Deliverable 1.2

Assessment of available knowledge on aquatic pollutants

Date: February 2023

The AquaticPollutantsTransNet partners have received funding from BMBF, ANR and SRC within the 2020 Transfer Project Call, implemented under the ERA-NET Cofund AquaticPollutants of the Joint Programming Initiatives (JPIs) on Water, Oceans and Antimicrobial Resistance (AMR).

Version History

WP	1. Demand for knowledge and knowledge gaps on aquatic pollutants from key water stakeholders
Task	1.2. Current state of knowledge on aquatic pollutants
Responsible partner	BRGM
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Reviewers	Kaitlyn Carter
Versions	1. Draft submitted to coordinator – 19.07.2023 2. Revisions after first review – 17.08.2023 3. Addition of German SoA – 13.10.2023 / 22.01.2024 4. Addition of Swedish SoA – 3010.2023 5. Revisions after second review – 02.10.2024 6. Revision after final review – 08.02.2024

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1. Introduction

Within the framework of ERA-NET Cofund AquaticPollutants, the three Joint Programming Initiatives (JPIs) on Water, Oceans and Antimicrobial Resistance (AMR) collaborated to implement a joint transnational call for research and innovation projects on risks posed to human health and the environment by pollutants and pathogens present in the water resources. In 2021, 18 research projects gathering researchers from the freshwater, marine and health sectors communities were selected under the 2020 Joint Transnational AquaticPollutants Call in order to develop multidisciplinary and practical solutions for the provision of safe drinking water and healthy aquatic environments (AquaticPollutants Funded Projects Booklet, 2021 - AquaticPollutants booklet — Water challenges for a changing world (waterjpi.eu)).

The AquaticPollutantsTransNet (TransNet) project aims to increase the impact of research results obtained by these 18 AquaticPollutants projects through effective knowledge, transfer, targeted dissemination, exploitation and communication. To enhance the transfer of the results from the AquaticPollutants projects to key water stakeholders, it is necessary to identify WHAT (knowledge) we need to transfer to WHO (relevant stakeholders). This is the objective of the first step of the TransNet project, which consists in identifying knowledge demands and knowledge gaps from key water stakeholders in the field of aquatic pollutants. This step is split into 3 main actions:

1) Profiling end-user groups and their knowledge demands (Deliverable 1.1);

2) Assessing the available knowledge and

3) Assessing the knowledge gaps (Deliverable 1.3).

This report deals with the second action, the "***assessment of the available knowledge"***, through identifying (expected) knowledge outputs from the 18 funded projects.

1.1. Deliverable Objectives

The main objectives of this report are to concisely present the AquaticPollutants projects' outputs and to position them with respect to existing scientific state of the art (SoA) in a way that is relevant for knowledge end-users (as identified in D1.1) as well as for the research projects themselves. By gathering information from scientific literature, past and current national and international research projects, and various scientific networks, not only can we establish an overview of the SoA, but we can also identify unanswered research questions or existing knowledge gaps in the SoA and align these to the expected outputs of the AquaticPollutants projects. The expected outputs are also discussed in relation to their expected impacts for different end-users. Identifying key water stakeholder target groups – policy, science, industry and society – is paramount in order to facilitate the knowledge transfer from the AP projects, which is the goal of the TransNet project.

1.2. Deliverable Structure

Chapter 2 explains the scope of the ERA-NET Cofund AquaticPollutants, focusing on the 18 research & innovation projects and the topics they address. Within this scope of AquaticPollutants, the context for this report is also highlighted. The methodology used to gather the state of the art (SoA) pertaining to CECs, AMR and pathogens is then explained, including the identification of the SoA themes and the subsequent data collection. **Chapter 3** lists the various sources used to construct the SoA summary: 1) SoA sections from the research projects' proposals and 2) projects and initiatives from international and national programs/projects. More detailed information from Chapter 3 is provided in *Annex A: European and National Supplemental Information*.

The SoA is therefore first explained in **Chapter 4**, which is structured by the themes identified in Chapter 2. The chapter is structured in that the SoA from literature (provided by the AP projects and via own desk research) and the SoA from national and international initiatives/programs is summarised and unanswered questions or knowledge gaps are identified. At the end of each section and/or sub-section in Chapter 4, the contributions from the AP projects to the respective themes is presented in call-out boxes.

Chapter 5 looks at the planned outputs from the AP projects, classifying them by type of output and framing them within the context of the unanswered SoA questions. **Chapter 6** provides an assessment of the deliverable, the process undertaken and the resulting outcomes. Needs for future updates of this report are discussed and the use of this report within TransNet and the AP Cofund are briefly highlighted.

2. Context & Methodology

2.1. Overview of the AquaticPollutants Research & Innovation Projects

The 18 research & innovation projects cover three themes related to addressing the risks posed by CECs, AMR and pathogens to human health and the environment.

Theme 1 *"**Measuring**: Environmental behaviour of CECs, pathogens and AMR bacteria in aquatic ecosyste*ms" includes two sub-themes:

- 1.1 - Assessment of the significance of different potential sources, reservoirs and pathways of CECs and pathogens including antimicrobial resistant bacteria
- 1.2 - Understanding and predicting the environmental and cumulative behaviours of CECs and pathogens including antimicrobial resistant bacteria, including the development of tools and digital solutions

The AP projects in Theme 1 (https://aquatic-pollutants.eu/measuring.html) include:

1. **ARENA** – Antibiotic resistance and pathogenic signature in marine and freshwater aquaculture systems
2. **FOREWARN** – Development of a smart forewarning system to assess the occurrence, fate and behavior of contaminants of emerging concern and pathogens, in waters
3. **MAPMAR** – Marine plasmids driving the spread of antibiotic resistances
4. **PAIRWISE** – Dispersal of antibiotic resistance and antibiotics in water ecosystems and influence on livestock and aquatic wildlife
5. **PARRTAE** – Probing Antibiotic Residues and Resistance Transfer in Aquatic Environments
6. **SARA** – Surveillance of emerging pathogens and antibiotic resistances in aquatic ecosystems
7. **SPARE-SEA** – Environmental spread and persistence of antibiotic resistances in aquatic systems exposed to oyster aquaculture

Theme 2 "***Evaluating**: Risk Assessment and Management of contaminants of CECs, pathogens and antimicrobial resistant bacteria from aquatic ecosystems (inland, coastal and marine) to human health and environment"* includes three sub-themes:

- 2.1 - Characterising the exposure routes and effects of CECs and pathogens including antimicrobial resistant bacteria, on aquatic ecosystems and on human health
- 2.2 - Development of integrated risk assessment and risk management procedures
- 2.3 - Parameters and strategies for monitoring potential antimicrobial resistant bacteria

The AP projects in Theme 2 (https://aquatic-pollutants.eu/evaluating.html) include:

1. **AIHABs** – Artificial intelligence-powered forecast for harmful algal blooms
2. **BIOCIDE** – Antibacterial biocides in the water cycle – an integrated approach to assess and mange risks for antibiotic resistance development
3. **CONTACT** – Consequences of antimicrobials and antiparasitics administration in fish farming for aquatic ecosystems
4. **PHARMASEA** – Presence, behavior and risk assessment of pharmaceuticals in marine ecosytems

Theme 3 "***Taking Actions**: Risk Assessment and Management of CECs, pathogens and antimicrobial resistant bacteria from aquatic ecosystems (inland, coastal and marine) to human health and environment"* includes three sub-themes:

- 3.1 – Implementation of strategies to reduce CECs and pathogens, including antimicrobial resistant bacteria at the source
- 3.2 – Development of methods for preventing the spread of CECs and pathogens, including antimicrobial resistant bacteria
- 3.3 – Assessment of management measures and technologies to reduce the impact of CECs and pathogens including antimicrobial resistant bacteria, on water quality

The AP projects in Theme 3 (https://aquatic-pollutants.eu/takingactions.html) include:

1. **NanotheC-Aba** – CECs and AMR bacteria pre-concentration by ultra-nano filtration and Abatement by ThermoCatalytic Nano-powders implementing circular economy solution
2. **SERPIC** – Sustainable Electrochemical Reduction of contaminants of emerging concern and Pathogens in WWTP effluent for Irrigation of Crops
3. **NATURE** – Nature-Based Solutions to Reduce Antibiotics, Pathogens and Antimicrobial Resistance in Aquatic Ecosystems
4. **AMROCE** – Nano-enabled strategies to reduce the presence of contaminants of emerging concern in aquatic environment
5. **PRESAGE** – Potential of decentralized wastewater treatment for preventing the spread of antibiotic resistance, organic micropollutants, pathogens and viruses
6. **GreenWaterTech** – Green Ultrafiltration Water Cleaning Technologies
7. **REWA** – Reduction and assessment of antimicrobial resistance and emerging pollutants in natural-based water treatment systems

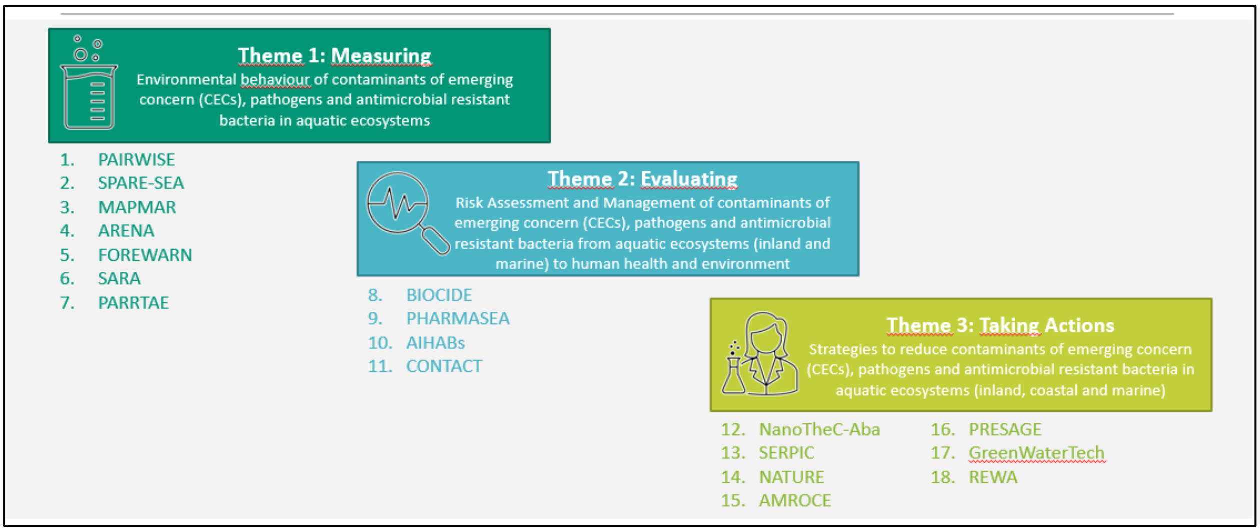

Figure 2-1. The 18 AquaticPollutants research & innovation projects by theme.

2.2. AquaticPollutants Projects' Alignment with Deliverable 1.2

The AquaticPollutants projects deal with developing research and innovation to better manage CECs, pathogens and AMR in the whole water cycle, from the source through the river basins and eventually to the estuaries and oceans. AP projects from the Theme 1 mainly deal with antibiotics and AMR, while AP projects from Theme 2 address mainly substances such as antibiotics, biocides, pharmaceuticals and cyanotoxins. For most projects, these contaminants are also considered for their role in the spread of AMR. Most of the AP projects from Theme 3 address at the same time, CECs, pathogens and AMR (see Table 2-1).

Table 2-1. Aquatic pollutant types addressed by the AP projects.

Theme	project	Aquatic pollutants types		
		Subtances (CECs & others)	Pathogens / Viruses	AMR (ARB / ARG)
1 - Measuring	PAIRWISE	antiobiotics		ARB & ARG
1 - Measuring	SPARE-SEA	antiobiotics		ARB
1 - Measuring	MAPMAR			ARG
1 - Measuring	ARENA	antiobiotics	microbial contaminants	
1 - Measuring	FOREWARN	antiobiotics	emerging viruses (SARS-CoV-2)	ARB & ARG
1 - Measuring	SARA		pathogenic viruses (SARS-CoV-2)	ARB
1 - Measuring	PARRTAE	antiobiotics		ARB & ARG
2 - Evaluating	BIOCIDE	biocide		ARB
2 - Evaluating	PHARMASEA	active pharmaceutical ingredients		
2 - Evaluating	AIHABs	cyanotoxins		
2 - Evaluating	CONTACT	antimicrobial/antiparasitics		ARG
3 - Taking Actions	NanoTheC-Aba	CECs	Pathogens	AMR
3 - Taking Actions	SERPIC	CECs	Pathogens	AMR
3 - Taking Actions	NATURE		Pathogens	AMR
3 - Taking Actions	AMROCE	antiobiotics		AMR
3 - Taking Actions	PRESAGE	CECs	Pathogens	AMR
3 - Taking Actions	GreenWaterTech	CECs	Pathogens	AMR
3 - Taking Actions	REWA	CECs, metals	Pathogens	AMR

In addition to covering the three call themes, the AP projects focus on different parts of the aquatic pollutant cycle from source to sea (see Figure 2-2). Within the three themes, each of the 18 AP projects seeks to address unanswered questions by providing information, data, tools, and/or various other outputs to various end-users or stakeholder groups. Three examples are presented in figure 2-3.

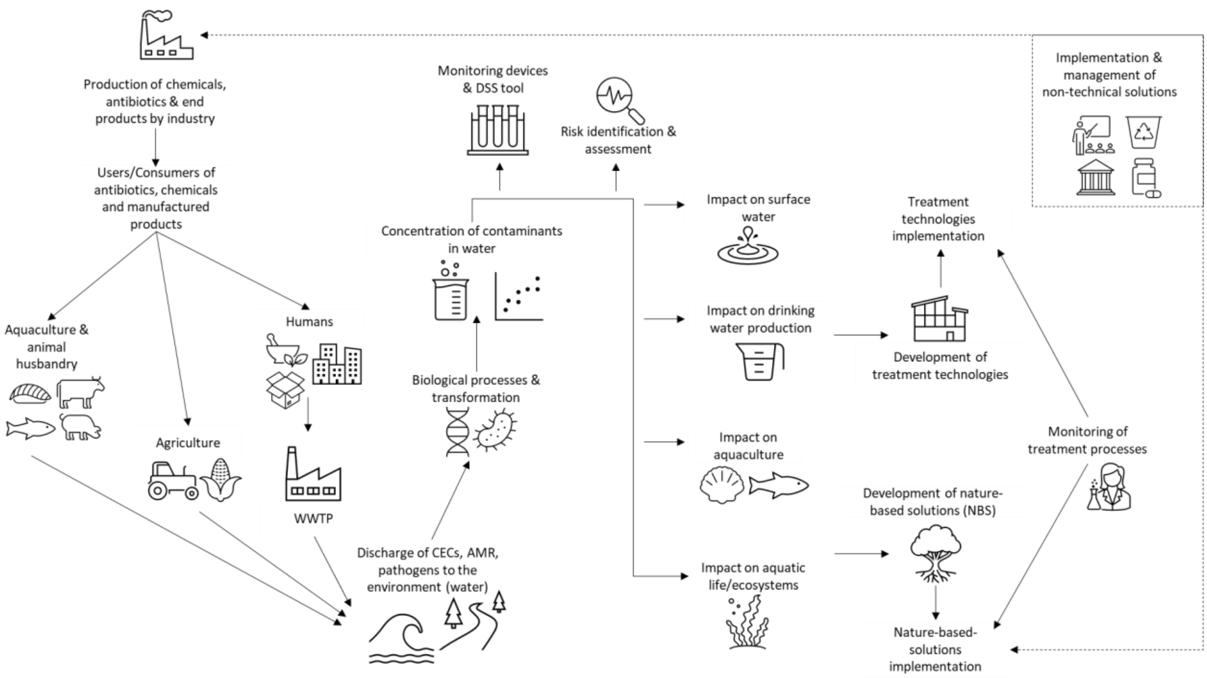

Figure 2-2. Ecosystem of the AquaticPollutants projects (Source: Maïté Fournier, ACTEON).

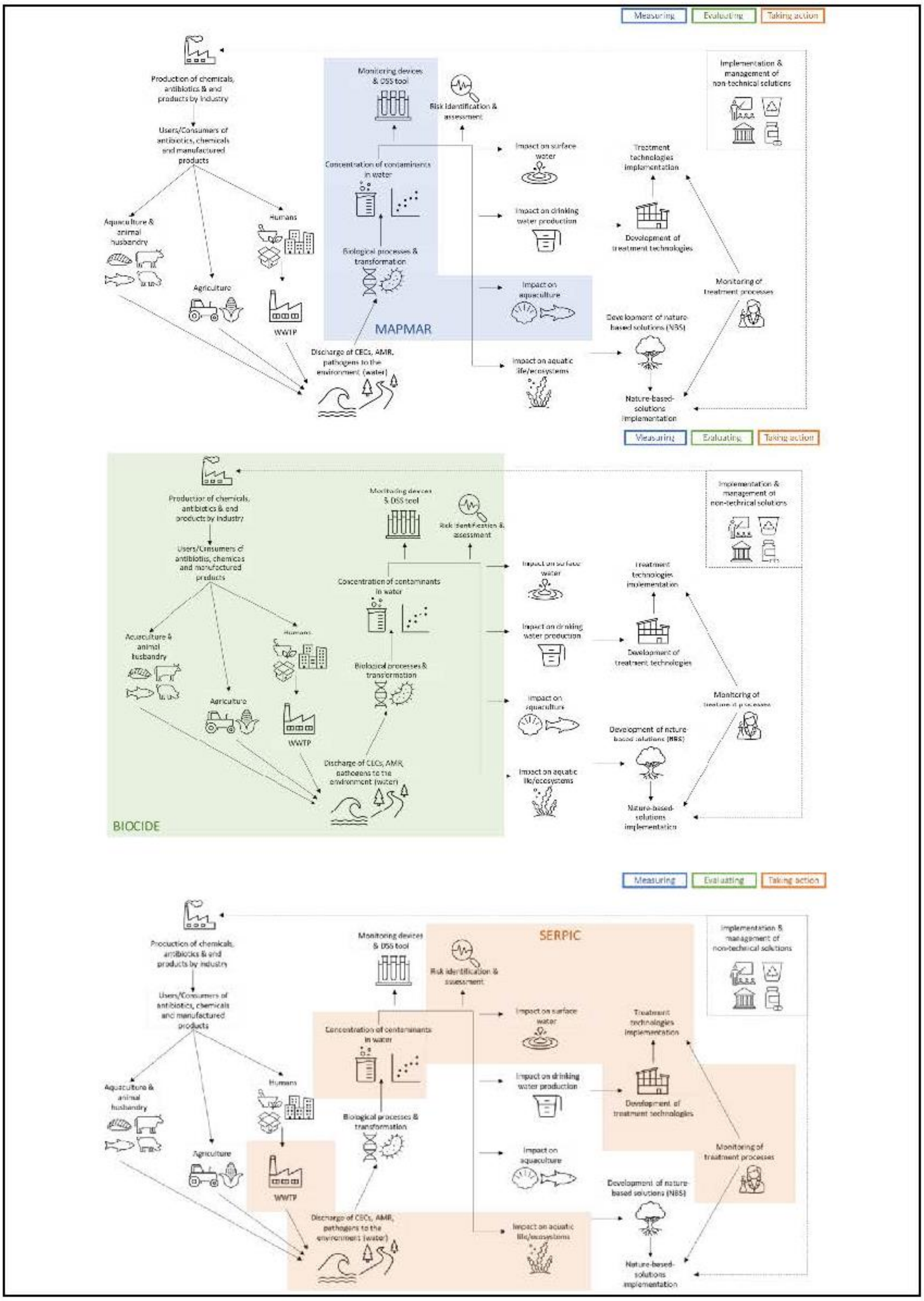

Figure 2-3. Sectors and themes addressed by AP projects. MAPMAR (Theme 1, blue), BIOCIDE (Theme 2, green) and SERPIC (Theme 3, orange), and are provided here as examples (Source: Maïté Fournier, ACTEON).

Using their provided research plans, this deliverable aims to determine and highlight the knowledge that will become available via the AquaticPollutants projects by defining overarching topics addressed by the AP projects, identifying knowledge gaps within these topics and aligning the projects' expected outputs with the SoA knowledge.

2.3. Methodology

The wide perimeter of these projects contributes to an integrated and cross-sectoral (freshwater, marine and health) approach to address the challenges of aquatic pollutants, which is very relevant. However, this wide perimeter presents a challenge for the assessment of knowledge, as summarizing and analysing the SoA at this scale is hardly feasible. In order to overcome this bottleneck, we adopted a pragmatic approach and focused on the following elements when describing the SoA:

- The SoA literature review is performed for the research fields that are addressed by the AquaticPollutants projects. Therefore, the summary of the current state of knowledge is **mainly based on state of the art provided by the funded projects of the AquaticPollutants Cofund**.
- Where relevant, a SoA review is completed for other recently funded projects or activities by Water JPI, JPIAMR and JPI Oceans, as well as other international and national programs and initiatives.
- Where relevant, the SOA review can be complemented by a wider literature review[1].

The following methodology was undertaken to perform the SoA assessment and to describe the AquaticPollutants outcomes:

1. Step 1:
   a) Collect information from the AP project coordinators, including their SoA proposal sections and a list of their expected outcomes, via a questionnaire (see Annex B for the questionnaire template). See Chapter 3.
   b) Identify the main themes addressed by the AP and the expected outcomes.

2. Step 2: Collect additional information from other international and national initiatives related to the main themes identified in Step 1b (focusing on French, German and Swedish initiatives that are available within the TransNet team). See Chapter 3.

3. Step 3: Compile the SoA provided by the AP projects (Step 1) and any relevant additional information from Step 2; assess and highlight the added value of the AP projects to the SoA. See Chapter 4.

[1] Bibliographic search in specific science databases, such as SCOPUS and Google Scholar, using a combination of keywords could be performed. Given the broad scope of the AquaticPollutants projects, this is not feasible in an exhaustive manner. However, some publications were selected to complement the state of the art provided by the AP projects.

4. Step 4: Describe the AP projects' expected outcomes provided via the questionnaire and the Funded Projects Booklet[2], specifying topics for political, environmental, economic and societal relevance. See Chapter 5.

This process is visually depicted in Figure 2-4. The SoA knowledge assessment includes a description of the knowledge that will be produced by the AP projects and a positioning of these outputs with regards to the SoA: *How do the projects address the questions raised.*

As a final step, outlined briefly in Chapter 5, the AP projects will review the information provided in Chapters 3 and 4 to confirm their alignment within the SoA context.

