRUE	0.00	0.00	0.23	1.20
29	3689-24-5	Sulfotep	2_Pest	TRUE	0.00	0.00	0.23	1.20
30	103-90-2	Paracetamol	1_Pharma	TRUE	0.00	0.00	0.23	1.20
31	95-51-2	2-Chloroaniline	3_REACH	TRUE	0.00	0.00	0.23	1.20
32	10311-84-9	Dialifos	2_Pest	TRUE	0.00	0.01	0.22	1.20
33	123-31-9	Hydroquinone	3_REACH	TRUE	0.00	0.00	0.22	1.20
34	108-95-2	Phenol	3_REACH	TRUE	0.00	0.00	0.22	1.20
35	2135-17-3	Flumetasone	3_REACH	TRUE	0.00	0.00	0.22	1.20
36	13121-70-5	Cyhexatin	2_Pest	TRUE	0.00	0.00	0.19	0.71
37	2439-35-2	2-(Dimethylamino)ethyl acrylate	3_REACH	TRUE	0.00	0.00	0.17	1.20
38	51-21-8	Fluorouracil	1_Pharma	TRUE	0.00	0.00	0.17	1.20
39	56-38-2	Parathion	2_Pest	TRUE	0.00	0.00	0.17	1.20
40	84-65-1	Anthraquinone	3_REACH	TRUE	0.00	0.00	0.17	1.20

41	112-02-7	Cetrimonium chloride	3_REACH	TRUE	0.00	0.00	0.16	1.20
42	540-97-6	Dodecamethylcyclohexasiloxane	3_REACH	TRUE	0.00	0.00	0.16	1.20
43	613-62-7	2-(Phenylmethoxy)naphthalene	3_REACH	TRUE	0.00	0.00	0.16	1.20
44	96-76-4	2,4-di-tert-Butylphenol	3_REACH	TRUE	0.00	0.00	0.06	1.10
45	128-37-0	2,6-di-tert-Butyl-p-cresol	3_REACH	TRUE	0.00	0.00	0.06	1.09
46	70124-77-5	Flucythrinate	2_Pest	TRUE	0.00	0.00	0.06	0.98
47	333-41-5	Diazinon	2_Pest	TRUE	0.00	0.00	0.06	0.86
48	793-24-8	N-1,3-Dimethylbutyl-N'-phenyl-p-phenylenediamine	3_REACH	TRUE	0.00	0.00	0.04	1.10
49	102-09-0	Diphenyl carbonate	3_REACH	TRUE	0.00	0.00	0.04	1.09
50	112-41-4	Dodec-1-ene	3_REACH	TRUE	0.00	0.00	0.04	0.76
51	112-72-1	Tetradecanol	3_REACH	TRUE	0.00	0.00	0.04	0.57
52	1073-69-4	(4-Chlorophenyl) hydrazine	3_REACH	TRUE	0.00	0.00	0.03	1.10
53	98-54-4	4-tert-Butylphenol	3_REACH	TRUE	0.00	0.00	0.03	1.10
54	101-77-9	4,4'-Methylenedianiline	3_REACH	TRUE	0.00	0.00	0.03	1.10
55	1570-64-5	4-Chloro-o-cresol	3_REACH	TRUE	0.00	0.00	0.03	1.10
56	123-30-8	4-Aminophenol	3_REACH	TRUE	0.00	0.00	0.03	1.10
57	298-02-2	Phorate	2_Pest	TRUE	0.00	0.00	0.03	1.00
58	106-51-4	P-Benzoquinone	3_REACH	TRUE	0.00	0.00	0.03	0.61
59	40487-42-1	Pendimethalin	2_Pest	TRUE	0.00	0.00	0.03	0.25
60	2032-65-7	Methiocarb	2_Pest	TRUE	0.00	0.00	0.03	1.06
61	15972-60-8	Alachlor	2_Pest	TRUE	0.00	0.00	0.03	0.70
62	134523-00-5	Atorvastatin	1_Pharma	TRUE	0.00	0.00	0.03	0.50
63	60168-88-9	Fenarimol	2_Pest	TRUE	0.00	0.00	0.03	0.61
64	67564-91-4	Fenpropimorph	2_Pest	TRUE	0.00	0.00	0.01	1.10
65	95-53-4	O-Toluidine	3_REACH	TRUE	0.00	0.00	0.01	1.10
66	145783-14-8	4,6-Dichloro-5-nitro-2-(propylthio)pyrimidine	3_REACH	TRUE	0.00	0.00	0.01	1.00
67	2996-92-1	Trimethoxyphenylsilane	3_REACH	TRUE	0.00	0.00	0.01	0.91
68	22224-92-6	Fenamiphos	2_Pest	TRUE	0.00	0.00	0.01	0.89
69	60111-54-8	3,3-bis[(Dimethylvinylsilyl)oxy]-1,1,5,5-tetramethyl-1,5-Divinyl-trisiloxane	3_REACH	TRUE	0.00	0.00	0.01	0.53
70	112-18-5	Dodecyldimethylamine	3_REACH	TRUE	0.00	0.00	0.01	0.38
71	102851-06-9	Tau-fluvalinate	2_Pest	TRUE	0.00	0.00	0.01	0.25
72	7786-34-7	Mevinphos	2_Pest	TRUE	0.00	0.00	0.01	0.20
73	886-50-0	Terbutryn	2_Pest	TRUE	0.00	0.00	0.01	0.81
74	41083-11-8	Azocyclotin	2_Pest	TRUE	0.00	0.00	0.01	0.25
75	99105-77-8	Sulcotrione	2_Pest	TRUE	0.00	0.00	0.01	0.19
76	3209-22-1	1,2-Dichloro-3-nitrobenzene	3_REACH	TRUE	0.00	0.00	0.00	1.10
77	2312-35-8	Propargite	3_REACH	TRUE	0.00	0.00	0.00	1.10
78	576-26-1	2,6-Xylenol	3_REACH	TRUE	0.00	0.00	0.00	1.10
89	120-83-2	2,4-Dichlorophenol	3_REACH	TRUE	0.00	0.00	0.00	1.10
80	106-49-0	P-Toluidine	3_REACH	TRUE	0.00	0.00	0.00	1.10
81	131983-72-7	Triticonazole	2_Pest	TRUE	0.00	0.00	0.00	1.09
82	67129-08-2	Metazachlor	2_Pest	TRUE	0.00	0.00	0.00	1.07
83	95-54-5	O-Phenylenediamine	3_REACH	TRUE	0.00	0.00	0.00	1.07
84	77-99-6	Propylidynetrimethanol	3_REACH	TRUE	0.00	0.00	0.00	1.03
85	636-30-6	2,4,5-Trichloroaniline	3_REACH	TRUE	0.00	0.00	0.00	0.97
86	86-50-0	Azinphos-methyl	2_Pest	TRUE	0.00	0.00	0.00	0.92