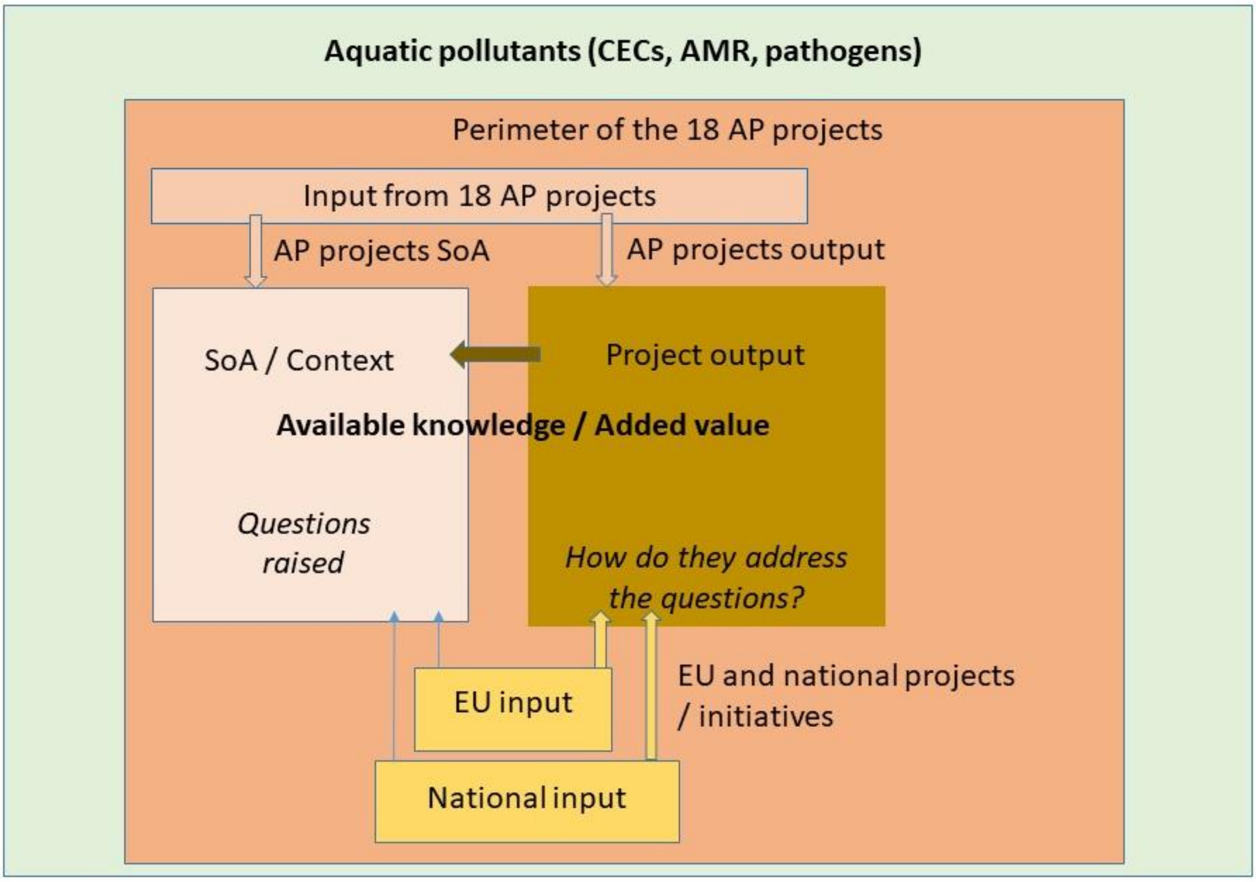

Figure 2-4: Schematic representation of the approach applied to identify available knowledge.

[2] JPI Funded Projects Booklet Joint Transnational Call 2020 (2021), available here: https://aquatic-pollutants.eu/Resources/Resources/_/AquaticPollutants%20RDI%20Funded%20Projects%20Booklet-1_bd.pdf

3. Data & Information Collection

3.1. Collection of SoA information from AP projects

Fifteen out of eighteen AquaticPollutants projects submitted completed questionnaires in the Fall of 2021. The information provided by each project for their SoA summaries were quite heterogeneous. Some SoA sections were quite complete (fair), some were very brief (see Table 3-1). In some cases, the information provided did not encompass any literature references.

Table 3-1. Questionnaire responses & state of the art collected in September 2021.

Call Theme	AP Project Acronym	Response to Questionnaire	State of the Art
1 – Measuring	PAIRWISE		Not received
1 – Measuring	SPARE-SEA	✓	Fair
1 – Measuring	MAPMAR	✓	Fair
1 – Measuring	ARENA		Not received
1 – Measuring	FOREWARN	✓	Fair
1 – Measuring	SARA	✓	Not received
1 – Measuring	PARRTAE	✓	Fair
2 – Evaluating	BIOCIDE	✓	Fair
2 – Evaluating	PHARMASEA	✓	Fair
2 – Evaluating	AIHABs	✓	Fair
2 – Evaluating	CONTACT	✓	Fair
3 – Taking Actions	NanoTheC-Aba	✓	Fair
3 – Taking Actions	SERPIC	✓	Fair
3 – Taking Actions	NATURE	✓	Fair
3 – Taking Actions	AMROCE	✓	Brief
3 – Taking Actions	PRESAGE	✓	Brief
3 – Taking Actions	GreenWaterTech		Not received
3 – Taking Actions	REWA	✓	Fair

- **Theme 1 “Measuring”**
  The SoA was provided by the 5 of the 7 AP projects via the questionnaire. The SoA sections were well documented even if bibliographic references were not always given.
- **Theme 2 “Evaluating”**
  The SoA sections provided by the AP projects were well documented even if the bibliographic references were not always given.
- **Theme 3 “Taking actions”**

The SoA sections provided by 6 out of the 7 AP projects were quite heterogeneous. They were specific to each AP project (addressing mostly relevant topics to each AP project) and the bibliographic references were not always given.

3.2. Identification of main themes and questions raised addressed by the 18 AP

The information available on the projects shows that their distribution is not homogeneous between the three themes of the call (see Figure 2-1) and their associated sub-themes, with Sub-Theme 2.3 hardly addressed and Sub-Theme 3.1 only addressed by one project. In addition, although the final objectives of the projects differs according to the three call themes, scientific questions common to all themes have been identified as specific objectives of the AP Cofund. This is why, rather than listing the scientific questions by sub-theme of the call for projects, **we propose a presentation by more cross-cutting scientific questions**.

This is the case for analytical and monitoring issues, which may be at the core of projects (mainly in Theme 1) or a prerequisite for projects addressing Themes 2 and 3. Similarly, questions related to the "source" of pollutants and "transfer routes/pathways" are common to all three themes. The risk assessment and effects of pollutants is fairly specific to Theme 2, while the remediation and mitigation aspects are specific to Theme 3. Finally, as most of the projects deal with antimicrobial resistance, a specific theme was deemed necessary. The presentation of the knowledge that will be produced by the projects can therefore be structured according to five categories:

1. **Analysis & monitoring:** How can we measure various contaminants in the different environmental compartments? How can methods and protocols of measuring and monitoring be harmonised?
2. **Sources and pathways:** What are the entry point (introduction routes) of CECs and pathogens and resistant bacteria and resistant genes into the environment? What are their pathways in aquatic environments?
3. **Mechanisms of AMR and of co-/cross-resistance:** How does antimicrobial resistance develop and transfer from aquatic ecosystems (inland, coastal and marine) to humans and environment?
4. **Effects on human health and environment:** Can we assess the effects of CECs and AMR on ecosystems and human health?
5. **Remediation and mitigation:** Can we reduce at the source CECs and pathogens, including antimicrobial resistant bacteria? Are current water treatment systems sufficiently effective for preventing the spread of CECs and antimicrobial resistant bacteria?

This outline also appears relevant if we consider the initial knowledge demands expressed by "external" stakeholders (see D1.1. p22-23), particularly with regards to the analytical and monitoring aspects and effects of pollutants on human health and environment. It fits also quite well to three out of four cross-cutting issues (CCI) defined by the partners involved in the 18 AP projects (AquaticPollutants | Cross-Cutting Issues (aquatic-pollutants.eu), projects having certain thematic and procedural commonalities among them. These CCIs are:

- CCI #2 Harmonization of methods, sampling procedures → linked to the 1st SoA category
- CCI #3 Mitigation technologies for CECs & AMR → linked to the 5th category
- CCI #4 Persistence, prioritization & impact of AMR/ARGs → linked to categories 1, 2 and 3

3.3. Collection of SoA information from other international & EU sources

In order to complete the SoA, we consulted the websites of the main funders of projects at the international and national levels (as identified in D1.1 – p15, p18-19) to search for projects about the 5 relevant categories we have identified in connection with the 18 AP projects (see section 3.2).

At the EU level, we examined the websites of the three organizing JPIs, namely Water JPI, JPIAMR, JPI Oceans, as well as other international sources, highlighted in the following sub-sections. This section lists the resources reviewed by TransNet and the most relevant projects or initiatives. More information on these relevant sources can be found in Annex A – section 2.

3.3.1. By funders – Joint Programming Initiatives

When consulting the JPIAMR, Water JPI and JPI Ocean websites, it is possible to gain access to completed projects on a one-by-one basis. Hubs (such as JPI-AMR hub and database (https://www.jpiamr.eu/jpiamr-supported-amr-research-database/#/) are a very good tool to consult data on projects funded by the JPIs. Although keyword searches provides a list of related projects, there is no compilation or synthesis of several projects' results. From this list, projects can only be viewed individually. Therefore, a compilation of project outputs was not readily available and could not be included exhaustively in the present SoA.

Some significant projects (StARE – Stopping Antibiotic Resistance Evolution (Water JPI – 2014-2017), MOTREM – Integrated Processes for Monitoring and Treatment of Emerging Contaminants for Water Reuse (Water JPI 2015-2017), REWATER – Sustainable and safe water management in agriculture (Water JPI 2017 -2020) are mentioned as examples in Annex A.

3.3.2. By funders – CORDIS

The Community Research and Development Information Service (CORDIS) is the European Commission's primary source of results from the projects funded by the EU's framework programmes for research and innovation, from FP1 to Horizon Europe. By using keywords „pollutant„, "emerging concern", 787 results are obtained. From this list, projects can only be viewed individually. Each project has its own dissemination plan. Therefore, a compilation of project outputs was generally not readily available and could not be included exhaustively in the present SoA.

A significant on-going project (TOXICROP – New methods to detect toxicity of cyanotoxins in water (Horizon2020 – Grant agreement ID: 823860) is particularly relevant to the themes of the AquaticPollutants Cofund and was included in Annex A, one. A compilation of nature-based solution projects projects (Nature-based Solutions State of the Art in EU-funded Projects (ISBN 978-92-76-17334-2 doi:10.2777/236007) is also of interest.

3.3.3. NEREUS (2014-2018)

The overall benefit of the COST Action NEREUS (ES1403) has allowed to consolidate the existing scattered data related to reuse of wastewater, and address the open challenges associated with it. It provides the platform for a systematic consolidation of data and standardization of methods for assessing emerging hazards associated with reuse. Its objective was to develop a multi-disciplinary network to determine which of the current challenges related to wastewater reuse are the most concerning ones in relation to public health and environmental protection, and how these can be overcome. Five working groups were created;

- WG1: Microbiome and mobile antibiotic resistome in treated wastewater and in downstream environments
- WG2: Uptake and translocation of organic microcontaminants and ARB&Gs in crops
- WG3: Effect-based bioassays required for wastewater reuse schemes.
- WG4: Technologies efficient/economically viable to meet the current wastewater reuse challenges
- WG5: Risk assessment and policy development

Several deliverables were produced (http://www.nereus-cost.eu/working-groups/deliverables/).

More specifically in relation to the themes addressed here, we can mention:

- **Deliverable 5** (November 2018): White paper on the implications of wastewater reuse on the spread of ARB&ARGs and adequate measures to minimize adverse impacts. This white paper presents and exhaustive review of the monitoring strategies, challenges and potential solutions relating to dissemination of antibiotic resistance elements from WWTP effluents.
- **Deliverable 15**: Harmonized protocols for effect-based in vitro biotests (bioassays) able to serve as routine tools for analysis and evaluation of the efficiency of the various treatment technologies to remove toxicological hazards and evaluate the quality of the wastewater to be reused.

The WG4 deliverables are unfortunately not available on the website.

3.3.4. NORMAN Network (2005 to date)

The NORMAN network (https://www.norman-network.net/) enhances the exchange of information on emerging environmental substances, and encourages the validation and harmonisation of common measurement methods and monitoring tools so that the requirements of risk assessors and risk managers can be better met. It specifically seeks both to promote and to benefit from the synergies between research teams from different countries in the field of emerging substances. A list of published peer-reviewed papers as well as position papers is available.

Activities are organised in 8 working groups and 2 cross-working groups. Their activities concern mainly analytical issues, monitoring, effects and wastewater. NORMAN organises the development and maintenance of various web-based databases for the collection & evaluation of data and information on emerging substances in the environment such as the NORMAN Substance database, NORMAN Antibiotic Resistance Bacteria/Genes Database and NORMAN EMPODAT Database – Chemical Occurrence Data. In the substance database information on chemical structure, chemical identifiers, accurate mass, measured or predicted toxicity information, predicted retention time and ionization efficiency, etc. is collected for many thousands of compounds. The NORMAN data base system also hosts MassBank Europe which collects high resolution mass spectra for thousands of chemicals searchable with open source or vendor specific software. To support suspect screening of emerging contaminants, the suspect list exchange collects priority lists with accurate mass information ready for use in screening studies, provided by the members of the NORMAN network. Much of the information provided is also linked with other chemical or toxicity data resources, such as the US EPA CompTox Chemical Dashboard.

One of the working groups have developed a methodology for prioritization of emerging contaminants based on the information in the database system and other available resources. Within the database system there is also a repository for high resolution chemical screening data, the digital sample freezing platform that facilitates re-evaluation of stored mass spectra data for identification of signals originating from chemicals. Statistical tools for trend analysis, etc. are associated with the digital sample freezing platform. It should be noted that contributions to these databases are voluntary and therefore heterogeneous. Considering the wide set of NORMAN data, it was not possible to include them in the SoA, as such.

3.3.5. PARC (2022-2029)

PARC (European Partnership for the Assessment of Risks from Chemicals – 2022-2029 - https://www.eu-parc.eu/what-we-do) aims to advance research, share knowledge and improve skills in chemical risk assessment. To date, PARC involves nearly 200 partners from 28 countries, as well as three EU agencies (the European Environment Agency – EEA, the European Chemicals Agency – ECHA and the European Food Safety Authority – EFSA). The partnership is on-going and there are many expected results. The expected results will be valorised in the form of scientific publications (https://www.eu-parc.eu/scientific-publications) as well as deliverables (https://www.eu-parc.eu/deliverables). In connection with the 18 AP projects, mention should be made of the activities of WP4 “monitoring and exposure” dealing with environmental and human exposures, by using conventional and innovative tools and strategies, WP 5 “hazard assessment”, WP6 “innovation in regulatory risk assessment” and WP9 “capacities building” including QA/QC criteria and standardization. A scientific watch on the activities of both PARC and NORMAN is suggested, with progress expected on the monitoring and risk assessment aspects.

3.3.6. Inventory summary

By consulting the websites of the various funders of European projects, lists of funded projects are accessible, and sometimes summaries and deliverables are available. This initial information is important, but it is then often necessary to consult the website of each project for more details. In our case, given the broad subject matter “aquatic pollutants”, this represents a huge amount of work. Summary documents by theme on the websites of project funders, bringing together different projects, would be welcome.

3.4. Collection of SoA Information from National Sources

In order to complete the SoA with results from other projects recently funded, we consulted information available for France, Germany and Sweden, either through national databases, R&D funders or existing relevant networks. The information is presented separately for CECs and antimicrobial resistance, as the two subjects are dealt with differently in each country.

3.4.1. France

This section provides a brief overview of some of the main actors within the French state of art knowledge context. This is by no means meant to be an exhaustive presentation of the current scientific status within France pertaining to CECs, AMR and pathogens, but merely a summary of the major information sources and foundational organizations. More detailed information on the actors presented below and further publications can be found in Annex A, Section 3.

Data sensus lato

HAL (https://hal.science/#), developed in 2001 in France, is a public, sustainable and responsible infrastructure. The environmental sciences section contains more than 178 000 results including peer-reviewed papers, theses, conference papers, videos, software, etc. By using "aquatic pollutants" as keyword, 198 documents are sorted out, some also being identified via Scopus and Google Scholar.

Projects Funded by ANR

ANR (Agence Nationale de la Recherche) is one of the main R&D funders. The Generic Call for Proposals is the French National Research Agency's (ANR) main call. It is directed towards all scientific communities and all public and private players involved in French research. It is designed to give researchers in various scientific fields access to co-funding in a large number of research themes (56 in 2023), basic or applied, in addition to their allocated recurrent funding.

The ANR website provides access to the list of funded projects, a summary and some deliverables. The information is therefore given on a project-by-project basis. Given the broad extent of the call, we cannot consider all of them. By using the keywords "aquatic pollutant", "emerging contaminant", "antiobioresistance", 7, 54, 33 projects are listed, respectively. Individual abstracts are available for these projects. Some projects relevant to the topics of AquaticPollutants are mentioned, based on public abstract, in Annex A.

(Pharm@ecotox – Pharmaceutical residues and ecotoxicology in seawater (2011- 2015); DETECMED – A novel sensing method for the detection of pharmaceuticals in water (2021-2025); RESISTE – Understanding the evolution of antimicrobials resistance in environmental vibrios (2020-2024); AMPERES - Analyse de micropolluants prioritaires et émergents dans les rejets et les eaux superficielles (funded by ANR PRECODD 2006-2009) ; ECHIBIOTEB – Méthodologies innovantes pour l'analyse chimique et biologique des substances des eaux traitées et boues (ANR ECOTECH 2010))

In some cases, the ANR publishes thematic reports. According to the AquaticPollutants scope, the most relevant is „Contaminants et environnements: constater, diffuser, décider" (Contaminants and environments: recognise, disseminate, decide) but it was not recently published (2012).

Projects from Other Funders

CECs & water initiatives are mostly supported by the French Office for the Biodiversity (OFB) in France. The OFB reported CECs occurrence in water in 2016[3] and in 2020[4]. The 2020 report indicated a watch substances list.

Before 2013, OFB funded research project on emerging compounds and water, for which output is not compiled. Some projects may be quite relevant to AP themes (e.g. ARMISTIQ 2010-2013 – Amélioration de la réduction des micropolluants dans les stations de traitement des eaux usées domestiques).

In 2013, the Ministry of Ecological Transition and the water agencies launched a project call on "Innovations and Changes of practices: fight against micro-pollutants in urban waters". Thirteen projects run between 2014 and 2019 were awarded under this call; eight deal with the theme of remediation and mitigation and are linked to theme 3 "taking actions" (see Annex A – section 3). A compilation of results produced by the 13 projects was carried out as well as an extended restitution. This work[5] was led by OFB. To our knowledge, this type of valorization and dissemination is rare. These results were structured as follow:

- Management of urban contamination
- Sources and fluxes of pollutants
- Innovative tools and methods to monitor or detect pollutants.

A document (in French) available to teachers, trainers, students, scientists, engineers and managers concerned with biodiversity was also produced on the basis of these projects, specifically considering pollutants emitted by health sector.

In addition to the program mentioned above, some water utilities such as the SEDIF[6] has run a program for 15 years to better understand CEC levels in water and to assess the efficiency of treatment technologies with respect to CECs. Unfortunately, no information was readily accessible to their website.

> As for the CECs, the French projects funded before 2013 and funded under the call "Innovations and Changes of practices" (2013) brought mainly results of micropollutants characterisation in wastewater and some results also into solutions for reduction at the sources. All these projects confirmed the presence of micropollutants in wastewaters and the need to develop reduction methods at the source. They hardly dealt with treatment technological development.

[3] results of CECs monitoring campaigns from 2011 & 2012 in all water media, https://www.eaufrance.fr/sites/default/files/documents/pdf/campex_201603.pdf

[4] results from surface water and sediment campaigns undertaken in the work programme 2018-2020 (Réseau national de Surveillance Prospective de la qualité chimique des milieux aquatiques entre l'OFB / Ifremer / BRGM / Ineris / INRAE / LNE / CNRS / ISA / Université de Bordeaux, https://www.ineris.fr/sites/ineris.fr/files/contribution/Documents/Substances%20Pertinentes%20%C3%A0%20Surveiller%20%28SPAS%29%20v3.pdf, https://www.ineris.fr/fr/campagne-emergents-nationaux-2018-emnat-2018-resultats-recherche-contaminants-emergents-eaux

[5] see https://professionnels.ofb.fr/node/15 for synthesis and link for the deliverables of each projects

[6] Service Public de l'Eau Ile-de-France https://www.sedif.com/parametres-emergents.aspx

National Plans and Other National Activities

CECs and AMR are dealt with according to specific programs.

CECs issues are addressed through at least three programmes: PNSE4, PNRM, Écoantibio 2.

- **PNSE4**: The 4th programme Health and environment (4^{e} Plan National Santé Environnement: « Un environnement, une santé » PNSE (2021-2025) is co-steered by the Ministries of Ecological Transition and Solidarity and Health. The elaboration of the PNSE every 5 years is included in the public health code (article L.1311-6). In this program (PNSE4), the terms mentioned are rather generic "chemical substances" with the exception of biocides which are clearly mentioned. The water compartment is not specifically mentioned as is air, but the term "exposome" is used. This national plan is intended to have regional variants.
- **PNRM**: Plan national sur les résidus de médicaments dans l'eau [PNRM] – National plan on pharmaceutical residues in water with one of the major objectives being to improve knowledge of their presence and potential effects on the environment and human health and to propose appropriate and proportionate management measures.
- **Écoantibio 2**: National plan to reduce the risk of antibiotic resistance in veterinary medicine (2017 – 2022) is led by the French Ministry of Agriculture.

AMR issues are addressed by the following national initiatives:

- In 2016, a first inter-ministerial committee for health builds a roadmap on the antibiotic resistance[7]. This committee aims to coordinate actions among three schemes: the antibiotic alerts scheme, the EcoAntibio scheme (dealing with use of antibiotics in farming) and the environment and health schemes (measure 12b). The "technical" actions proposed by the roadmap aimed mainly to better understand the antibiotic resistance, scale the issue and improve its monitoring. This roadmap did not especially mentioned the water compartment.
- In 2017, a synthesis of the presence of antibiotics and antibiotic resistance in environment (including water) was undertaken by the Ministry of Environment (Thema, 2017). It showed that waters were contaminated by antibiotics and bacteria and genes of antibiotic resistance, one of the main source being urban wastewaters. Besides, there are not many French available publications on the occurrence of antibiotics and AMR in environment and no information was found on solutions for AMR management and treatment.
- In 2020, the Ministry of Research and Innovation and the General Secretariat of Investment launch a priority research programme on antibiotic resistance. Eleven projects (48-72 months duration) were awarded under the theme "understanding, innovation and action" and aimed to better understand the antibiotic resistance, not especially focussing on the water media. One of them (Dyaspeo) dealing with the transfer, persistence and evolution trends of AMR between human beings, animals and environment may deal with the water compartment. Three projects were awarded under the "structuring" theme. These projects aimed to store, integrate, analyse and share data through designing a dedicated platform (ABRomics_PT), structure antibiotic resistance actors network (PROMISE) and monitor societal aspects of antibiotic resistance through the development of an observatory (Dosa-Observatoire).
- Two missions were recently carried out on a national scale for several years now provide information on the consumption of antibiotic in health institutions and on the surveillance of

[7] https://solidarites-sante.gouv.fr/IMG/pdf/feuille_de_route_antibioresistance_nov_2016.pdf

bacterial resistance in health institutions, home health care and establishment for dependent elderly people (Missions SPARES and PRIMO).

- In February 2022, the French ministry of Environment released a national strategy for infection prevention and antibiotic resistance. This strategy includes two pillars: prevention actions for infection control and promotion of good use of antibiotics.

As for AMR, there are on-going national research programmes, which aim to scale up the issue of antibiotic resistance. Moreover, a recent strategy for infection prevention and antibiotic resistance has been released. These actions do not focus on the water media and mainly frame the issue, focusing on reduction at the source of antibiotics use.

3.4.2. Germany

This section provides a brief overview of some of the main actors within the German state of art knowledge context. This is by no means meant to be an exhaustive presentation of the current scientific status within Germany pertaining to CECs, AMR and pathogens, but merely a summary of the major information sources and foundational organizations. More detailed information on the actors presented below and further publications can be found in Annex A, Section 4.

In Germany, projects usually involve a combination of CECs and AMRs. RiSKWa funding measure described below illustrates this.

German Federal Ministry of Education and Research (BMBF)

The Federal Ministry of Education and Research (BMBF) is responsible for education, research, and innovation in Germany. The tasks of BMBF include funding research projects, promoting educational institutions and programs, supporting innovations in science and business, and developing education and research policies at the federal level. Among other things, BMBF funds research projects in the field of environmental sciences and technologies that deal with the identification, monitoring and elimination of trace substances. This is done within the framework of research programs aimed at developing solutions to environmental problems and promoting scientific understanding in this field.

Funding Measure RiSKWa (2011 – 2016)

The funding measure "RiSKWa" stands for "Risk Management of New Pollutants and Pathogens in the Water Cycle" is an initiative of BMBF (https://riskwa.de/). The aim of this funding measure was to assess, manage and reduce the risks posed by new pollutants and pathogens in the water cycle.

Under RiSKWa, 13 research projects were funded to address various aspects of risk management related to contaminants and pathogens in water. This included the development of new analytical methods for the detection of pollutants and pathogens, the study of the impact on the environment and human health, the development of measures to reduce risks, and the development of recommendations and guidelines for dealing with these risks. These 13 projects are therefore linked to the 3 themes of the call (“Measuring”, “Evaluating” and “taking actions”.

In the end, a so-called ‘best practices manual’ was developed, summarizing the results of the funding measure (https://riskwa.de/Downloads/_/RISKWA_Handbook_of_good_practice.pdf).

The following 13 collaborative projects were funded in RiSKWa (see details in Annex A, section 4):

- *AGRO (Risk management of trace substances and pathogens in rural karst catchments)*
- *ANTI-Resist (Investigation of antibiotic inputs and the development of antibiotic resistance in urban wastewater as well as the development of suitable strategies, monitoring and early warning systems using the example of Dresden)*
- *ASKURIS (Anthropogenic trace substances and pathogens in the urban water cycle: Assessment, barriers and risk communication)*
- *PRiMaT (Preventive risk management in drinking water supplies)*
- *RiMaTH (Risk management in domestic drinking water installations - rapid detection methods for bacterial contamination and monitoring of remediation projects)*
- *RiskAGuA (Risks from effluents from intensive livestock farming for groundwater and surface water in agricultural areas)*
- *RISK-IDENT (Assessment of previously unidentified anthropogenic trace substances and action strategies for risk management in the aquatic system)*
- *SAUBER+ (Innovative concepts and technologies for the separate treatment of wastewater from healthcare facilities)*
- *SchussenAktivPlus (Reduction of micropollutants and germs to further improve the water quality of the Lake Constance tributary Schussen)*
- *Sichere Ruhr (Bathing water and drinking water for the Ruhr area)*
- *TOX-BOX (Hazard-based risk management for anthropogenic trace substances to safeguard drinking water supplies)*
- *TransRisk (Characterizing, communicating, and minimizing risks from emerging contaminants and pathogens in the water cycle)*
- *HyReKA (Biological or hygienic-medical relevance and control of antibiotic-resistant pathogens in clinical, agricultural and municipal wastewater and their significance in raw water)*

Atlas of Water Innovations

The "Atlas of Water Innovations" is also an initiative of BMBF. This Atlas, or inventory of innovative water solutions, provides a comprehensive overview of water research and innovation in Germany.

The Atlas serves as an information platform and database that informs researchers, companies, policy makers and the public about ongoing projects, developments and innovations in the field of water. It contains information on research institutions, companies and projects dealing with various aspects of water management, water supply, water quality, water treatment, wastewater treatment and other water-related topics, such as the challenge of CECs and AMR. Users can visit the site to research available solutions, strategies, technologies and more by specific water resource matrices and application sector.

National plans and other national activities

The Federal Environment Agency

The Federal Environment Agency (UBA) in Germany is responsible for environmental protection and sustainability, working under the Federal Ministry for the Environment. Their recent analyses suggest that comprehensive environmental protection requires a combination of measures in production, use, and wastewater treatment. In the context of pharmaceuticals, they emphasize disclosing environmental data during approval, promoting environmentally friendly active ingredients, and educating both medical professionals and patients about proper disposal.

For plant protection products (chemical pesticides), the UBA recommends reducing their use by expanding organic farming and adopting precautionary measures in conventional farming, with an emphasis on minimizing water pollution. Biocides, used for pest control and disinfection, should also be minimized, and comprehensive data collection and guidelines are essential. Wastewater treatment should include a fourth stage to reduce micropollutant inputs, which may require additional costs. The UBA is considering levies on pesticides and pharmaceuticals, as well as amendments to the wastewater levy to finance advanced purification technologies.

The Federal Environment Agency has published various publications on measures to reduce the input of micropollutants into water bodies (Phase 1, Phase 2, Organic micropollutants in waters (Fourth treatment stage for fewer inputs), Reduce chemical inputs to water - Trifluoroacetate (TFA) as a persistent and mobile substance with many sources). Additionally, UBA maintains several national databases, which can be found listed in Chapter 4.3 of D1.1 Mapping of end-user groups and governance and synthesis of their demands for knowledge.

The German Centre for Micropollutants

In 2016, the Federal Ministry for the Environment initiated a stakeholder dialogue with the goal of developing a federal strategy to reduce the presence of trace substances in water bodies. The strategy focuses on a combination of source-oriented and application-oriented measures to effectively reduce trace substances. The process involved multiple phases, including the development of initial recommendations by stakeholders in a policy paper by June 2017, followed by the refinement of these recommendations by March 2019 through four working groups. The results were presented to the Minister in March 2019.

A pilot phase was conducted until December 2020 to test the practicality of the proposed activities. After the pilot phase, an evaluation took place, and the results were presented to stakeholders in March 2021, marking the establishment of the Federal Centre for Trace Substances. Since 2022, this Centre has been working on identifying relevant trace substances in collaboration with experts and facilitating discussions between manufacturers, the water industry, and federal states. They also advise on input pathways, substance properties, and the introduction of advanced wastewater treatment methods. Additionally, the Centre promotes information exchange between federal states, municipalities, competence centers, and universities regarding source-oriented and downstream reduction measures.

Micropollutants Competence Centre BW (KomS BW)

The Micropollutants Competence Centre (KomS) Baden-Württemberg (BW), established in 2012, is an institution in Baden-Württemberg, Germany, focused on researching and managing trace substances in the environment.

The Competence Center has several tasks, including research, consultation and education, solution development and networking. KomS BW plays a crucial role in advancing the understanding and management of micropollutants in Baden-Württemberg and beyond, with a particular focus on improving the environmental and public health aspects of these trace substances.

In Baden-Württemberg, several wastewater treatment plants have so far been retrofitted with an additional treatment stage for the removal of trace substances based on the findings from semi-technical investigations. For the removal of micropollutants, activated carbon processes have been used so far, which are currently regarded as suitable processes for the elimination of trace substances in wastewater in addition to ozonation.

German Association for Water, Wastewater and Waste (DWA)

The German Association for Water, Wastewater and Waste (DWA) is a German-based organization that deals with issues relating to water management, wastewater treatment and waste management. It is a cross-industry association composed of professionals, companies, government agencies and research institutions. Among other things, the DWA deals with the research, monitoring and reduction of trace substances in water and wastewater systems. It develops guidelines, recommendations and standards for the analysis and handling of trace substances (e.g. DWA position paper "Anthropogenic trace substances in water"). This serves to minimize environmental impacts and protect drinking water quality and water quality.

In Germany and other countries, efforts to reduce trace substances in water bodies and wastewater treatment plants are of great importance, and DWA plays an important role in developing guidelines and measures to address this environmental problem.

The German Antibiotic Resistance Strategy (DART 2030) is an initiative of the Federal Ministry of Health, developed in collaboration with other ministries. The strategy was adopted by the Federal Cabinet in April 2023 and is intended to further deepen the results achieved with the predecessor strategy “DART 2020”. The goals and measures for combating antibiotic resistance at the national level and in international cooperation are presented in six areas of action (Prevention, Surveillance and Monitoring, Appropriate use of antibiotics including laboratory diagnostics, Communication and Cooperation, European and International Cooperation, Research and Development).

Relevant events in Germany

The exchange of information between German scientific and research actors occurs also via established events and exchange platforms. Some examples related to micropollutants are listed below, though this is by no means a comprehensive list:

- SETAC GLB (Society of Environmental Toxicology and Chemistry – Europe German Language Branch e.V.)

- DECHEMA's conference on "Trace substances and pathogens in the water cycle" (Spurenstoffe und Krankheitserreger im Wasserkreislauf; SUK)
- KomS Webinars
- KomS Congress on Trace Elements in the Aquatic Environment
- DWA Training Courses and Webinars on the topics of wastewater treatment, water protection, etc.

3.4.3. Sweden

This section provides a brief overview of some of the main actors within the Swedish state of art knowledge context. This is by no means meant to be an exhaustive presentation of the current scientific status within Sweden pertaining to CECs, AMR and pathogens, but merely a summary of the major information sources and foundational organizations. More detailed information on the actors presented below and further publications can be found in Annex A, Section 5.

Major funders for research on CECs, antimicrobial resistance, and pathogens in Sweden are the Swedish Environmental Protection Agency (Swedish EPA), the Swedish Agency Marine and Water Management (HaV), the Swedish research council for sustainable development (FORMAS), the Swedish research council (VR), the Swedish foundation for strategic environmental research (Mistra) as well as the trade association for drinking water producers and sewage treatment plants (Svenskt Vatten).

Publications from screening and mitigation studies on CECs, AMR and pathogens can be found through the Swedish digital scientific archive DiVA, which can be searched using the DiVA portal (http://www.diva-portal.org). Searches can be performed based on agency (i.e. NV, Swedish Museum of Natural History, etc), University or Research Institute or individual publications or authors or type (article in journal, book, dissertation etc.). The Swedish EPA regularly finances screening studies on CECs in the Swedish environment for different compound groups.

CECs

Although there are collections of data and screening of occurrence on several different groups of CECs, most research concerning remediation has focused on pharmaceuticals or PFAS. Pharmaceuticals is a suitable group of chemicals for studying advanced treatment in WWTPs as there is a constant release of into the sewage system of substances with a wide range of physiochemical properties: pKa values, (bio)degradability, hydrophobicity, etc..). HELCOM[8] has published a knowledge summary on pharmaceuticals in the Baltic Sea with information on both prescription volumes, environmental occurrence, concentrations in WWTPs, reductions efficiencies, etc. Non-target screening studies on effluents from WWTPs and target screening studies on a wide range of compounds exist, but are not extensive.

The Swedish EPA, in collaboration with HaV has funded research concerning advanced treatment techniques for removal of micropollutants (mostly pharmaceuticals) through a research programme

[8] The Baltic Marine Environment Protection Commission – also known as the Helsinki Commission (HELCOM) – is an intergovernmental organisation

spanning 2014 – 2017, and pre-studies or implementation tests for advanced treatment during the period 2018 – 2023. In total, more than 30 M€ has been provided for evaluating solutions and performing tests at various scales. A summary for the eight projects funded 2014-2017 was published by HaV in 2018. Techniques using physical separation techniques such as reversed osmosis, ultra- or nanofiltration, sorption, advanced oxidation, biological or enzymatic degradation, as well as combinations of these were evaluated both for the potential to degrade micropollutants and for feasibility of implementation using life cycle cost- and assessment methods. A list of micropollutants recommended for following up the efficacy of the advanced treatment techniques was created by the Swedish EPA including a number of pharmaceuticals, PFAS, hormones, phenolic compounds as well as the use of effect tests for estrogenic activity (YES-test) and Ames test. No synthesis report has been made for the projects funded during the period 2018 – 2023.

One of the largest national research programmes on CECs in the environment was the MISTRA-funded MistraPharma (MistraPharma – Mistra). The project had a duration of eight years (2008 – 2015) and one of the primary aims was improving the understanding of environmental effects from pharmaceuticals and novel methods for prioritization of substances. It also evaluated remediation techniques for pharmaceuticals in both lab and pilot scale, including tests at four different WWTPs. The tests evaluated both ozonation, sorption on carbonaceous particles and moving bed bioreactor (MBBR) steps in different arrangements. Ecotoxicological effects of treated wastewater was also studied in connection with the treatment tests. The project included research concerning the promotion of antimicrobial resistance in the environment and development of analysis methods for AMR genes. Mixture effects of steroidal pharmaceuticals were also studied. The programme resulted in the creation of a **knowledge centre for pharmaceuticals** and the environment, located at the Swedish Medical Products Agency.

Funding for studies have also been provided through the EUSBSR policy area Hazards and European Regional development fund through the Baltic Sea Pharma Platform. Six projects were funded over the period 2017 – 2020: MORPHEUS - Model Areas for Removal of Pharmaceutical Substances in the South Baltic, CWPharma – Clear Water from Pharmaceuticals, GrePPP – Green Public Procurement of Pharmaceuticals for the Baltic Sea Region, MicroWasteBaltic – Impact of micropollutants emitted from municipal wastewater treatment plants on Baltic Sea ecosystems and assessment of cost-benefit of advanced treatment technologies in a regional perspective, NONHAZCITY – Innovative management solutions for minimizing emissions of hazardous substances from urban areas in the Baltic Sea Region. Sweden´s first permanent full-scale tertiary WWTP, Tekniska verken in Linköping, comprising ozonation and MBBR steps was evaluated as a part of the CWPharma project.

Stockholms Municipality also funded an evaluation of advanced treatment techniques during the period 2009 – 2014, including the evaluation of ozonation, UV/Ozone, sorption on carbonaceous material, ultrafiltration and biological degradation methods. This provided the basis for the implementation of ultrafiltration for all of Stockholms wastewater.

EU and national funding in the Baltic sea region has also funded several large projects through the joint Baltic Sea research and development programme BONUS. These include the BONUS Micropoll, that evaluated the optimization of onsite wastewater treatment plants and BONUS Clearwater, that investigated both ozonation and a range of solutions for biological degradation of micropollutants.

A lot of research has also been performed on the remediation or removal of PFAS, both from contaminated sites and from sewage or drinking water. Techniques employed have been for instance sorption using ion exchange, functional framework materials or carbonaceous particles, foam fractionation, filtration, phytoremediation, electrochemical degradation.

AMR

The Swedish Research Council (VR) is leading a ten-year national research agenda for antimicrobial resistance. It is in turn based on the same topics as the Joint Program Initiative for Antimicrobial Resistance (JPIAMR), and strategic calls are mostly made through the JPIAMR. Sweden has been successful in keeping a low prescription rate of antibiotics through a long running strategic programme against antibiotic resistance (Strama | Samverkan mot antibiotikaresistens), formed in 1995. The Strama network aims at a responsible use of antibiotics and has subnetworks for medical practitioners, veterinaries, and dentists. Through education campaigns and information to policy makers a continuous awareness of the risks of antibiotic resistance has been maintained. Annual goals of reduced prescriptions have resulted in long term negative trends for the use of antibiotics.

National data on antibiotic sales as well as the occurrence of antimicrobial resistant bacteria is published yearly through a collaboration of the Public Health Agency of Sweden and the National Veterinary Institute (Swedres-Svarm - SVA). The reporting collects both human and animal data. Similar reports are published on EU data by the European Medicines Agency, e.g. the 'JIACRA' (joint inter-agency antimicrobial consumption and resistance analysis) reports that collect data on a three year basis.

An international network on action against antimicrobial resistance was formed in Uppsala, sweden in 2005; React (Antibiotic resistance – ReAct (reactgroup.org)). The network has been mainly funded by Swedish International Development Cooperation Agency (SIDA) and Uppsala University. The network aids countries in setting up national plans on antibiotic resistance and publishes yearly reports (JIACRA III - Antimicrobial consumption and resistance in bacteria from humans and animals (europa.eu))

A centre for AMR research exists at Gothenburg University (GU) and is a collaboration between GU, Chalmers Technical University and Sahlgrenska Hospital and Region Västra Götaland. Researchers at the centre are part of several large international projects, for instance EMBARK (9th transnational call JPIAMR, 2019), EDAR (VR call), and BIOCIDE (Aquatic Pollutants call).

3.4.4. Inventory summary

A brief overview of some of the main actors within the French, German and Swedish state of art knowledge context is given. This is by no means meant to be an exhaustive presentation of the current scientific status within these three countries pertaining to CECs, AMR and pathogens, but merely a summary of the major information sources and foundational organizations.

It appears that:

- CECs have generally been studied more extensively and more historically than AMR.
- The results/outputs obtained are generally presented project by project, either directly on the project website or via the funders' websites, sometimes by keyword or via a tool enabling a more detailed selection, e.g. by type of outputs (e.g. Atlas of Water Innovation in Germany).
- Synthesis outputs/reports on a specific theme or scientific issue summarizing outputs from multiple projects are rather rare.
- Of the five categories we defined, the category "mechanisms of AMR" has the least information available. The implementation of plans and strategies at national level should make it possible to fill the knowledge gaps in coming years.
- As for AMR sources and pathways, efforts to date have focused mainly on reducing the use of antibiotics or others antimicrobials.
- As for CECs, there are numerous studies on the effectiveness of treatment plants (category 5 – Remediation & Mitigation).

3.5. Collection of Information on AP project outputs

As for the **AP projects' outputs**, the number of outputs was estimated from the initial questionnaire (from the answer given to question: "*What key knowledge gaps are you addressing with your project*?"; see Annex A) and the AP Funded Projects Booklet (Figure 3-1). The number of outputs (key knowledge gaps addressed and knowledge that will be available when projects are finished) were often different from the information collected in the questionnaire or in the booklet. Moreover, it was sometimes difficult to estimate the number of outputs.

Theme	AP project	Response to questionnaire	Number of outputs from questionnaire / from booklet
1 - Measuring	PAIRWISE		not received / unclear
1 - Measuring	SPARE-SEA	✓	5
1 - Measuring	MAPMAR	✓	5 / 3
1 - Measuring	ARENA		not received /unclear
1 - Measuring	FOREWARN	✓	[2] / 7
1 - Measuring	SARA	✓	8 / unclear
1 - Measuring	PARRTAE	✓	[3] / 4
2 - Evaluating	BIOCIDE	✓	not completed / 4
2 - Evaluating	PHARMASEA	✓	5 / 4
2 - Evaluating	AIHABs	✓	[2] / 1
2 - Evaluating	CONTACT	✓	not completed
3 - Taking Actions	NanoTheC-Aba	✓	3
3 - Taking Actions	SERPIC	✓	1
3 - Taking Actions	NATURE	✓	3
3 - Taking Actions	AMROCE	✓	5
3 - Taking Actions	PRESAGE	✓	5 / 3
3 - Taking Actions	GreenWaterTech		not received / 1
3 - Taking Actions	REWA	✓	not completed / 5

Figure 3-1 – Questionnaire / Booklet feedback on number of outputs for each AP project

The information on the AP projects provided through the questionnaire and the booklet is very relevant, but remains uneven in content. In order to manage this heterogeneity, **it is important to consider the content of this report as draft and evolving**. The content may be updated with future additional information (e.g. AP project results / outputs as they are delivered) beyond WP1.

4. State of the Art: Knowledge Available and Expected

In addition to a general introduction on aquatic pollutants, this section compiles available knowledge from the AP projects and other sources of information according to the following SoA categories (as defined in Section 3.2):

1. Analysis & monitoring
2. Introduction routes: Sources and pathways
3. Mechanisms of AMR and of co-/cross-resistance
4. Effects on human health and environment
5. Remediation and mitigation

This selection of the above-listed themes results from the compilation of information provided by the AP projects in relation to the scientific questions raised and **the expected knowledge (outcomes of the project).** This section is therefore not exhaustive but focuses on the expected results of the 18 projects.

For each category, the SoA is briefly described for each type of aquatic pollutants (chemicals, AMR and pathogens) and the added values of AP projects are stated. The contributions from the AP projects is presented in call-out boxes.

The bibliographical references used are provided in Annex C.