87	626-43-7	3,5-Dichloroaniline	3_REACH	TRUE	0.00	0.00	0.00	0.86
88	114-07-8	Erythromycin	1_Pharma	TRUE	0.00	0.00	0.00	0.81
89	110-65-6	But-2-yne-1,4-diol	3_REACH	TRUE	0.00	0.00	0.00	0.78
90	95465-99-9	Cadusafos	2_Pest	TRUE	0.00	0.00	0.00	0.69
91	24279-39-8	2,6-Dichloro-4-trifluoromethylaniline	3_REACH	TRUE	0.00	0.00	0.00	0.69
92	58-08-2	Caffeine	1_Pharma	TRUE	0.00	0.00	0.00	0.69
93	41198-08-7	O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate | Profenofos	3_REACH	TRUE	0.00	0.00	0.00	0.63
94	98-83-9	2-Phenylpropene	3_REACH	TRUE	0.00	0.00	0.00	0.61
95	108-80-5	Cyanuric acid	3_REACH	TRUE	0.00	0.00	0.00	0.60
96	78-97-7	Lactonitrile	3_REACH	TRUE	0.00	0.00	0.00	0.60
97	29964-84-9	Isodecyl methacrylate	3_REACH	TRUE	0.00	0.00	0.00	0.57
98	118-60-5	2-Ethylhexyl salicylate	3_REACH	TRUE	0.00	0.00	0.00	0.56
99	68855-18-5	Heptanoic acid, ester with 2,2-dimethyl-1,3-propanediol	3_REACH	TRUE	0.00	0.00	0.00	0.44
100	95-82-9	2,5-Dichloroaniline	3_REACH	TRUE	0.00	0.00	0.00	0.39

** PEC – predicted environmental concentration; for details see Section 4.3*