4.1. Introduction to "Aquatic Pollutants"

4.1.1. Contaminants of emerging concern (CECs)

Contaminants of emerging concern (CECs) are typically divided into chemicals, as they are properly called, and biological CECs, such as pathogens. CECs comprise a vast array of contaminants that have only recently been analysed in water, or that are of recent concern because they have been detected at concentrations significantly higher than expected, and/or their risk to human and environmental health may not be fully understood. CECs span natural and artificial chemical substances and their by-products, comprising pharmaceuticals (for human and animal consumption), personal care products (PPCPs), flame retardants (FRs), pesticides (i.e. plant protection products and biocides), artificial sweeteners (ASWs), nanoparticles, microplastics and their transformation products, but also antibiotic resistant bacteria (ARB), antibiotic resistant genes (ARG) and, more recently, the SARS-CoV-2 virus and even natural toxins (Pastorino and Ginebrada, 2021 ; Richardson and Kimura, 2020).

4.1.2. Antimicrobials and antimicrobial resistance

Antimicrobials – including antibiotics, antivirals, antifungals and antiparasitics – are medicines used to prevent and treat infections in humans, animals and plants. Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi and parasites change over time and no longer respond to medicines making infections harder to treat and increasing the risk of disease spread, severe illness and death. As a result of drug resistance, antibiotics and other antimicrobial medicines become ineffective and infections become increasingly difficult or impossible to treat.

Among antimicrobial resistance, antibiotic resistance is increasing in pathogenic, commensal, and environmental bacteria.

4.1.3. Harmful algal blooms

Harmful algal blooms (HABs) represent a natural phenomenon caused by a mass proliferation of phytoplankton (cyanobacteria, diatoms, dinoflagellates) in waterbodies. Blooms can be harmful for the environment, human health and aquatic life due to the production of harmful toxins and the consequences of accumulated biomass (oxygen depletion). These blooms are occurring with increased regularity in marine and freshwater ecosystems and the reasons for their substantial intensification can be associated with a set of physical, chemical and biological factors including climate changes and anthropogenic impacts.

4.2. Analysis & Monitoring

This category includes scientific questions and AP project outcomes pertaining to both the monitoring and surveillance of CECs, AMR and pathogens/microorganisms and others pollutants, as well as analytical methods.

4.2.1. Organic Contaminants - CECs

During the last couple of decades, monitoring studies to assess the occurrence, fate, and behaviour of CECs have been based on liquid and gas chromatography (LC and GC) coupled to mass spectrometry (MS) (Petrovic et al., 2020). More recently, high-resolution-mass spectrometry (HRMS) coupled to LC for identifying unknown contaminants is increasing in popularity, especially for the detection of transformation products and disinfection by-products. These new techniques include Orbitrap, time-of-flight (TOF), quadrupole (Q)-TOF, and sometimes Fourier transform (FT)-ion cyclotron resonance (ICR) mass spectrometers. Another emerging approach for monitoring is the development of sensors and biosensors, some of them for on-site remote operation. Biennial review covers developments in water analysis for emerging environmental contaminants are published (Richardson and Kimura, 2020 – period of October 2017−October 2019 and Richardson and Ternes, 2022 – period of September 2019−September 2021).

Despite analytical advances, knowledge of contamination levels remains fragmented for some organic contaminants. Thus, data on levels of biocides in European aquatic ecosystems is limited, partly because analytical methods are missing.

BIOCIDE will develop state-of-the art chemical analysis protocols for a range of antibacterial biocides applicable to different sample types and **generate screening data** for the presence and levels of antibacterial biocides in different environmental matrices from a set of different aquatic ecosystems in Europe and Africa.

To estimate the antibiotic contamination in aquaculture context, **ARENA** will develop a novel methodology based of flow cytometry for a rapid detection of antibiotic residues in edible products from fish farming.

4.2.2. Microorganisms and AMR

PCR (polymerase chain reaction) and molecular techniques are the techniques of choice for virus analyses. However, these molecular methods cannot discriminate between inactivated and infectious viruses. Recent studies have proposed the use of viability markers incorporated into qPCR-based methods (Quantitative PCR) for assessing infectivity of several human enteric viruses in waters of different origin.

Antiobiotic resistance is transferred from bacteria to others. One of the main vehicles for gene transfer are small circular pieces of DNA, i.e. plasmids (see section 4.4.). Tools and methods for plasmid typing and identification (Fernandez-Lopez et al., 2017), including the development of bioinformatic pipelines for the analysis of plasmids from genomic (i.e. genetic information of a single organism) and metagenomic data (i.e. mixture of DNA from multiple organisms and entities) (Vielva et al., 2017; Lanza et al., 2014) have been developed. These efforts led to the first global cartography of plasmid "species", or PTUs (Plasmid Taxonomic Units), which were described in a recent publication (Redondo et al., 2021). The global map can be found here (https://castillo.dicom.unican.es/PlasmidID/). Several methods for the functional analysis of plasmids (Tal et al., 2012) were also developed. Preliminary results indicate that MAPs form specific plasmid clusters are capable of oceanic-wide horizontal gene transfer, however, are poorly characterized.

To better monitor the transfer of viruses and AMR or ARG in different environmental compartments or matrices, analytical methods are required.

ARENA will develop sensor tools, based on impedimetric detection, for the selective on-site quantification of bacterial pathogens and contamination monitoring in aquaculture (environmental and biological samples).

PRESAGE will standardize the analysis and quantification of target ARM and ARG for water and sludge of wastewater treatment plants. Selected ARGs will be quantified in a consistent manner using high-throughput culture-independent methods based on qPCR or ddPCR (Droplet Digital PCR) after validation.

MAPMAR will use metagenomics, data science and single-cell sequencing to obtain a catalog of most prevalent and transmissible Marine Plasmids, these MAPs being able to transmit ARGs across oceanic distances.

4.2.3. Algae and cyanobacteria

Remote sensing methods for the study of algae and cyanobacteria are essentially based on the analysis of pigments as a means for the quantification of chlorophyll and suspended solids amount in water. To better locate, track, and understand a threat, a method to distinguish the potentially harmful organisms from the harmless, even though they look virtually identical to the human eye, is needed.

One potential method to achieve this is the hyperspectral imaging for which each kind of object has a unique spectral signature.

The recent availability of new satellites with a higher resolution data (i.e., EnMAP, PRISMA, Sentinel-2 and Landsat-8) is expected to provide a quantum leap for Earth Observation EO in the years to come. Recently, the Ocean and Land Color Instrument (OLCI) on-board the Sentinel-3 satellite has been launched. Its spatial resolution of 300 m is still far from what is required for fine detail analysis. In comparison, the SENTINEL-2 Multispectral Instrument (MSI) has four bands at 10 m resolution.

Microcystins are the most worldwide extended and common toxins produced by cyanobacteria in freshwater. Microcystin-leucine arginine (MC-LR), associated with the most toxic incidents involving microcystins, are within the cyanobacteria (intracellular) until released into the surrounding waters (extracellular) during cell lysis. Therefore, the relationship between intracellular and extracellular cyanotoxins allows a comprehensive risk of cyanobacteria-containing waters, preventing disease and improving human safety. A portable microfluidic sensing platform for the simultaneous detection of free (extracellular) and total MC-LR (intracellular and extracellular) was previously developed by the International Iberian Nanotechnology Laboratory (INL). The integrated system contains the sample processing and detection modules capable of performing the chemical lysis, filtration, sample mixing with antibodies, and electrochemical detection of MC-LR based on an indirect strategy. These immunosensors have a linear dynamic range between 10^{-4} and 10^{-7} g L^{-1} and a limit of detection of 6.6x10-10 g L^{-1} (dos Santos et al., 2019).

Cyanobacteria in excessive levels cause harmful algal blooms (HABs). They are also well-known producers of secondary metabolite called cyatoxins that are in most cases toxic to humans and other organisms. Microcystins are the most worldwide extended and common cyanotoxins in freshwater.

To better locate, track, and understand a threat by cyanobacteria in inland and coastal waters, a method to distinguish the potentially harmful organisms from the harmless, is needed.
AIHABs will introduce novel colour difference algorithms and texture feature extraction based on Deep Learning (DL) and Convolutional Neural Networks (CNNs) to exploit the full potential of higher data resolution from new satellites**.**

Besides, **the existing system** developed by the International Iberian Nanotechnology Laboratory (INL) allowing the simultaneous detection of free (extracellular) and total Microcystin-leucine arginine (intracellular and extracellular) **will be improved during the AIHABs for operation in the field.**

4.2.4. Wastewater-based epidemiology (WBE)

Wastewater-based epidemiology (WBE) assesses the presence or quantity of a chemical or biological signal in a pooled sample of sewage, taken from the sewer network or wastewater treatment plant (WWTP), to gain information on various aspects of public health. Concentrations of chemical or biological signals are measured to indicate drug or substance consumption patterns in a population or provide indicators of chemical exposures or illness. Other human biomarkers or biological signals such as viral RNA can indicate the level of disease or spread of pathogens within a population. WBE programs consist of sampling, sample preparation, analysis, data processing and interpretation, and reporting steps, each of which can introduce uncertainty into the final interpretation of the data (O'Keeffe, 2021).

WBE appears as a useful complement to "conventional" established monitoring tools to chemical or biological contamination or uses. But appropriate methods are needed for WBE implementation. **SARA** aims **to define best practices** for sampling, the standard operation protocols for the analysis of cultural parameters, and to determine the sample preparation for the molecular biological methods. **SARA** also consider SARS-CoV-2 detection in raw wastewater as a biomarker of COVID-19 cases and enteric viruses, antibiotic resistance and microbial source tracking.

4.3. Sources and Pathways of aquatic pollutants

This category includes scientific questions and AP project outcomes pertaining to both sources of the various aquatic pollutants and their pathways.

Anthropogenic emission sources include both point and diffuse sources, and hotspot reservoirs that receive, then further transmit pollutants. The term 'pathways' refers to the routes or mechanisms by which substances undergo dissemination/migration from the source to other environmental compartments as well as the exposure routes or mechanisms, including the exposure media, that characterize their dissemination. For a single substance, there can be multiple exposure or intake routes.

Despite multiple studies carried out during the last decade, there is still a lack of information about certain groups of CECs and pathogens and the impact of mixtures, specifically about their sources, transport, persistence, fate and behaviour under different climatic and environmental conditions.

4.3.1. Organic contaminants - CECs

Introduction routes into the aquatic environment for CECs consist of point and diffuse sources. Figure 4-1 shows the multiplicity of sources and complexity of the pathways. Within the context of the AP projects, this diagram 4-1 should be completed to take into account marine waters as other receptors.

Major point sources include wastewater treatment plants (WWTPs) in different contexts, urban areas, industries, agricultural activities and hospitals and therefore concern different CECs.

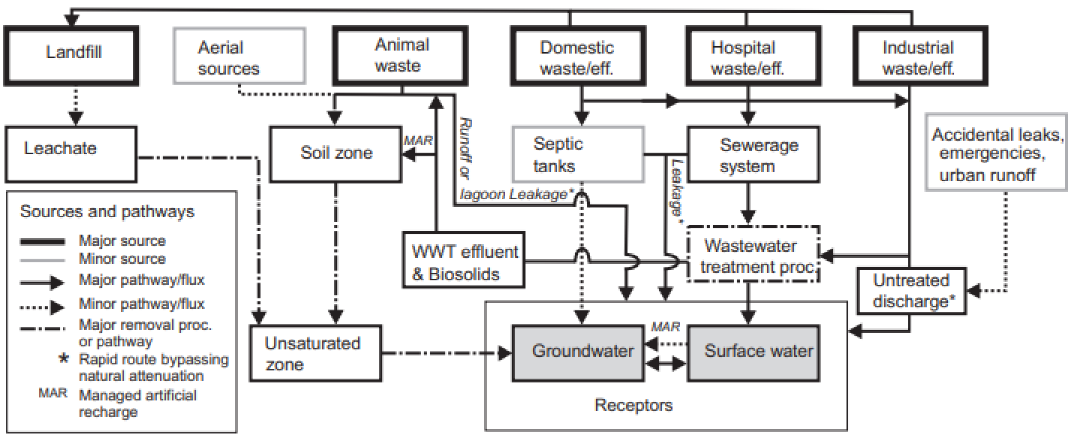

Figure 4-1 : Schematic diagram, using the source-pathway-receptor approach, highlighting potential sources and pathways of CECs (from Lapworth et al., 2012)

Depending on the pollutant, this diagram can also be adapted, underlining the difficulty of presenting a single source/transfer pathway diagram. The specific case of pharmaceuticals including antibiotics and aquaculture will be described below as examples.

Drivers and sources of CECs

Antibiotics present in ecosystems and aquatic compartments have different origins linked to different uses. One origin is the use of antibiotics in livestock farming. Uses vary from country to country. It is known that antibiotic use in Belgian livestock farming is high compared to the European average. The use of antibiotics in animal farming is even more alarming in Spain, which ranks among the EU countries with highest use of antibiotics per kg (Armstrong et al., 2018).

A second origin of antibiotic pressure is the use of antibiotics in aquaculture. A common issue with the fish cage nets used in aquaculture is the formation of biofoulings and biofilms, acting as a bacteria reservoir and reducing the water flow inside the cages. This implies frequent net cleaning and replacement, or the use of expensive antifouling agents that increase the operating costs of fish farming. 40% of the antibiotics used in the **intensive animal farming** to prevent/treat infections and promote animal growth, are excreted into the environment.

A third origin is linked to the consumption of human and veterinary medicines. Consumption of human and veterinary medicines in conjunction with improper disposal at domestic sites is a major cause for the release of pharmaceuticals into aquatic environments. Hospitals have been identified as an important source of CECs, generating between 4- and 12-times higher wastewater flows than households, significantly loaded with microorganisms, pharmaceuticals and other toxic chemicals.

The use of antibiotics varies widely from one country to another depending on veterinary uses or on population densities and their transfer into aquatic environment and their transfer into the environment is influenced by environmental conditions (climate, soil, etc.).

To characterise this variability, **PARRTAE aims** to study bacteria, antibiotic resistance genes (ARGs) and **antibiotic residues** in groundwater, surface water, marine water environments in the North Sea and Atlantic including ports and aquaculture facilities from **sites with high and low suspected loads of antibiotic residues**.

Pathways of CECs

Major pathways of CECs include wastewater treatment plants (WWTPs), industrial and hospital discharges, aquaculture facilities, animal farming and runoff from soils (Klatte et al., 2017; Yang et al., 2017). Antibiotics enter surface water compartments mainly with hospital and urban wastewaters, industrial wastewaters (in particular from pharmaceutical production facilities), leachate from waste disposal sites, agricultural runoff from fields fertilized with manure and aquaculture practice (Niegowska et al., 2021 - Figure 4-2). Figure 4-3 shows different sources and pathways and the connections between the different environmental compartments and figure 4-4 shows antibiotic concentrations measured in selected aquatic environments.

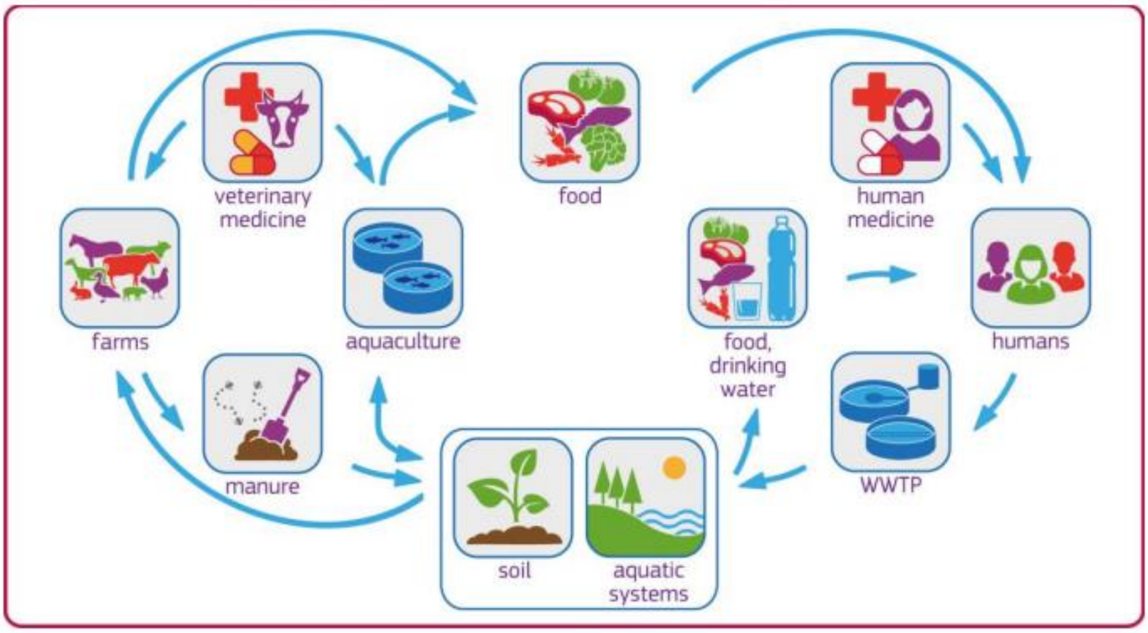

Figure 4-2 : Schematic representation of the environmental routes for antibiotics from human and veterinary uses (Sanseverino et al., 2018)

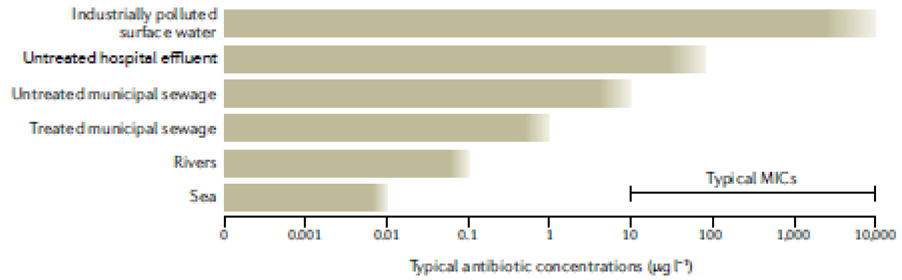

Fig. 2 | Antibiotic concentrations in selected aquatic environments. Different types of sources of antibiotic pollution typically give rise to different levels of exposure to aquatic bacterial communities. This, in turn, provides a reflection of the probability of environmental selection. Although very much a simplification, the ranges of typical antibiotic concentrations in aquatic environments exposed to excreted antibiotics from human use are depicted for the sea, rivers, treated and untreated municipal sewage effluents and untreated hospital effluents. Sea and river environments refer to those contaminated with treated municipal sewage. In addition, surface waters polluted directly by wastewater from drug manufacturing are included. As a comparison, typical minimal inhibitory concentrations (MICs) for many antibiotic–pathogen combinations often fall within the 10–10,000 μg l^{-1} range. As both depicted environmental concentrations and typical MICs are simplified illustrations representing many different antibiotics, an overlap between the two is not necessarily evidence of selection, unless there is overlap also for individual antibiotics. Note also that selection may occur at concentrations below the MIC.

Figure 4-3. Antibiotic concentrations in selected aquatic environments (Larsonn and Flach, 2022).

The contribution by domestic WWTPs depends on their technological design. Conventional systems have been developed to easily or moderately remove biodegradable carbon, nitrogen and phosphorus compounds as well as microbial organisms, using primary and secondary wastewater treatment processes. However, the majority of medicines are only partially removed by such conventional processes, and the application of more advanced treatments, such as active carbon filtration, ozonation and UV treatment (or combinations thereof), has only been implemented in very few European pilot WWTPs (Quintana et al., 2010; Bulloch et al., 2015; Huang et al., 2020; Oliveira et al., 2020). More information can be found in Section 4.6 on technical solutions for remediation.

As a consequence, pharmaceuticals still continue to be released into freshwater and marine environments (Figure 4-4) and the occurrence of pharmaceuticals is extensively documented in aquatic ecosystems worldwide (Rodil et al., 2012; Moreno-González et al., 2014; Madikizela et al., 2017; Desbiolles et al., 2018; Mezzelani et al., 2018; Almeida et al., 2020). Beside freshwater systems, such compounds have recently also been documented in coastal areas, where the dilution effect of marine water had been assumed to prevent a similar occurrence.

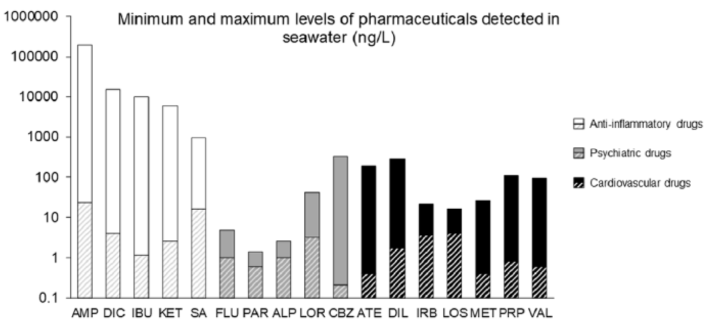

Fig. 1. Minimum (striped colored bars) and maximum (solid colored bars) concentrations (ng/L) of anti-inflammatory, psychiatric and cardiovascular drugs detected in seawater: AMP Acetaminophen, DIC Diclofenac, IBU Ibuprofen, KET Ketoprofen, SA Salicylic Acid, FLU Fluoxetine, PAR Paroxetine, ALP Alprazolam, LOR Lorazepam, CBZ Carbamazepine, ATE Atenolol, DIL Diltiazem, IRB Irbesartan, LOS Losartan, MET Metoprolol, PRP Propranolol, VAL Valsartan.

Figure 4-4 : Minimum and maximum levels of pharmaceuticals in seawater (Source: Mezzelani et al., 2018).

Monitoring studies are useful for evaluate the concentration in different aquatic compartments but they only provide pictures of past situations, while studies under controlled conditions only inform about specific cases. Modelling schemes that collect available and new information (including climate patterns, geological data and anthropogenic indicators) can contribute to better understand their transport and persistence, and be able to prevent contamination episodes, bacterial resistance transfer, and to determine areas under risk thereby improving the decision-making process.

Known sources of CECs into the aquatic compartments include runoff and seepage from landfills, agriculture, aquaculture, hospital, industrial and domestic wastewater. CECs discharged into the environment via the wastewater flow is often considered to be the main source. **Depending on the contaminant, knowledge on the sources and pathways varies. FOREWARN, SERPIC, PHARMASEA, BIOCIDE will produce new knowledge notably for pharmaceuticals including veterinary drugs, biocides and antimicrobials (see below for details).**

There have been several recent studies that demonstrate the potential for using machine learning and statistical techniques to predict the emergence of CECs and related events from other data and characteristics. After the data collection on CECs and pathogens (*in silico* case studies from past and on-going EU project), **FOREWARN will establish models of occurrence, transport and resilience to relate their presence with environmental and anthropogenic parameters**.

As a first step to select target CECs for treatment technology development and test, **SERPIC** will investigate **spread and transformation of CECs** (ARB, ARG and other chemicals compounds) **in different matrices** (water from raw municipal wastewater and treated effluent, soil and crops) at four regional showcases.

PHARMASEA will assess the distribution and characterization of pharmaceuticals in water and sediments of regional marine coastal areas, on wide regional range in order to unravel spatial and temporal variations.

As a first step to determine how antibacterial biocides (i.e. chemicals with antibacterial properties that are not used for treating infections) contribute to the development and spread of antibiotic resistant bacteria**, BIOCIDE** will generate screening data for the **presence and levels of antibacterial biocides** from a set of different aquatic ecosystems in Europe and Africa, enabling knowledge of sources and pathways in aquatic environments of this type of CECs.

4.3.2. AMR, Resistant bacteria and resistant genes

Local and catchment scale

Several pathways, including hospital effluent, agricultural waste, and wastewater treatment facilities, have been identified as potential routes for the spread of resistant bacteria and their resistance genes in soil and surrounding ecosystems.

The occurrence of antibiotic resistance is a natural phenomenon but it is enhanced by the use of antibiotics, as antibiotic resistance is selected under antibiotic pressure.

A natural background resistance exists but is poorly documented. **PARRTAE** will sample sites with high and low suspected loads of antibiotic residues to analyze the selective pressure promoted by antibiotics. **PARRTAE** will determine common ARG plasmids circulating in European waters.

FOREWARN will determine the presence and diversity of pathogens as well as the presence and spread of ARG in order to assess the contribution of WWTPs to the environmental and human resistome.

PAIRWISE aims to advance knowledge of antimicrobial resistance as a pollution in aquatic environments, wildlife, and livestock. PAIRWISE focuses on dispersal and dynamics of antibiotic resistant bacteria, antibiotic resistance genes and antibiotics in aquatic environments affected by WWTPs.

SARA will study the spread of viruses, ARB, ARGs and MST markers from wastewater, WWTP effluent, streams and rivers to the sea in different regions to take into account the effect of environmental factors such as climate.

MAPMAR will provide a description of the pathways of ARG spread in freshwater and marine ecosystems (major routes and accumulation sinks of ARGs), since their origin in hospitals, farms and urban settlements until their eventual return to the food chain via marine products.

Case of aquaculture / mariculture

Number of studies in the last decade demonstrated the primary role of aquaculture in promoting the spread of AR and its transmission to humans. Pathways of antimicrobial resistance (AMR) genes from closed and open aquaculture systems into the water are illustrated in figure 4-5.

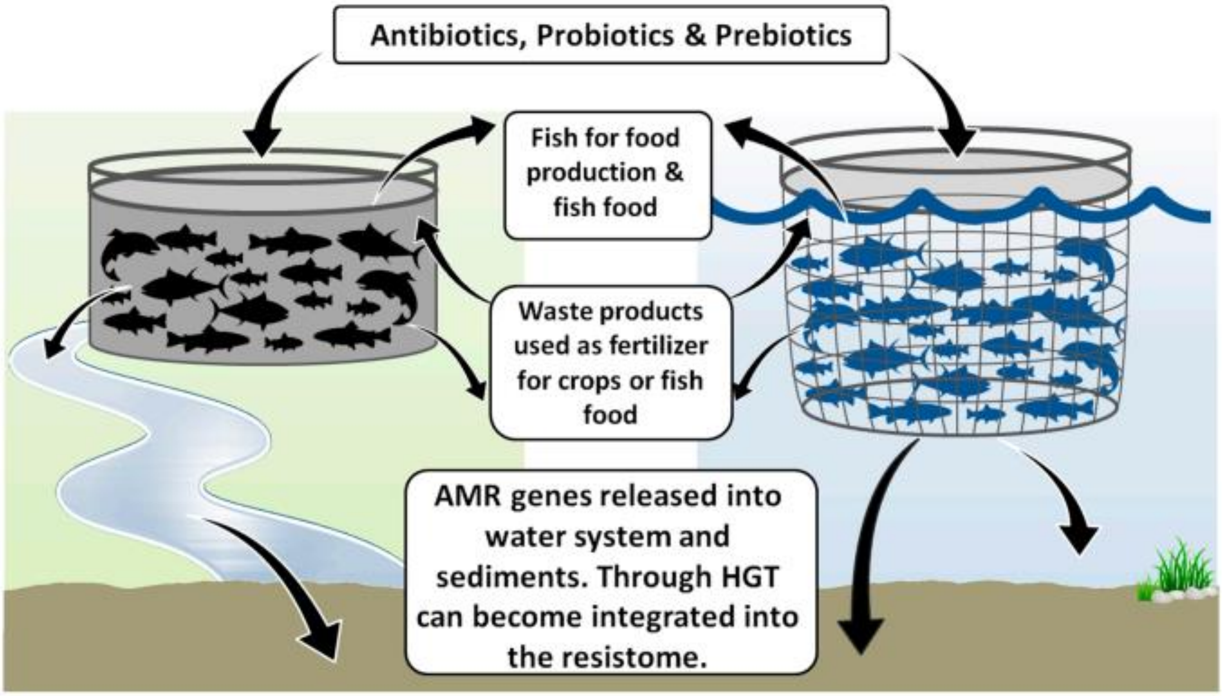

Figure 4-5 : Pathways of antimicrobial resistance (AMR) genes from closed and open aquaculture systems into the water and sediment environmental resistome (Watts et al., 2017). (HGT = Horizontal gene transfer).

AR in aquaculture are not just causing environmental pollution and economic damage to aquaculture but can also pose a serious threat to human health due to the high concentrations of ARGs in diverse resident pathobiomes. In particular, bacterial pathogens belonging to the genus *Vibrio* and their associated diseases are emerging in the coastal marine environment mainly as a consequence of the ongoing warming of marine waters representing a growing threat for human and animal health. AR is widespread in notorious *Vibrio* pathogens like *V. Cholerae* and *V. vulnificus*, but also infections contracted by seafood consumption contain resistant strains. Accumulation of pathogenic microorganisms in bivalve tissue from fecal contamination (e.g.*Salmonella* spp., *Escherichia coli*) is another concern for the shellfish industry, since resistance to a broad range of antibiotics are widespread and can readily be acquired via horizontal gene transfer. Evidently, aquaculture systems contain highly abundant and diversified bacterial communities, and the extensive use of antibiotics in shrimp aquaculture has already led to the emergence of resistant pathogens causing mass mortalities. One of the major aquaculture industries focussing molluscs is the production of Pacific oyster *Crassostrea gigas* and consumption of raw oysters also represents one of the major sources of food-borne infections (often caused by *Vibrio* species). **Yet, the relationship of aquaculture production of oysters and antibiotic resistance in associated *Vibrio* pathogens has only rarely been investigated despite the widespread use of antibiotics in hatcheries (SPARE-SEA).**

Recent studies, however, have shown evidence of ARG accumulation in mariculture products, even in settings where antibiotic administration is heavily regulated. This suggests that fish and shellfish may concentrate associated microbiota containing ARGs in the seawater they are grown with. Oceanic ARGs may have thus a return pathway, closing a vicious cycle that could boost the prevalence of antibiotic resistant infections in humans.

Aquaculture is expected to increase its importance in the global food chains. However, aquaculture has been identified as a gateway for antibiotic resistance either as they are exposed to contaminants due to discharges of chemical, either by antibiotics or others molecules used for the production.
As the variety of systems and fish species used for fish farming all over the world is manifold (estimates suggest more than 600 fish species raised in systems reaching from simple earthen ponds to highly mechanized Recirculating Aquaculture Systems), a generalization of data on source and pathways into aquatic systems of compounds used in aquaculture (i.e. antimicrobials and pharmaceuticals) is difficult.

One goal of **CONTACT** is to improve understanding of the consequences of antimicrobials on ARG emergence and dissemination **from aquaculture** activity into the aquatic ecosystems with special respect to major routes of entry, hotspots of emergence and spread and potential co-selection e.g. due to additional application of antiparasitics. Two "models" i.e. selected compounds with expected contrasted fate and behavior will be studied: florfenicol as a model for antimicrobials and emamectin benzoate as an antiparasitic.

SPARE-SEA focusing on a bivalve aquaculture (oyster farming) will characterize the oyster resistome and will identify environmental drivers and pathways of AR spread within and between environmental compartments including known and emerging pathogens.

PAIRWISE will consider the dispersal of microbial resistance through aquatic birds by producing ground-breaking knowledge about how different point-sources affect occurrence in aquatic birds, and the role birds play in transfer of pollutants between aquatic habitats, regionally and between countries and continents.

ARENA will address the source and pathways of antibiotic resistance in mariculture systems and in recirculating systems (using low or no antibiotics) in different periods (seasonal variations).

MAPMAR will provide a description of the pathways of ARG spread in freshwater and marine ecosystems.

4.3.3. Harmful algal blooms

Climate change pressures will influence marine planktonic systems globally, and it is conceivable that harmful algal blooms may increase in frequency and severity. These pressures will be manifest as alterations in temperature, stratification, light, ocean acidification, precipitation-induced nutrient inputs, and grazing, but absence of fundamental knowledge of the mechanisms driving harmful algal blooms frustrates most hope of forecasting their future prevalence. What currently is known and not known about the environmental conditions that favor initiation and maintenance of harmful algal blooms was summarized in 2015 (Wells et al., 2015). The occurrence of harmful algal blooms (HABs), particularly those producing toxins, is not well documented in many places across Europe (Wietz et al., 2019). However, monitoring and early detection of HABs is essential for the establishment of effective water governance policies.

To document the occurrence of harmful algal blooms (HABs) across Europe, reliable analytical techniques for analysis the toxins should be developed, long-terms studies should be conducted to allow identifying **the factors driving the formation for HABs, and methods for early detection and predictive models** should be developed. These are the objectives of the **AIHABs project.**

4.4. Mechanisms of AMR and Co-/Cross-resistance

This category includes scientific questions and AP project outcomes pertaining to antimicrobial resistance development. The aim of research in this area is to examine mechanistically how resistance is created and how it can be transferred to different environmental compartments.

4.4.1. AMR Mechanisms

As bacteria and genes often cross environments and species boundaries, it is critical to understand and acknowledge the connections between the human, animal and environmental microbiota (the One Health Concept) to manage this global health challenge (Larsson and Flach, 2022). Antibiotic resistance can arise both from mutations in the pre-existing genome of a bacterium and from the uptake of foreign DNA. Figure 4-6 illustrates the evolution of ARGs (antibiotic resistance genes) and the link between environment and/or the human/domestic animal microbiota. **How common the different evolutions are is still largely unknown.**

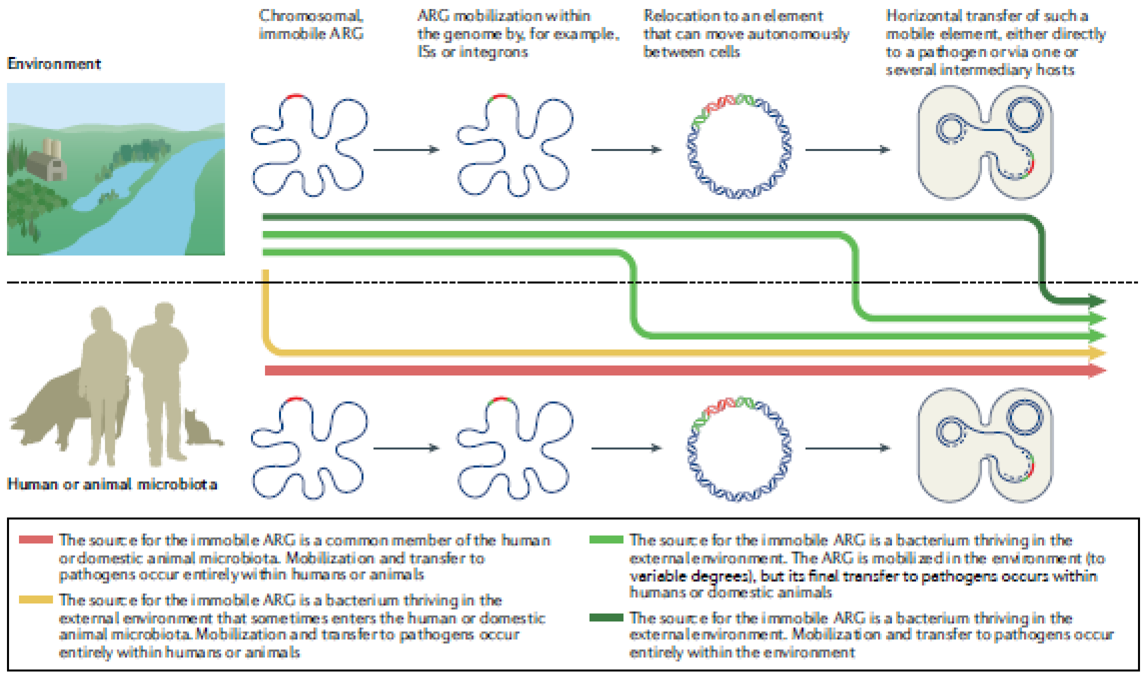

Fig. 1 | **The role of the environment in the emergence of new resistance genes in pathogens.** Conceptual illustration of how evolution leading to the emergence of a new antibiotic resistance gene (ARG; red) in pathogens can involve the environment and/or the human/domestic animal microbiota to different extents. The evolution typically occurs in steps, as indicated by the grey arrows. The first can be the association of a chromosomal ARG (red) with, for example, insertions sequences (ISs; green), which provide intracellular mobility. Intracellular relocation to, for example, a plasmid allows the ARG to move horizontally across strains and species. The mobilized ARG can then be transferred to a pathogen in one or several steps. In the most extreme cases, all genetic steps occur in either the environment (top) or in the human or domestic animal microbiota (bottom). However, at any stage bacteria carrying the ARG may move physically from the environment to the human or domestic animal microbiota, as illustrated by the differently coloured, thick arrows. The genetic reservoir is considerably larger in the environment, suggesting that the source for new ARGs is often environmental bacteria. By contrast, reoccurring, strong antibiotic selection pressures and close contact with pathogens are more common in humans and domestic animals, although some external environments also share those drivers. Environmental release of faecal bacteria may also boost the evolutionary process by providing genetic elements that are adapted to capture and transfer ARGs. How common the different depicted scenarios are is still largely unknown. A better understanding of how often the different evolutionary steps occur in the environment versus the human or domestic animal microbiota and what drivers are most important would enable more efficient resource allocation to limit or delay the emergence of new ARGs in pathogens.

Figure 4-6 - The role of environment in the emergence of new resistance genes in pathogens (Larsonn and Flach, 2022)

The dominating routes for transmission of (resistant) pathogens are between humans, between domestic animals and sometimes between animals and humans. These transmission routes can be direct or indirect via the external environment. There are also rarer and less predictable evolutionary events where new resistance factors are recruited to pathogens **by horizontal gene transfer** from the diverse, environmental microbiota. Such transfer events may occur either in the environment or within the human or domestic animal microbiota. The consequences of single gene transfer events may be vast and are irreversible.

One of the key reasons behind the fast propagation of antibiotic resistances is the ability of bacteria to transfer antibiotic resistance genes (ARGs) from one cell to the other (de la Cruz et al., 2000). These ARGs are shuttled by different mobile genetic elements (MGEs), gene platforms with the ability to colonize different strains and species. Among bacterial MGEs, plasmids stand out due to their ability to invade different species, therefore being fundamental agents of the spread of ARG through human and animal microbiomes (Smilie et al., 2010). **Plasmids are key vectors of horizontal gene transfer** (Figure 4-7).

In environmental water or contaminated drinking water, pathogenic bacteria may potentially meet and mix with resident aquatic bacterial communities and exchange of genetic material might occur

(Arias and Murray, 2009). ARGs are often located on mobile genetic elements such as plasmids or transposons. Thus, the dissemination of ARGs occurs by horizontal transfer mechanisms, i.e., transformation (bacteria take up DNA from their environment – this genetic material often comes from adjacent lysed bacteria and can include plasmid DNA or fragmented DNA released into the environment), transduction (Bacteriophages (bacterial viruses) move genes from one cell to another), and conjugation (Bacteria directly transfer genes to another cell). Vertical gene transfer also occurs when genetic information is transferred from one generation to the next. Synergy of genes transfer is represented in Figure 4-7.

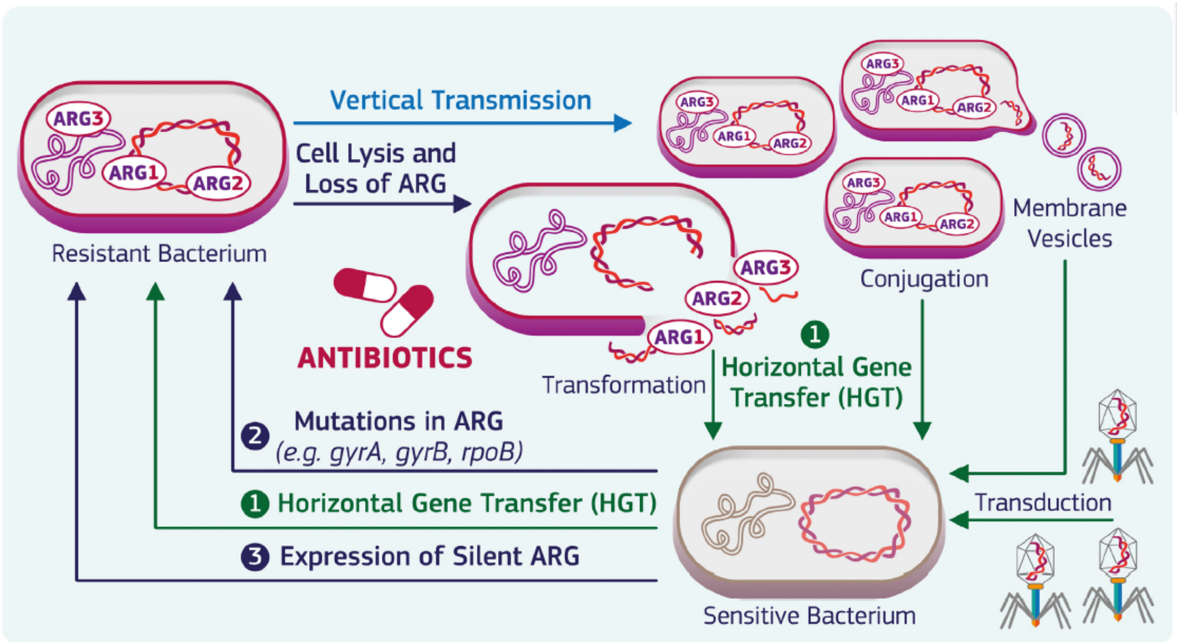

Figure 1. Synergistic effects of antibiotics and ARGs on the spread of AMR. ARGs can be acquired by sensitive bacteria following exposure to antibiotics present in the environment. This may occur through several pathways such as 1. HGT through conjugation with already resistant bacteria, transformation with plasmids or membrane vesicles carrying ARGs, transduction by phages and/or uptake of free-floating extracellular DNA; 2. Mutations selecting for resistance in genes encoding key bacterial proteins which are antibiotic targets (e.g. mutations in *gyrA* and *gyrB* which are responsible for resistance to fluoroquinolones, and mutations in *rpoB* which confer resistance to rifampicin) while other genes may encode enzymes able to actively degrade antibiotics (e.g. β-lactamases); and 3. Expression of silent resistance genes. Much more severe pressure due to antibiotic use in human and veterinary medicine also results in the expression of resistance in exposed microorganisms. ARGs released from lysed cells may be uptaken from waterbodies by sensitive microorganisms or those already bearing other resistance genes, in the latter case leading to multidrug resistance. Vertical gene transfer due to the proliferation of resistant bacteria further contributes to the spread of AMR.

Figure 4-7 - Synergistic effects of antibiotics and ARGs on the spread of AMR (Niegowska et al., 2021)

Aquaculture and raw seafood consumption has been identified as a main gateway for spread of antibiotic resistance of clinical concern (e.g. beta-lactamases). Clearly, also the use of the same antibiotics used to treat humans in oyster hatcheries has potential to increase the abundance of ARB in diverse oyster microbiomes, since **aquaculture systems in general have been designated as “genetic hotspots” for gene transfer**. Not only are oysters cultured in highly anthropized environments, but as filter-feeders they can act as bio-reactors that concentrate contaminants and microorganisms, thereby accelerating horizontal gene transfer and the spread of ARGs among microbial communities. Indeed, genes related to mobile genetic elements, particularly those involved in conjugative transfer, are highly induced in vibrios during oyster colonization. Similarly, biofilms in mixed bed biofilters are also a reservoir for antibiotic resistance genes.

Aquaculture led to the massive use of antimicrobials but also antiparasitics. Aquaculture and raw seafood consumption have been identified as main gateways for the spread of antibiotic resistance.

In the context of aquaculture**, CONTACT** will assess the spread of ARGs and mobile genetic elements (MGEs) into aquatic environments and throughout all trophic layers, the shift in the structure and function of free-living microbiomes as well as specific host and food web structures. Freshwater and marine water and different climatic regions will be considered.

PARRTAE will determine the type of plasmids from different environments for transmission frequency and ARG profiles. Their ability to transfer to recipients (marker species E. coli, S; algae, Vibrio spp) will be analysed.

SPARE-SEA will determine how human activities like the input of chemical contaminants activate MGEs in aquaculture and the role of oyster bioreactor in the transfer of ARGs. The project will determine where, when and why ARBs circulate in ecosystems exploited for oyster culture.

MAPMAR will identify and evaluate the transmissibility of marine plasmids, their association to ARGs and their dispersal through oceanic waters.

SARA will focus on the identification of ARGs in plasmids and determination of the dissemination potential of ARGs by conjugation and evaluation of the prevalence of key ARGs in non-bacterial fractions from the water environment.

4.4.2. Co-/Cross-resistance Mechanisms

Pollutants involved in co/cross-resistance

Alongside antibiotics, **heavy metals** from natural and anthropogenic sources are widely distributed in water environments. Elevated environmental exposure to metals has raised an ecological and public health concern linked to the effects of metal toxicity (Bradl, 2005). Increasing scientific evidence demonstrates the relationship between molecular strategies developed by microbial species to resist pressure from a range of metals, antibiotics and the spread of AMR (Imran, et al., 2019; Komijani et al., 2021; Pal et al., 2017; Poole 2017). It has been shown that metal resistance and antibiotic resistance in microorganisms are governed by shared genetic mechanisms and can be encoded by the same genetic elements (Randall et al., 2015). Similar findings have been described for **cross-resistance to antibiotics and biocides** (e.g. triclosan) (Carey and McNamara 2015; Gilbert and McBain, 2003; Webber et al., 2017). In particular, selection for heavy metal tolerance is often accompanied by antimicrobial drug resistance, since ARGs and chemicals resistance genes are often co-located on mobile genetic elements, co-regulate their expression or employ common resistance mechanisms (such as efflux pumps). Such "co- selection" has been observed in wastewater treatment plants and can lead to the spread of ARGs even in absence of the specific stress (antibiotic presence), increasing resistance complexity in the environment and the potential risk for human health. In particular, the **relationship between environmental concentrations** of antibiotics and the acquisition of ARGs by antibiotic-sensitive bacteria **as well as the impact of heavy metals and other selective agents on antimicrobial resistance** (AMR) need to be defined (Niegowska et al., 2021).

Mechanisms of co-resistance or cross-resistance

There are at least two main mechanisms by which biocides may **co-select** for antibiotic resistance. The first involves **co-resistance**, where the biocide resistance genes are present on the same, mobile genetic element (e.g., a plasmid) as the antibiotic resistance genes. Such co-localization will indirectly select for antibiotic resistance. The second is referred to as **cross-resistance** where the biocide and the antibiotic share a common resistance mechanism (e.g., up-regulation of efflux pumps). For both these mechanisms, exposure to biocides will directly and inevitably enrich already co-resistant strains, and thus promote their dissemination and ultimately increase **transmission** risks. In addition, there is evidence that the immense genetic diversity of the environmental microbiome contributes to **emergence** of novel mobile antibiotic resistance determinants in pathogens; these are less common evolutionary events but with potentially profound consequences. Here, selection from biocides could very well play a key role if cross-resistance is involved, while this is not the case for co-resistance – unless the different genes are linked from the very beginning. **Therefore, understanding the relative roles of co- versus cross-resistance mechanisms in co-selection is essential to assess and manage different risk scenarios.**

The role of biocides in the development of antibiotic resistance must therefore be carefully gauged and controlled. Biocide use is controlled by the EU Biocidal Products Regulation (BPR, Regulation EU 528/2012), which also asks to evaluate and manage the risks for development of resistance or cross-resistance (Art. 19.1.b.ii). **However, currently resistance risk is not addressed satisfactorily because the scientific foundation is incomplete**, and accordingly there are no harmonized procedures. In addition, many antibacterial substances have their primary use in areas that circumvent the BPR, such as in plant protection product, food preservation and personal care products. There is still an opportunity to regulate or control exposure via e.g. the Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD). However, for no chemical so far has the risk of antibiotic resistance promotion played a role in its regulation. However, this would be feasible, as supported by the recently proposed Environmental Quality Standards (EQS) for ciprofloxacin (an antibiotic) prepared by consultants to the Swedish Agency for Marine and Water Management (Sahlin et al., 2018).

Recent data indicate that pollution at low levels of antibiotics, detergents, heavy metals or biocides in water might facilitate the transfer of ARGs from one bacterium to another**. It is not only antibiotics that can drive resistance development, but also co-selection.**

CONTACT will evaluate the risk of co-selection of ARGs and MGEs due to administration of antiparasitics and other confounding factors such as disinfectants or heavy metals in aquaculture.

BIOCIDE's overall aim is determining how antibacterial biocides (i.e. chemicals with antibacterial properties that are not used for treating infections) contribute to the development and spread of antibiotic resistant bacteria in different aquatic/marine ecosystems, and to inform and enable measures that ultimately protects human health and safe water resources for both humans and wildlife. **BIOCIDE** will directly assess co-selection by competition experiments in complex aquatic communities using a subset of biocides and quantify the potency of selected biocides to induce horizontal transfer of antibiotic resistance genes via conjugation and transformation.
SPARE-SEA, thanks to an experimental approach, will assess whether the horizontal transfer of ARG is enhanced in the oyster bioreactor and how the cumulative effects of antibiotics and agrochemicals (heavy metals from vineyards, pesticides from rice culture) can select for the spread of ARGs.

REWA will study the co-selection potentials to antibiotic resistance in polluted waters before and after treatment and in aquatic reference habitats.

4.5. Effects on Human Health & Environment

This category includes scientific questions and AP project outcomes pertaining to effects of aquatic pollutants (CECs and AMR) on ecosystems and human health.

4.5.1. Effects on human health

The effects of aquatic pollutants on human health are widely mentioned in the projects, whatever the type of contaminant, but none of the projects deal directly with this aspect.

In an indirect way, AMROCE will consider the effects on human health of a mitigation solution developed to reduce antimicrobial uses. Antimicrobial/antibiofilm fish cage nets and wastewater filtration membranes will be developed through polymer and surface nano-engineering. Marine-derived antimicrobial agents and antibiofilm enzymes will be nano-formulated as an alternative to antibiotics for fish and animal feed supplement. Human and environmental nanosafety during the manufacturing and use of the novel nanotechnology-embedded products will be continuously evaluated to anticipate nanosafety issues.

4.5.2. Effects on the environment

The environmental consequences of pharmaceutical residues have recently emerged as a major research area in marine science. The available information is still fragmented for non-target species (i.e. species that is not intentionally targeted by the use of a specific compound e.g. antibiotics), with a prevalence of studies focusing on NSAIDs[9] and psychiatric drugs as opposed to cardiovascular and lipid-regulating drugs, synthetic steroidal hormones, and antibiotics. Well-documented effects of single classes of pharmaceuticals, dosed at low, environmentally realistic concentrations, evidenced marked similarities in modes of action between target and non-target species, with the same cellular pathways involved in metabolism or the onset of adverse consequences.

However, a key feature to consider when assessing the impact of pharmaceuticals on marine species is the typology and conditions of exposure. Target organisms typically ingest specific compounds intended to alleviate a particular disturbance at a defined dosage and time of treatment. By contrast, under field conditions non-target species are exposed, potentially for their entire life cycles, to low doses of several classes of co-occurring drugs that modulate a variety of pathways and metabolic processes. A recent review (Mezzelani and Regoli, 2022) highlighted the complexity of such interactions), revealing either synergistic or antagonistic effects on the same cellular targets. Notably, the intricate network of mechanisms regulating organism responsiveness to pharmaceuticals might be further challenged by the simultaneous presence of other types of chemical pollutants (trace metals, polycyclic or halogenated hydrocarbons, microplastics, biotoxins, etc.) or environmental stressors (such as ocean acidification and temperature increases) that affect the same cellular pathways.

An increasing body of evidence highlights the **impairment of multiple biological processes** including reproduction, growth, metabolism, immunity, feeding, locomotion, colour physiology and behaviour in fish, molluscs and other **aquatic invertebrates exposed to pharmaceuticals** at environmentally relevant concentrations but the **available information is still fragmented for non- target species** in marine contexts.

The project PHARMASEA will allow i) in-depth studies on Active Pharmaceutical Ingredients (API) distribution, effects and risks in four European coastal areas (marine areas), ii) occurrence, uptake and trophic transfer along regional marine food webs; iii) bioaccumulation/excretion kinetics, **potential ecotoxicological effects from molecular to individual levels**, and characterization of modes-of-action in model and selected marine species; and iv) development of specific risk assessment procedures for APIs.

The project BIOCIDE will generate a **dose-response matrix for a large number of biocides** and bacterial species in order to predict No Effect Concentrations (PNECs) for growth that will also be protective against antibiotic resistance co-selection in different aquatic/**marines ecosystems.**

Accumulation of ARGs in fish and shellfish produced by mariculture represents a serious threat to human health. **MAPMAR** will study the feasibility of minimizing the spread of AR (different interventions including chemical compounds use) in order to attain a safer employment of continental and sea waters for human nutrition.

PRESAGE aims to evaluate the ecotoxicity of effluents from various Waste Water Treatment Plans (hospital, urban, …).

[9] *Non-steroidal anti-inflammatory drugs*

4.6. Remediation & Mitigation

This category includes scientific questions and AP project outcomes pertaining to the remediation and mitigation of aquatic pollutants. On the one hand, the aim is to assess whether existing technologies (treatment plants) or nature-based solutions are sufficiently effective in preventing the spread of CECs and AMR. On the other hand, the aim is to evaluate whether solutions that enable reduction at the source exist.

4.6.1. Wastewater Treatment: Case of water use or discharge to the environment

Conventional wastewater treatment plants (WWTPs) are designed to remove pathogens and coliforms and to reduce loads of carbon, nitrogen, and phosphorus. The removal of many expected and emerging (i.e. not yet regulated) contaminants, including pharmaceuticals and personal care products, hormones, and other industrial chemicals is, however, incomplete. **Hence, WWTPs have been identified as a source of such pollutants in the aquatic environments** (Loos et al., 2013; Golovko et al., 2021)**.**

It has also been shown that conventional wastewater treatment **processes remove antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) at limited efficiencies**, leading in certain cases to their increase in final effluents (see references in Niegowsha et al., 2021).

Thus, wastewater treatment facilities do not always fulfil the minimum security and environmental requirements for water reuse defined on national or European scale. This point was highlighted by of a group of international experts, members of the NEREUS COST Action ES1403. Their main conclusion is that a single advanced treatment method is not sufficient to minimize the release of chemical CECs and ARB/ARGs and make wastewater reuse for crop irrigation safer, but a smart combination of them (Figure 4-8) and a suitable monitoring program would be necessary (Rizzo et al. 2020).

Importantly, **in some cases, a solution to one target hampers another**: Some processes favouring CECs degradation may lead to increased AMR. Therefore, we can anticipate that it is not always possible to reach jointly the objective of minimising the discharge of the different contaminants of emerging concern (CECs), both CECs and AMR.

When water is polluted and decontamination becomes necessary, the best treatment should be chosen to reach the **decontamination objectives as established by legislation.** Treated wastewater can be discharged into the environment (e.g. surface waters) or reused. Three main types of water reuse are identified: 1) direct potable reuse, where appropriately treated waste water is fed into the water supply network; 2) indirect potable reuse, where treated waste water is released into surface waters and groundwater used as drinking water sources; and 3) reuse for non-drinking purposes, including agricultural irrigation, industrial use (for instance, as processing or cooling water), recreational use (for example, for snowmaking or golf course irrigation), environmental use (for instance, for groundwater recharge or wetlands restoration), and urban use (for example, for irrigation of public parks, fire protection systems or street cleaning.

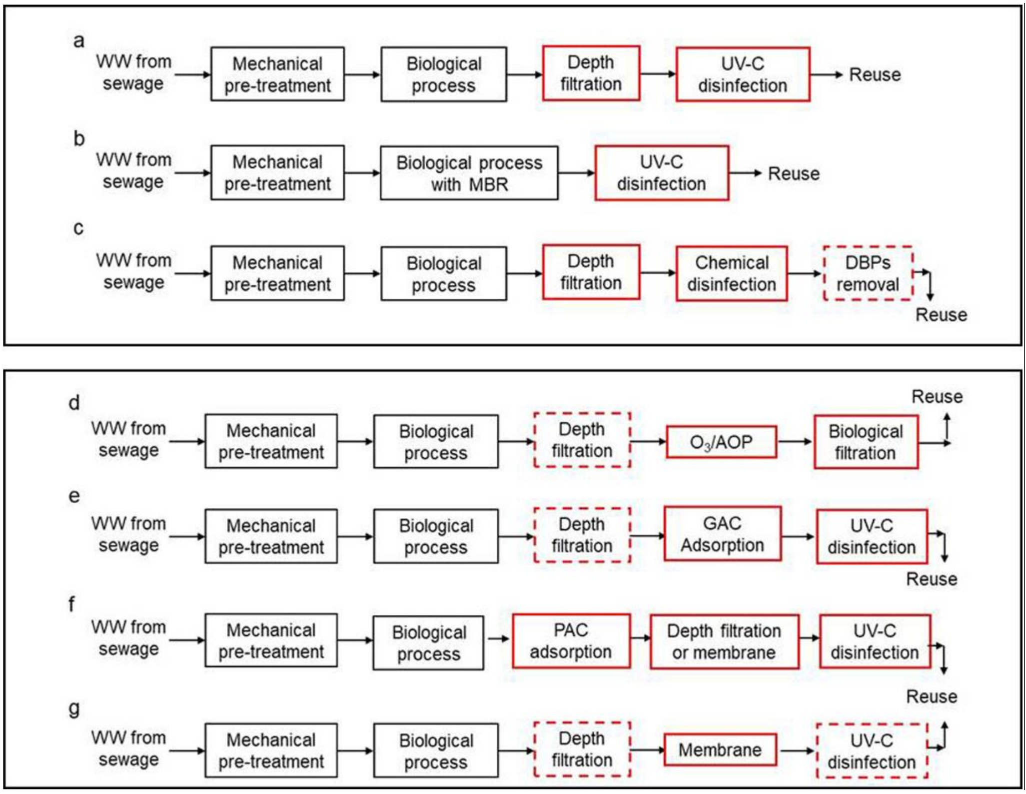

Figure 4-8 - Different options of treatment trains for urban wastewater reuse to address traditional parameters set in wastewater reuse regulation and guidelines (e.g., BOD, COD, TSS, E. coli etc.) (a, b, c) and to effectively remove CECs in addition to the typical parameters (d, e, f, g) (Rizzo et al., 2020).

Besides water use / discharge to the environment, treatment processes have also to be developed and selected according to the wastewater type (e.g. influent from industries, hospitals, black and grey water, etc).

SERPIC will propose a technology to treat the effluents of wastewater treatment plants (municipal source) for irrigation crops (REUSE) and for discharge to the aquatic system.

The other AP projects propose to develop treatment processes or strategy of several wastewater types for discharge to the aquatic system:

- GREENWATERTECH will validate new water cleaning methodologies (combination of muddles for decentralised uses) relevant for wastewater from industries and hospitals discharges.

- NanoTheC-Aba targets wastewater treatment system, covering different wastewater origins such as hospitals, tanneries, aqua-mariculture, industrial wastewater, agriculture, utilities.

- PRESAGE technologies will be validated at 4 demo sites treating black and grey water, and effluents from hospitals and an antibiotic industry.

- REWA technologies (regarding wastewater) will be validated for urban wastewater treatment plants as well as metallurgical wastewater industry. REWA will also address surface water treatment.

4.6.2. Which technologies for which objectives?

Reuse of treated wastewater for irrigation in agricultural context

Recent reports on more than 20 conventional wastewater treatment systems situated in the UK, Europe and South Africa applying tertiary treatment showed e.g. reduction of analgesics (60 – 99 %), except for Tramadol and its breakdown products (<1 %); antibiotics (42-96 %); anti-inflammatories (75-95 %), except diclofenac (18 %); other pharmaceuticals (60 – 99 %), except venlafaxine (23 %). The efficiency of these technologies is also challenged by the variance in CEC reduction within a specific CEC class. In this context, combined treatment can be necessary. A multi-barrier treatment approach was recently proposed in the NEREUS COST Action ES1403 (Deliverable 17), which discusses the best available technologies for wastewater treatment related to the reduction of CECs and resistant microorganisms in the context of irrigation of crops. In this sense, the combination of membrane filtration and the electrochemical production of powerful oxidants (peroxosulfate and chlorine dioxide) activated by deep UV (UVC), emerges as a promising combination to reach a process with a very high reliability to degrade CECs and disinfect not only bacteria but also viruses, together with a limited formation of hazardous by-products and with limited cost.

The main conclusion of a group of international experts (NEREUS COST Action ES1403) , is that a single advanced treatment method is not sufficient to minimize the release of chemical CECs and ARB&ARGs and make wastewater reuse for crop irrigation safer. A prototype treatment plant will be setup and test through the **SERPIC** project. **SERPIC** combining membrane nano-filtration technology and a residual disinfection using chlorine dioxide produced electrochemically to the stream used for crop irrigation (Route A) or light driven electro-chemical oxidation when discharged into the aquatic system (route B).

Case of aquaculture: a need for quality water inlet and outlet

Aquaculture both necessitates water of suitable quality for aquafarming and generates / releases (waste) water from its practices. In most of the cases wastewater-dominated streams/rivers discharge their water into coastal or estuarine areas with high aquaculture development. In this regard, the occurrence of CECs in seafood receiving seawater intrusion was observed as also inland drainage from agriculture and wastewater within the premises of the seafood production farming area (Gadelha et al., 2019). The correlation between seasonality and human activities highlighted the need for environmental protection and sustainable resource exploration for safe seafood production.

The **NATURE** project tackles the issue of water quality for aquaculture **(inlet)** by assessing and promoting the resilience to aquatic pollutants in estuarine areas. This project will assess **nature-based solutions (NBS) as management option** for water treatment on the catchment scale including conventional and high-end constructed wetlands, river re-naturalization, and restoration of wetlands will cover the continuum from urban sources to coastal biota in estuaries.

The performance of conventional wastewater treatment units in treating CECs compound in **aquaculture effluents** was reviewed and assessed (Ahmad, 2022). Many conventional wastewater units can remove CECs from aquaculture effluents. The most reported CEC was oxytetracycline, and the most reported treatment units were adsorption and constructed wetland. Oxytetracycline is commonly used as an antibiotic in aquaculture for treating fish disease caused by bacterial infection (Choi et al., 2020). Increase in oxytetracycline concentration in wastewater is highly related to the introduction of the compound into ponds for the treatment and prevention of diseases. Only a small amount of the introduced compound is actually consumed, whereas residues remain in systems and are released during water discharge (Nguyen Dang Giang et al., 2015).

Veterinary products and other types of compounds are used in aquaculture and can be incompletely treated by current technologies. To reduce this source of contamination (**effluent**), new technologies are needed.

NanoTheC-Aba develops several wastewater treatment systems, one being dedicated to aqua-mariculture waste water.

AMROCE develops **wastewater filtration membranes** through polymer and surface nano-engineering to reduce antibiotic pollution and AMR bacteria spread **in the context of aquaculture.**

Drinking water supply

Humans are at significant risk of contracting antibiotic resistance via exposure to contaminated waters, including drinking water, and there is thus a strong need for environmental solutions to mitigate the looming antibiotic resistance crisis. A major challenge in many countries is to provide safe and sufficient amount of drinking water. Especially, people living in rural areas may not have direct access to safe drinking water supply. In addition, today's water plants combine practices that are designed in treatment stages, where each individual treatment is likely to be suboptimal. This complexity constitutes an enormous challenge, particularly due to the system's inherent dependence on a vast array of parameters that are of a continuous, integral and categorical nature. Importantly, in some cases, a solution to one target hampers another—e.g., disinfection by chlorination generates hazardous organic contaminants and can even concentrate various ARGs and other contaminants of emerging concern (CECs).

To protect human health, obtaining high-quality potable water necessitates research and development of new and effective technological approaches, which are also affordable in rural areas.

REWA project will offer solutions for **surface water treatment** largely based on use of specifically prepared nature-based materials (clays, carbon-based materials and wood residues), which are well **available worldwide**.

4.6.3. Treatment types and technologies

Decentralised water treatment

Decentralised treatment of wastewater is often based on compact technologies, such as membrane bioreactors (MBRs). They work at high solid retention times (SRTs), which derives in high solid/biomass concentrations and greater microbial community diversity. These operating conditions lead to high quality effluents in terms of suspended solids, protozoa and coliform bacteria and, under optimal conditions of some viruses. Also the removal of moderately biodegradable OMPs has demonstrated to be enhanced.

On the contrary, some studies evidence that working with enhanced primary metabolic activity and long SRTs favour the development of ARMs. Therefore, we can anticipate that it is not always possible to reach jointly the objective of minimising the discharge of the CECs, both CECs and AMR. **In such**

cases, the development of ecotoxicological indicators to determine the global effect of the final effluents on the aquatic system, supposes a promising alternative to define the optimal treatment strategies.

Decentralised WWTPs, based on photocatalyst, can prove valuable to treat the more recalcitrant CECs (e.g. the DEWATS test facility in Durban, treating effluent from only 80 households).

PRESAGE aims to develop sustainable decentralized treatment processes based on anaerobic and aerobic systems. The technologies will be validated at 4 sites treating various effluents in particular by taking effluent ecotoxicity into account.

GREENWATERTECH develops a water cleaning concept, amenable for decentralized use, using non-critical and non-toxic materials to eliminate contaminants of emerging concern (CECs) and pathogens, including antibiotic resistant bacteria (ARB).

The WWT systems developed by the other **AP projects** address central WWTPs.

Adsorption processes

Adsorption on activated carbon, implemented for the treatment of drinking water, has recently been used at full scale for municipal wastewater reclamation. The process bottleneck is the limited efficiency in the reduction of CECs.

Sorption using biobased sorbents is a feasible alternative due to their sustainability, low cost and abundance. Biosorbents are also easy to apply on many scales. For example, sawdust has shown promising potential for removing different classes of antibiotics from water.

The wider literature review enable to have feedback on the promising metal-organic frameworks (MOFs) and MOF-based nano-adsorbents (MOf-NAs) to remove CECs (Joseph, 2019). MOf-NAs have very **high-performance adsorption**. However, adsorption of different CECs varies significantly depending on the physicochemical properties of the compounds, the physicochemical properties of the MOf-NAs themselves, and the water quality. Moreover, **many challenges remain such as long-term stability, high cost and disposal**.

REWA project will offer solutions for water (surface water and sewage effluent) treatment largely based on use of specifically prepared **sorbents** made out of nature-based materials (clays, carbon-based materials and wood residues), which are well available worldwide**.**

Filtration

Rapid sand filtration followed by UV irradiation represents a widely applied treatment sequence, able to reduce suspended solids, bacteria and viruses, but **with limited efficiency to some CECs and without a persistent disinfection effect**.

Membrane processes, commonly applied as a barrier for pathogens, have the potential to reduce CECs. Especially nanofiltration (NF) and reverse osmosis (RO) have been reported to reduce ARGs below levels of detection. NF, being less energy intensive than RO, seems to be more promising for the reduction of CECs. However, **the accumulation of the rejected constituents in the membrane concentrate is not sufficiently solved yet and remains a problem**. Nanofiltration (NF) solutions were proposed for polluted water treatment, although the AMR pathogens and CECs abatement remains a challenge. NF membranes have typically pore size between 1-2 nm. NF membranes have lower ion rejection than RO membranes, but can offer several advantages such as low operating pressure and high permeability, resulting in relatively low investment, operation and maintenance costs. **NF membranes can reduce hardness, and remove small organics molecules, virus and bacteria from aqueous systems**. Thus, NF membranes are used for water depollution and detoxification. At the present, NF market is dominated by polymeric membranes. But these membranes typically suffer from swelling, biofouling, scaling and poor thermal and chemical resistance, which limit their operation time and make difficult to clean them. Compared with polymeric membranes, ceramic membranes have higher thermal, mechanical and chemical resistances. Among the ceramic materials, silicon carbide (SiC) has outstanding chemical stability in harsh conditions, for example in corrosive and high-temperature environments. Compared with polymeric and with oxide materials such as titania and zirconia, SiC membranes are very hydrophilic and exhibit low fouling. However high sintering (frittage) temperatures and the addition of sintering aids are usually required their production, implying: (i) expensive processing and (ii) difficulty to fabricate thin SiC layers in NF range by partial sintering. Thus, the use of SiC membranIs is rather limited today and it is mostly relegated to microfiltration processes. Nevertheless, alternative processing routes for the preparation of mesoporous SiC at reduced temperatures, based on the conversion of metal organic polymers to SiC, the use of sintering additives, as well as the use of nanoparticles (NPs) are now available (Nano-Carb).

Kim (2018) undertook an extensive review on CECs removal by filtration. He stated the following CEC removal trend: (i) the removal efficiency for the **membranes follows the declining order: RO ≥ FO > NF > UF**; (ii) the retention of CECs by RO and FO membranes is mainly governed by size/steric exclusion, while high retention can still be achieved due to hydrophobic (adsorption) and electrostatic (attraction) interactions for NF and UF membranes; (iii) more polar, less volatile, and less hydrophobic organic CECs have less retention than less polar, more volatile, and more hydrophobic organic CECs; (iv) while, in general, FO and RO membranes show significant metal/toxic anion retention (> 95%) regardless of water quality and operating conditions, metal/toxic anion retention by NF and UF membranes is more efficient at neutral and alkaline conditions than at acidic values; and (v) while UF alone may not effectively remove CECs, it can be employed as a pretreatment step prior to FO and RO. However, numerous studies were limited to a few membranes (e.g., FO, RO, NF, or UF), focused on synthetic solutions, or examined only a few compounds under limited solution pH/conductivity ranges and operating conditions. A systematic retention assessment of various CECs was deemed necessary as insufficient information was currently available about FO, RO, NF, and UF membrane processes to allow full-scale implementation.

SERPIC will combine membrane filtration and light driven electro-chemical processes to reduce CECs from WWTP effluent.

Destruction

The application of **chlorination** is developed at full-scale to produce drinking water, but has limited efficiency for the abatement of CECs in wastewater. Furthermore, the formation of chlorinated by-products as the hazardous trihalomethanes and haloacetic acids is known. Hence, obtaining high-quality potable water necessitates research and development of new and effective technological approaches, which are also affordable in rural areas.

Ozonation has been shown at full scale in Germany and Switzerland to reduce a wide spectrum of CECs; however, by-products formation, cost-intensive installations and high-power consumption are major impediments. **Photocatalytic ozonation** is an efficient process to remove persistent organic compounds while, most of the time, not increasing the toxicity of the effluent (as reported by 86% of the studies). Due to the wide variation in experimental set-ups, no clear correlation between reaction conditions and toxicity was determined, however, V. fischeri acute toxicity assays and chronic/sublethal tests appeared most sensitive to transformation products. Future studies need to a) incorporate multiple toxicity tests to produce a more reliable and inclusive ecotoxicity assessment of treated effluent and b) investigate immobilized catalysts and energy efficient radiation sources (i.e. solar and LEDs) for industrial applications (Lashuk, 2021). **Hybrid ozonation-ultrasonication** process has been identified to be able to address those concerns with early successes demonstrated. However, the extent of degradation efficiency varies depending on the features of the pollutant, system configuration, operational parameters and water quality. The synergy between the two effects is also still poorly understood. Besides, a few recommendations for future research direction with regards to the CECs reactivity, suitable system set up, operational cost etc. are also made. (Hussein et Abdullah, 2020).

Other technologies are currently being researched, such as photo-Fenton or electrochemical oxidation. The results of these investigations have shown limited reduction efficiencies, limited scalability and large energy consumption, respectively. The Water JPI-project REWATER (2016-2020) studied the integration of electrochemical and biological technologies for urban wastewater [13] while another Water JPI-project (2013) MOTREM studied advanced bio-oxidation process and photodegradation method. However, there is need for cost-effective and sustainable systems to improve the removal of CECs and pathogens, which do not require high investing costs.

The performance of currently applied biological treatment methods for secondary treatment was analysed by Krzeminski et al. (2019). Technological solutions including conventional activated sludge (CAS), membrane bioreactors (MBRs) and moving bed biofilm reactors (MBBRs), were compared for the achievable removal efficiencies of the selected CEC and their potential of acting as reservoirs of ARB&ARGs. With the aim of giving a picture of real systems, this review focuses on data from fullscale and pilot-scale plants treating real urban wastewater. CAS process is the most investigated process for the removal of CEC. However, the conventional layout (i.e. aerobic process) is ineffective while operation at high SRT or with sequential anoxic-aerobic phases can ameliorate the performance for some pharmaceutical compounds. Thus, research should be devoted to the optimization of the process performance by modifying the operating parameters (when possible), and/ or investigating the combination with more powerful technologies to be applied as tertiary treatment. MBR technology has been extensively investigated for the removal of CEC, but the mechanisms have not yet been fully unravelled. Further research is needed to understand removal mechanisms of the CEC and microbiological contaminants such as ARB&ARGs. For example, fouling layer interaction and the role of deposits on the membrane surface as potential additional barrier increasing CEC removal is needed. In addition, identification of CEC removing bacterial species and/or enzymes, unravelling optimal operating conditions, and elucidation of the metabolites produced during MBR treatment is required. These products may possess different structural characteristics compared to the parent compounds, making them toxic once they are filtered and end up in the clarified MBR effluent. Finally, cost-effective integrated MBR systems providing synergistic effects of combined technologies, should be further developed with emphasis on system optimization, scaling up, and full-scale validation. The Water JPI-project (2013) StARE showed that the wastewater treatment plants (WWTP) reduce AMR but the load of the resistance is still significant in effluents. It has been also shown that conventional activated sludge treatment (CAS) does not remove pharmaceuticals effectively. CAS and recently installed membrane bioreactor (MBR) in the Oulu's WWTP do not remove CECs efficiently although MBR removes very efficiently bacteria.

The use of **TiO2 photocatalysis** for water and waste treatment, treating contaminants of emerging concern (CECs), pesticides, endocrine disrupters (EDs) and bacteria using both UV and visible light irradiations was reviewed (Murgolo et al.,2020; Byrne et al., 2018. Feng et al., 2020). TiO2 has the ability to photocatalytically inactivate bacteria by damaging their cell walls and then oxidizing their internal components. However, this process is quite new and still need further investigation (toxicological assessment, reactor engineering studies, upscaling from lab-scale to full scale, cost and sustainability assessment) to be implemented.

In **SERPIC project**, further to a nanofiltration (NF) step, a residual disinfection using chlorine dioxide produced electrochemically will be added to the stream used for crops irrigation. The CECs in the polluted concentrate (retentate) stream will be reduced by at least 80 % by light driven electro-chemical oxidation, prior to discharge in the aquatic system.

REWA project develops novel concept for surface water treatment combining photocatalysis and adsorption process (using clay-polymer nanocomposites and tailored-made specific sorbents).

PRESAGE promotes packed disinfection systems to minimise the amount of biocide needed and thus a reduction of this risk. The overuse of biocides being related to the generation of ARMs.

Antibacterial products

Nanomaterials emerged as novel antimicrobial agents with demonstrated efficacy against AMR bacteria. Antimicrobial ZnxCu1-xO nanoparticles (NPs) showed strong bactericidal effect against multi-drug resistant bacteria. A sonochemical technology was applied for single step synthesis and permanent embedding of metal oxide (MeO) NPs onto fibrous structures that retained their antibacterial efficacy even after 100 washings at 75 ºC. Nanoformulated antimicrobials, such as aminocellulose, vancomycin, and penicillin to achieve high antibacterial activity at minimum inhibitory concentration (MIC), coupled to balanced biocompatibility. Anti-infective and antibiofilm enzymes are used to generate hybrid metal-enzyme nanoaggregates and multilayer nanocoatings able to efficiently impede bacterial colonisation and biofilm growth on surfaces. These enzymes make bacteria less virulent and more susceptible to lower antimicrobial dosage, reducing the infection incidence. Antimicrobial lipids and peptides (AMP) able to disrupt the bacterial cell membrane precluding the appearance of resistance mechanisms are another alternative against AMR. Microalgae are a valuable prospection source for novel AMP and lipids due to their biodiversity and simple growth needs (AMROCE).

AMROCE develops (antimicrobial/antibiofilm fish cage nets and) wastewater filtration membranes through polymer and surface nano-engineering to reduce antibiotic pollution and AMR bacteria spread in the context of aquaculture.

NanoTheC-Aba, combining different treatment stages, generates antibacterial membranes for AMR pathogens abatement by using nanoparticles.

4.6.4. Nature-based solutions

Nature-based solutions (NBS) have been used for several years to improve water quality, reducing the release of organic matter and nutrients into water bodies. More than being green infrastructure, NBS are define as “living solutions” inspired by nature to address various societal challenges in a resource-efficient and adaptable manner, while delivering simultaneously economic, social, and environmental benefits. NBS are included in the EU R&I agenda as an essential component to greening the economy and achieving sustainable development (https://ec.europa.eu/research/environment/index.cfm?pg=nbs).

Constructed wetlands (CWs) are some of the most frequently applied NBS for wastewater treatment. CWs, either as horizontal flow (HF) or vertical flow (VF) systems have been studied by the members of this consortium, demonstrating that vertical systems provide the greatest attenuation of contaminants of emerging concern (CECs) due to their prevalent aerobic conditions (Gorito et al., 2017; Lyu et al. 2018; Matamoros et al., 2007). Other configurations such as surface flow (SF) CWs have been used as tertiary treatment systems to attenuate pesticides and CECs from secondary-treated wastewater, obtaining reclaimed water of high quality (Matamoros et al., 2008). In the last years, a novel Microbial Electrochemical-based CW (METland) has been developed. The METland system attenuates regulated pollutants substantially better than reported for CWs (Ramírez-Vargas et al., 2019), but its efficiency to remove CECs is yet unknown. NBS have also been used as management measures in river basins to reduce sediments, nutrients, and pollutants entering streams, lakes, groundwaters, and coastal waters. The creation or restoration of wetlands and renaturalization of river basins, with the aim to reinstall ecosystem services, can increase the attenuation of certain CECs (Matamoros et al., 2012; Matamoros et al., 2017), reducing their release into the coastal and estuarine ecosystems. This is relevant since in most of the cases these wastewater-dominated streams/rivers discharge their water into coastal or estuarine areas with high aquaculture development. In this regard, the occurrence of CECs in seafood receiving seawater intrusion, but also inland drainage from agriculture and wastewater within the premises of the seafood production farming area has been observed (Gadelha et al., 2019). The correlation between seasonality and human activities highlighted the need for environmental protection and sustainable resource exploration for safe seafood production. Recently, concerns are increasing on the occurrence of antibiotics (ABs), antimicrobial resistance (AMR) together with the presence of microbial pathogens including viruses in aquatic ecosystems.

Nature based solution (NBS) have been used for several years to improve water quality, reducing the release of organic matter and nutrients into water bodies. Constructed wetlands (CWs), some of the most frequently applied NBS for wastewater treatment, provide attenuation of contaminants of emerging concern (CECs).

So far, **there is still little information about the capability of using NBS to reduce impact of antibiotics, antimicrobial resistance, pathogens** including viruses on water quality.

NATURE project will assess **the effectiveness of already implemented NBS** for reducing the prevalence of ABs, AMR, and pathogens in aquatic ecosystems from inland to coastal areas.

4.6.5. Reduction at the Source

By their nature, aquaculture systems contain high numbers of diverse bacteria, which exist in combination with the current and past use of antibiotics, probiotics, prebiotics, and other treatment regimens—singularly or in combination. In addition to the use of antibiotics, other pharmaceuticals and metal-containing products are often used in aquaculture to prevent fouling, and to feed and treat fish, in order to limit the spread of infections (Watts et al., 2017).

In aquaculture, a common issue with the fish cage nets is the formation of biofoulings and biofilms, acting as a bacteria reservoir and reducing the water flow inside the cages. This implies frequent net cleaning and replacement, or the use of expensive antifouling agents that increase the operating costs of fish farming and increase the risk of water contamination and/or antimicrobial resistance development and spread. Antimicrobial agents are usually administered to fish, mixed with food. Not only can residues of antimicrobials remain in fish products, but undigested food and fish faeces containing unabsorbed antimicrobials and secreted antimicrobial metabolites can remain in the water and sediment around fish farms for an extensive period of time, depending on their concentrations and biodegradability (Watts et al., 2017).

In aquaculture, a common issue with the fish cage nets is the formation of biofoulings and biofilms, acting as a bacteria reservoir and reducing the water flow inside the cages. A solution today is antimicrobial uses which have undesirable effects.

AMROCE develops antimicrobial/antibiofilm fish cage nets through polymer and surface nano-engineering to reduce the use of antifouling agents. Marine-derived antimicrobial agents and antibiofilm enzymes will be nano-formulated as alternative to antibiotics for fish and animal feed supplement.

5. Synthesis and key outputs of the 18 funded projects

5.1. Assessment of available knowledge by categories and their expected impacts

This chapter briefly outlines the environmental, social, regulatory, technical and economic impacts relating to aquatic pollutants, which motivated the funding of 18 research projects by ERA-NET Cofund AquaticPollutants.

The results and outputs expected from these 18 projects will provide answers to a number of scientific questions listed here in a succinct and non-exhaustive manner. The details are presented in chapter 4. This work corresponds to the available knowledge that could be transferred to different stakeholders.

A state-of-the-art, based on funded projects and extended by reviewing national and European projects that have been completed or are ongoing, highlights existing knowledge.

This knowledge can be grouped into 5 categories:

1. **Analysis & monitoring:** How can we measure various contaminants in the different environmental compartments? How can methods and protocols of measuring and monitoring be harmonised?
2. **Sources and pathways:** What are the entry point (introduction routes) of CECs and pathogens and resistant bacteria and resistant genes into the environment? What are their pathways in aquatic environments?
3. **Mechanisms of AMR and of co-/cross-resistance:** How does antimicrobial resistance develop and transfer from aquatic ecosystems (inland, coastal and marine) to humans and environment?
4. **Effects on human health and environment:** Can we assess the effects of CECs and AMR on ecosystems and human health?
5. **Remediation and mitigation:** Can we reduce at the source CECs and pathogens, including antimicrobial resistant bacteria? Are current water treatment systems sufficiently effective for preventing the spread of CECs and antimicrobial resistant bacteria?

Our work reveals that the **expected outputs are of various formats and are aimed at different water stakeholders and end-users**. A summary is given here based on the information available, i.e. the answers to a questionnaire elaborated by Transnet, the list of planned deliverables and a brief description of the technical programmes of each of the 18 AP.

WHAT ARE AQUATIC POLLUTANTS? WHICH ONES ARE STUDIED HERE?

According to the definition of Water JPI Knowledge Hub of CECs, contaminants of emerging concern (CECs) is a designation that can be attributed to compounds that may have been present in the aquatic environment in the past but which only recently have raised concern about their ecological or human health impacts. Although CECs refers most commonly to chemicals, the broad perception herein presented applies also to microorganisms, such as antibiotic resistant bacteria and their antibiotic resistance genes, or particles, such as nanoparticles or microplastics (the latter not being included in the call).

The 18 AP projects address a wide range of CECs (*sensu lato*), which are essential for organic compounds, but in some cases also for metals. These CECs include antibiotics, pharmaceuticals and biocides. At least 7 projects also address pathogens. The majority of the projects (15) also consider the issue of antimicrobial resistance (AMR). One project tackles the issue of toxins linked to harmful algal blooms in waterbodies.

REGULATORY CONTEXT

Chemicals pollutants

The European Water Framework Directive (WFD), the Marine Strategy Framework Directive (MSFD) and the newly adopted European Green Deal, all require the European Union member states to reach good environmental status of their aquatic ecosystems. The 'ecosystem' of the Cofund AquaticPollutants covers the entire chain of aquatic pollution, from pollution sources to pollutant transformation, to risk management and treatment. So that the scope is extremely broad and regulatory /policy contexts also. Detailed information on the regulatory context is available in the deliverable DL1.1. (p45-85) at EU and national levels (France, Germany, and Sweden).

It appears that **some CECs are regulated at European level, others are not**. For regulated CECs, the Member States remain responsible for defining and implementing the means to reach the EU objectives; this means that implementations differ from one member state to another.

Briefly, biocides, plant protection products and pharmaceuticals have their own pieces of legislation ruling their production, marketing and use, considering the goals set by the environmental legislations regarding the chemical quality of water bodies.

The answers to the questionnaires addressed to the 18 AP (question "*Will your project test, implement, improve or revise current regulations*") are more or less precise. Most indicate "current regulations without any details" or "support the implementations of current regulations", others give more concrete information and mention that they will give recommendations to policymakers (MAPMAR) and/or help to revise list of priority substances (AIHABs).

The regulations most concerned are environmental directives (i.e. Water Framework Directive and its related directives and Marine Strategy framework directive). A few projects expect impact of "products" directives (REACH, biocidal products regulation). In some case there is no regulation directly linked to the project content (e.g. for oyster production and aquaculture in the context with antibiotic use – SPARE-SEA).

For examples, the project NATURE will tackle relevant remaining gaps in EU and national policies in the water, marine, health and agricultural and environmental sectors. It involves specific water policies (EU Water Framework and Urban Waste Water Treatment Directives) and the EU aquaculture policy (building resilience to aquatic pollutants in estuarine areas).

Overall, many Theme 3 projects, which are essentially based on technology developments, do not expect to have a direct impact on existing regulations. AMROCE proposes solutions to reduce the prevalence of CECs at the source by developing new fish cage nets and filtration membranes containing metal oxide nanoparticles (free of antibiotics). They will identify parameters related to the safety of the nano-formulation process for both workers and users and gaps existing in legislation and norms. SERPIC will facilitate water reuse for agricultural irrigation in line with the recently adopted European Parliament legislative resolution and regulation on minimum requirements for water reuse.

Antimicrobial resistance (AMR)

AMR is not yet integrated into binding legislation at the EU level. However, the European One Health Action Plan against AMR supports the EU and its Member States in delivering innovative, effective and sustainable responses to AMR, especially to reduce the emergence and spread of AMR and to increase the development and availability of new, effective antimicrobials inside and outside the EU.
At national level, the way in which the risk of AMR is taken into account and studied varies from country to country.

- ⇨ As for the CECs, the expected impacts on regulations/policies varies considerably from project to project, whether in terms of "environmental" or "products" regulations.
- ⇨ AMR is not yet integrated into binding legislation at the EU level; the projects will initially have an impact on the national level, sometimes in response to national plans or programmes being implemented.

ENVIRONMENTAL AND HUMAN HEALTH ISSUES, AND ECONOMIC ISSUES

Chemicals pollutants

CECs are detected in the aquatic environment at low concentrations. The spectrum of these pollutants is broad and they derive from anthropogenic sources, especially from consumer products, urban areas, agriculture, animal husbandry farms, industry and maritime activities, and consequently end up in our rivers, estuaries and coastal ecosystems. These CECs and their transformation products could be persistent and mobile, consequently widely distributed. They have been qualified as a risk to human health and environmental ecosystems that urgently needs to be addressed. Knowledge is therefore expected on different aspects dealing with 5 different categories we have defined.

Analysing and monitoring (category 1) CECs are important questions, in particular how and which CECs should be monitored*.* BIOCIDE will develop state-of-the-art of chemical analysis protocols for biocides in different matrices, ARENA a method for a rapid detection of antibiotic in edible products.

A better understanding of sources and pathways (category 2) is also a major challenge. New knowledge will be produced notably for pharmaceuticals including veterinary drugs, biocides and antimicrobials (FOREWARN, SERPIC, PHARMASEA, BIOCIDE).

CECs are of recent concern because they have been detected at concentrations significantly higher than expected, and/or their risk to human and environmental health may not be fully understood. ***A better understanding of effects on human health and ecosystems (category 4) is required***. Thus, effects should be assessed by generating data Predicted No effect concentration of biocides on bacteria for instance (BIOCIDE), of pharmaceutical products on different aquatic species (PHARMASEA) or non-target biota in aquatic ecosystems (CONTACT).

Toxicity of by-products originating from the treatment processes of water shall be assessed and minimised for water dedicated to agricultural re-use (SERPIC). PRESAGE shall undertake an ecotoxicological evaluation of the effluent produced at the different demo sites.

Phycotoxins

A second identified issue is Harmful Algal Blooms (HABs). They represent a natural phenomena caused by a mass proliferation of phytoplankton (cyanobacteria, diatoms, dinoflagellates) in waterbodies. Blooms can be harmful for the environment, human health and aquatic life due to the production of harmful toxins and the consequences of accumulated biomass (oxygen depletion). The adverse effects on human health of HABs occur primarily through the impacts of natural phycotoxins via various exposure routes including the ingestion of contaminated seafood, inhalation or direct skin contact. Phycotoxins are potent organic compounds produced by dinoflagellates, other flagellated phytoplankton, and cyanobacteria that inhabit marine, brackish, or freshwater environments.

Many bloom episodes have significant impacts on socio-economic systems. Fish mortality, illnesses caused by the consumption of contaminated seafood and the reluctance of consumers to purchase fish during HABs episodes represent only some of the economic impacts of HABs (EU JRC, 2016).

To better track cyanobacteria, AIHABs will develop a new method based on the exploitation of data from satellites. An existing system will be also improved to be used in field to analysis microcystins. These outputs relate to category 1.

Antimicrobial resistance

An identified risk to humans is AMR. AMR has been classified by the World Health Organization as one of the three most significant threats to public health in the 21st century and the latest report from the UK Review on Antimicrobial Resistance, estimates that the 700,000 annual deaths currently attributable to infections by drug-resistant pathogens will increase, if unchecked, to 10 million by 2050 (Robinson et al., 2016). AMR has a direct impact on human and animal health and carries a heavy economic burden due to higher costs of treatments and reduced productivity caused by sickness. AMR is responsible for an estimated 33,000 deaths per year in the EU. It is also estimated that AMR costs the EU €1.5 billion per year in healthcare costs and productivity losses.

The problem has been escalated by the overuse of antibiotics, causing selective pressure on bacterial populations, both in antibiotic-treated human and (farmed) animal hosts and by contamination of residues in the environment. The emergence and global dissemination of antibiotic resistant bacteria has developed into a severe threat to public health, jeopardizing our ability to treat bacterial infections, cure cancer and perform advanced surgery, all of which are dependent on effective antibiotics. In 2017, the World Health Organization published its first ever list of antibiotic-resistant “priority pathogens”. The list includes 12 families of bacteria that have developed resistance against the existing antibiotics, including fluoroquinolones, carbapenem, clarithromycin, penicillin and others.

A serious risk for freshwater and marine ecosystems, and consequently human health, derives from the occurrence of pathogens (parasites and bacteria), especially ARB and Antibiotic Resistance *Gene (*ARGs), which are now widespread throughout the aquatic environment and pose a serious emerging risk for aquatic organisms (especially fish) and human health.

Understanding how antimicrobial or antibiotic resistance develop and how transfer (category 3) between aquatic ecosystems or to humans are question addressed by several projects (CONTACT, BIOCIDE, SPARE-SEA, REWA).

- ⇨ By evaluating and assessing the environmental behaviour and effects of aquatic pollutants on (human health and) ecosystems, environmental impact is expected.
- ⇨ By developing new monitoring tools or strategies of monitoring, projects will have an economic impact.
- ⇨ By understanding how antimicrobial or antibiotic resistance develop and how transfer, environmental and sanitary impacts are expected.

Focus on Aquaculture and Mariculture

Human population expansion is accompanied by a worldwide increase in needs for food with the challenge to maximize production yields while minimizing negative impacts on the environment. Transition towards high-protein diet further fosters the increase in protein demand and the intensification of production systems, particularly in low- and middle-income countries. Aquaculture is considered one of the most sustainable sources of animal protein and currently accounts for around 10% of all animal protein consumed globally, expected to increase by 50% by 2030. In this context, aquaculture provides nearly half of the world food fish supply, with a production valued at over US$250 billion. Yet being the fastest growing food sector, in order to meet global demand, production must further expand by 50% by 2050. So, there has been a shift from extensive and semi-intensive culture to intensive and super-intensive systems, as these culture systems can increase fish production.

In intensive systems, practices lead to poor water quality and disease outbreaks (see references in Hemamalini et al., 2022). This concerns both CECs and AMR.

Indeed, this intensification and increase in production is accompanied by the massive use of multiple antimicrobials and further veterinary drugs such as antiparasitics to either fight or prevent the spread of pathogens, or as surrogates for hygiene on farms. For example, a continuous increase in the amount of antimicrobials applied only in Chilean salmonid farms from 300 tons in 2010 to more than 700 tons in 2016 has been observed. During 2016, companies utilized approximately 0.53 kg of antimicrobials per ton of harvested salmon.

Although the use of antimicrobials in aquaculture follows clear regional patterns, there is only a limited number of veterinary drugs used worldwide, as so far only a relatively small number of antibacterial agents are registered for use in aquaculture. For Europe, Canada and the U.S. these include members of the following classes of drugs: macrolides (erythromycin), β-lactams (amoxicillin), amphenicols (florfenicol), tetracyclines (oxytetracycline), quinolones (oxolinic acid), fluoroquinolones (flumequine) and potentiated sulphonamides (sulfadimethoxine/ormetoprim, sulfadiazine/trimetoprim).

Some of the antimicrobials being used are listed as “critically important”, “highly important,” and “important” for human medicine by the World Health Organization and FDA.

Thus, when aquaculture and mariculture are often considered **as receptors** of contamination due to e.g. incomplete treatment of wastewater, they are also identified **as gateway** for antimicrobial resistance (via aquatic compartment or food consumptions). Aquaculture and mariculture are therefore both sources and receptors of contaminants and antibiotic resistance genes and therefore appear to be a relevant example of the **concept of one health**.

At least 6 projects (SPARE-SEA, ARENA, CONTACT, NanoTheC-Aba, AMROCE, NATURE) deal with aquaculture/mariculture.

As examples, AMROCE proposes to tackle the pollution at the source by lowering the consumption of antibiotics in aquaculture industries (and for livestock) by designing novel antibiotic-free antimicrobial strategies.

Environmental safety and economic benefits for aquaculture shall be promoted by NATURE through the introduction of Nature Based Solutions (NBS) for water treatment and management (including the safety of food products in estuarine areas).

> ⇨ In the context of aquaculture/mariculture, expected outputs will have economic (securing the supply chain, reducing the costs associated with AMR), technical and also social (food) and health and environmental impacts.

RISK MANAGEMENT

Some of the projects help to improve risk assessment and management of aquatic pollutants (CECs and AMR).

Particularly solutions are proposed to reduce the flow of contaminants including ARGs and thus reduce the risk of AMR from waste water treatment plants. These technical solutions aim to be safe and sustainable. Thus the ecotoxicity of discharges (treated waste water) is taken into account (GreenWaterTech; PRESAGE; SERPIC) and efforts are made to reduce energy consumption (GreenWaterTech) or use natural or green materials (GreenWaterTech; NATURE).

Several solutions are developed by the AP projects to manage risks to human health and the environment from aquatic pollutants. These solutions include treatment technologies development (SERPIC, REWA, GreenWaterTech, PRESAGE, NanoTheC-Aba), protection of ecosystem and public health by transferring of NBS practices to key stakeholders such as government and public authorities, water utilities, health authorities (NATURE) or by reduction at the sources (AMROCE). In addition management strategies to reduce risks may be proposed; e.g. PRESAGE will propose optimal and competitive management strategies for hospital, pharmaceutical industry and domestic waste water that reduce the risk of CECs spread in the urban aquatic environment, evidencing its important economic and societal challenges. Data on reachable effluent qualities through competitive developments will be very relevant for water authorities to develop future policies.

The projects, mainly those in Theme 3 dealing with solutions for water treatment, have expected technological impacts (AMROCE, GreenWaterTech, NanoTheC-Aba, PRESAGE, REWA, SERPIC). During the development of technical solutions, economic aspects are closely considered. Indeed, technical progress is expected to improve the efficiency of water treatment plants (urban hospitals, industrial) for various contaminants including ARG, but today significant costs for the community remains and should be reduced.

AMROCE will estimate the sustainability and cost effectiveness of their solutions by applying life cycle assessment and costing (LCA/LCC) to optimize costs as well as environmental and social impacts. NanoTheC-Aba will apply a circular economy principle for the development of their membrane, by recycling the ceramic scraps deriving by filters production. REWA will prefer sorption process on biosorbents because of their sustainability, low cost and abundance. SERPIC will develop a new technology and test a prototype that will use regenerative, sustainable energy (photovoltaic modules).

AMROCE will alleviate the costs of biofouling in aquaculture by 90%. Additional cost burdens related to the reduction fish welfare, including the presence of biofilm associated pathogens, low oxygen concentrations due to poor water exchange and increased stress during net changing, will be mitigated.

NATURE shall consider socio-economic, environmental and legal constraints to assess environmental safety and economic benefits of NBS for water treatment and management.

GreenWaterTech will pay attention to employ cost-effective and sustainable (non-toxic and green) materials and to minimize energy consumption in investigating and developing materials and techniques to make a modular reactor comprising several steps for cleaning of water, possessing a broad-spectrum CEC and ARB action.

- ⇨ Several projects (at least 6) dealing with remediation will have technological impacts by producing new solutions for water treatment.
- ⇨ These technological solutions as well as nature based- solutions will have environmental impact.

OTHER SOCIAL IMPACT OF THE PROJECTS

Several projects have planned to develop training seminars for master and doctoral students (NanoTheC-Aba, REWA, PRESAGE, FOREWARN) and/or educational workshops (NATURE).

- ⇨ Communication and dissemination actions will have a social impact particularly by targeting young people.

5.2. Types of Outputs

To foster the transfer of knowledge, it is necessary to draw up an inventory of the expected results (WHAT) and to know in what formats they will be produced (type of Outputs). Indeed the formats is likely linked to the target audience (WHO). Secondly, it is necessary to consider whether there is a match between the demand and the knowledge produced (DL1.3.), and also how this knowledge is to be transferred. **The assumption is that disseminating results in different formats through the right communication channels will increase the transfer to other stakeholders**.

Information Sources & Communication Channels of different stakeholders have identified thanks to questionnaire and national workshops (see DL1.1. p. 36, 40-44).

The topics and purposes of the 18 projects are therefore quite different. The expected outcomes (as collected by the questionnaires addressed to the coordinators of Cofund projects) are also varied. A summary is provided in section 5.2.2. Deliverables (194 deliverables identified for 15 projects) are also expected (up to 20 per project).

To follow the progress of the deliverables, you can consult the website of TransNet (https://aquatic-pollutants.eu/Resources/Research+Project+Results.html**)** or the websites of each project, the links to which can be accessed via https://aquatic-pollutants.eu/Projects.html**.**

5.2.1. Definitions

In a very concise and simplified way, the AP projects outputs were presented according to the following types:

- **De novo knowledge:** New fundamental knowledge which enables to better understand, assess and manage the issues associated with aquatic pollutants. This knowledge yields to science-based knowledge mainly dedicated to experts and R&D community (scientific publications).
- **Report / study / review:** Compilation of existing knowledge or new knowledge through scientific report or study. Reports are the most common deliverables of (EU) projects.
- **Case study:** Study which enables the demonstration and implementation of a tool, a process, a technology or a strategy on a real case. This is a step towards a more operational phase.
- **New guidelines or standards:** Documents which delineate the use and the implementation of analysis, tool, process, technology, methodology or strategy by describing all the protocol and steps to follow. These are the documents which enable an adequate implementation, guaranteeing normative and best performance. The category refers to recommendations on good practices, approaches and solutions which are not limited to policymakers, but also target a wider range of stakeholders, including practitioners, river basin managers, drinking water companies, industry actors, and NGOs.
- **Numerical tools:** Modelling or software which enables to predict the occurrence, the fate or the risk of aquatic pollutants. The level of expertise of numerical tools conditions the type of end-users.
- **New technologies / processes / prototype:** A new technology is any set of productive techniques which offers a significant improvement over the established technology for a given process in a specific historical context. Prototype: the category refers to original models constructed to include all the technical characteristics and performances of a new product.
- **Training:** is defined as the organized procedure by which people learn knowledge and/or skill for a definite purpose. Trainings can be aimed at the academic or research communities, but also at a more varied audience of stakeholders.
- **Others:** other types of results have been identified. Although just as important, they were only mentioned in a few projects. This is why they are grouped together under the term "others".
    - Decision support system (DSS): a computerized system that gathers and analyses data, synthesizing it to produce comprehensive information reports. DDS is of special importance is to develop a tool to facilitate decision makers with the complex process of selecting optimal solutions e.g. for removal of emerging contaminants.
    - Database: a structured set of data held in a computer, especially one that is accessible in various ways.
    - Demonstration event: it encompasses trade shows, or other events where new products and services are displayed, demonstrated and discussed. They are focused on or involve business, future investors, academic and research communities.
    - Early warning system: mechanism for detecting, characterizing and providing notification of a source water contamination event.

5.2.2. Classification

De novo knowledge

De novo knowledge produced by AP projects include:

- Mechanisms involved in the antimicrobial resistance development (BIOCIDE, REWA, MAPMAR) and spread (SARA)
- Role of various contaminants (e.g. metals, biocides) on antimicrobial resistance development (BIOCIDE)
- Effects on bacterial species and non-target biota (CONTACT)
- Source of emerging contaminants (FOREWARN, SERPIC, PHARMASEA, BIOCIDE)
- determination of common ARG plasmids circulating in european waters (PARRTAE, SARA, MapMAR)
- …..

Report/study/review

The information collected via the questionnaire (Annex B) does not allow this section to be filled in correctly, as there is not a clear distinction between peer-reviewed publications and other reports, studies, or reviews. However, some technical reports were provided:

- Description of reactor construction (GreenWaterTech)
- Results for synthesis of antimicrobial $Ti0_2$ (NanoTheC-Aba)
- Report on model calibration (FOREWARN)

In addition, as the projects are R&D oriented, peer-review publications are expected and many projects listed scientific papers as deliverables. It is known that publication through peer-reviewed journals are the preferred way for most scientist to communicate their results. For an updated list of published papers, visit the TransNet website: https://aquatic-pollutants.eu/Resources/Research+Project+Results.html.

It is likely that other types of scientific publications will be produced (policy brief, conference proceedings etc.) but we have no details of the actions planned.

Case studies

Fourteen AP projects are based on case studies. They may be study cases in which new investigations will be conducted (ARENA, FOREWARN, MAPMAR, PAIRWISE, SARA, AIHABs, BIOCIDE, CONTACT, PHARMASEA, GreenWaterTech, NATURE, PRESAGE, REWA, SERPIC) and/or in silico case studies (FOREWARN, MAPMAR).

The case studies are located not only in different European countries, but also in Africa (Tunisia and Uganda - PAIRWISE; BIOCIDE; Uganda, Mozambique, South Africa - SARA), Brazil (PRESAGE) and Israel (REWA, MAPMAR, SARA). The case studies, which vary in scale, will make it possible to respond to the various themes by improving knowledge of the sources and routes of transfer or contamination. In connection with Theme 3, the case studies correspond to different types of wastewater treatment plants in different contexts (urban, hospital, industry, agriculture including aquaculture).

New guidelines/standards

AP projects propose to develop the new guidelines and standards as follows:

- Methods harmonization (sampling, standard operation protocols for analysis of cultural parameters and sample preparation for molecular biological methods) (SARA)
- List of most transmissible marine plasmids (MAPMAR)
- Protocols for cultivation and verification of antibiotic resistant bacteria DNA-extraction, antimicrobial resistance gene quantification, microbiological source tracking, analysis of antibiotics and biocide (PAIRWISE)
- Pre-normative research which might be of utility to develop reliable normative guidelines and appropriate strategies for environmental risk assessment for implementation into European Directives (PHARMASEA)
- state-of-the art chemical analysis of antibacterial biocides (BIOCIDE)
- identification of diagnostic indicators for cost-future monitoring (NATURE)
- standardization of the analysis and quantification of AMR and ARG i.e. Standardized operation procedures (SOP) (PRESAGE)
- optimized protocols for microbial indicators/ assessment (REWA)
- guideline on co-selection potentials (REWA)
- guidelines to mitigate AMR (REWA)
- produce an evaluation scheme in collaboration with relevant authorities on how resistance risks formally could be incorporated in existing regulatory framework (BIOCIDE)
- recommendations for monitoring parameters / genomic techniques to identify resistome diversity (SARA)
- Establishment of conceptual models of the understanding on the consequences of antimicrobials on ARG emergence and dissemination from aquaculture activity into the aquatic ecosystems. They will be based on two molecules worldly used and belonging to different chemical families studies (florfenicol as antimicrobial / emamectin benzoate as antiparasitic) (CONTACT)
- test mitigation strategy (Israel) by using plant-based biofilters to reduce therapeutics administration (CONTACT)
- strategy for detection and monitoring of Active Pharmaceutical Ingredients in water systems (PHARMASEA)

Training

Aimed at the academic community

- Training seminar for master and doctoral students (NanoTheC-Aba, PRESAGE, FOREWARN)
- e-learning course (REWA)

Aimed at other stakeholders

- Various measures will be taken to implement capacity building activities (SARA)
- A workshop/training event for industrial stakeholders that can be advocate toward the need of using water system capable to abate CECs and AMR pathogens (NanoTheC-Aba)
- Information transfer to local and international stakeholders (i.e. public health, environmental, aquaculture farmers, and wastewater treatment plant managers), educational workshops and training events, scientific publications, and an international symposium on NBS (NATURE)
- Establish a sustainable network of scientists among EU countries working at the frontiers of NBS researcher and development (NATURE)

Modelling / software (Numerical tools)

- Scientifically sound software-assisted tool for Environmental Risk Assessment (PHARMASEA)
- Forecasting system to predict the occurrence and spread of Harmful Algal Blooms (AIHABs)
- Machine-learning methods to model transfer and behaviour of CECs (namely antibiotics) (FOREWARN)
- Modelling of antimicrobial resistance dispersal by gull (PAIRWISE)

New technologies /new process

New technologies

- Tool for rapid detection of antibiotics residues (flow cytome-try based) + microbial pathogens (sensor-based) for environmental and biological samples (ARENA)
- Remote sensing and *in situ* sensing for monitoring toxic cyanobacterial blooms in coastal and freshwater (AIHABs)
- Combination of ultra-stable silicon carbide (SiC) UltraFiltration/ NanoFiltration (NanoTheC-Aba)
- Novel, energy-efficient wastewater treatment NBS (Microbial Electrochemical-based Constructed wetlands) (secondary treatment) (NATURE)
- Nanoformulations for fish and animal feed with antibiofilm and bactericidal efficacies (AMROCE)
- Development of materials and techniques to make a modular reactor with low energy consumption employing cost-effective and green materials / produce hydrids materials with high permeability, bearing immobilized oxidative enzymes for persistent pharmaceutical pollutants and pathogens / nanoporous adsorbents / photocatalytic coatings (GreenWaterTech)
- Pilot (to treat ~ 1 m^3/day) (GreenWaterTech)
- Particle Bed Biocidal Reactor for WWTP as decentralized treatment (PRESAGE)
- Demonstration of the use of wood-based biosorbents and carbon-based materials for sewage effluent polishing and biocoagulants for metal-rich effluents (REWA)
- Development of technology to reduce CECs from WWTP effluent by membrane filtration and light driven electro-chemical processes (SERPIC)
- A prototype treatment plant validated in relevant environment (TRL5) and powered by photovoltaics (SERPIC)
- Nano-safety assessment and LCA/LLC will facilitate the industrial adoption of the novel technology (AMROCE)

Novel process

- Novel concept for surface water treatment using clay-polymer nanocomposites, photocatalysis and tailored-made specific sorbents (REWA)
- (UF/NF) membrane for pre-concentration,+ nano-enabled thermocatalytic energy efficient packed-bed reactor (TPBR) + nano-enabled antimicrobial MicroFiltration (MF) membrane (NanoTheC-Aba)

Others

- Decision support system (DSS) for the selection of wastewater treatment (FOREWARN)
- Catalog of the marine plasmidome (MapMAR)

- Contribution to the Harmful Algal information system (HAIS) and the Copernicaus database (new datasets of selected inland and coastal water bodies in Europe) (AIHABs)
- Updating of BacMet database (BIOCIDE)
- Early warning systems for bacterial contamination (ARENA)
- Early warning system (artificial intelligence) (AIHABs)
- Demosites could be used as showcase spaces for other industries than those acting as associated partner (PRESAGE)

6. Synthesis and Lessons Learned

6.1. Knowledge assessment content & outreach

This deliverable gives an overview of the current SoA on "Aquatic Pollutants" **focusing on the three themes of the Cofund AquaticPollutants and on research specific subjects** which are addressed by the 18 **AP projects**. Being aware of the variety of the AP projects' aims and expected research results (various scales, TRLs, etc.), this report attempts to present AP projects' ambitions and expected outcomes in an overarching and encompassing manner. This is done, first, through positioning the AP projects with respect to scientific SoA and related categories (analysis & monitoring, source & pathways, mechanisms of antimicrobial resistance, effects on human health and environment, remediation & mitigation), see Chapter 4. Secondly, the AP projects are put into perspectives of the socio-economical and regulatory context (Chapter 5, Section 5.1.). Finally, AP project outputs are presented according to their types and formats, which very often helpful to pre-identify the types of end-users targeted (Chapter 5, Section 5.2.).

This **multi-approach presentation of the AP projects** may enable the AP Cofund funders and researchers to have **an overview of the diversity of AP outputs content and format** and be informed of added values produced by the AP projects. This overview may encourage the identification of **synergies** between AP projects, establish potential **links** between AP project outputs and identify potential **feeding processes** from one project to another along the AP life cycle.

The present knowledge assessment report can also be used as a summary of existing knowledge and **provide global information on the added value of the AP projects for external stakeholders**.

This deliverable presenting the expected outcomes of the projects is a **steppingstone to assess the potential matching of the demands expressed by the stakeholders** (collected through national workshops and interviews, see DL1.1 and DL1.3) and the AP project results. A methodology will be co-developed with the AP projects in order to propose matchmaking of outputs and demands (DL1.3).

6.2. Lesson learned & perspectives

The wide scope of the AP Cofund presented a real **challenge** to summarise the SoA and the construction of this deliverable. Indeed, the 'ecosystem' of the Cofund AquaticPollutants covers the entire chain of aquatic pollution, from pollution sources to pollutant transformation, to risk management and treatment. This chain is assessed for three main types of aquatic pollutants having very specific fate, transport and toxicological properties: contaminants of emerging concern (CECs), pathogens and antimicrobial resistance (AMR). Both the diversity of the aquatic pollutants and considering the whole pollution chain results in an extremely wide scope, making it difficult to summarise the state-of-art knowledge on "aquatic pollutants".

One way to tackle this challenge was to mainly **focus** on the **SoA related to specific subjects tackled by each individual AP project**. The SoA of the AP projects were collected from the very start of the project through a questionnaire and resulted in **some heterogeneity of the information** obtained. This certainly leads to different level of details for the SoA from one subject to another. To reduce the heterogeneity of the compiled SoAs, the SoAs provided by the AP projects were supplemented **with EU and national SoA** based on **information readily available**. This exercise showed that, because of the large number of funders, the number of existing projects on such a broad theme, etc., it is not easy to propose a state of the art. It also showed that the deliverables are often intended for scientists and less frequently for other end-users.

The expected AP projects outcomes were established based on information collected from the AP project questionnaire and the AP booklet. These collection means (questionnaire and booklet) yield to obtain short & concise information, which also lead **to some imprecision** and potential inaccuracy (from the authors of this deliverable) **in describing the outputs**. In order to reduce imprecision (in description or understanding) associated with this deliverable, **AP projects feedback could be included** in this report.

SoA and AP projects output were solely compiled from data collected through AP questionnaire and booklet. This resulted somehow in imprecisions in SoA and AP projects output description in this deliverable. Therefore, data collection is a crucial step in knowledge assessment. This is why we consider that the questionnaire and the booklet are good primary sources of information but shall be completed by a **more qualitative data collection from the AP projects**. In the matching phase of our work we intend to **cooperate tightly with the AP projects** in order to get more qualitative information on the AP project outputs.

Although this deliverable undoubtedly makes it possible to propose an initial analysis of the knowledge that should be available at the end of the funded projects, it **should be updated** in order to **validate** the description as it has been drawn up here on the one hand, and on the other, according to the **progress of the projects** (outcomes achieved, adapted or finally not achievable). This involves **close cooperation between the 18 AP projects and TransNet**.