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# Deliverable Report
# **D1.1 Target CECs**

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# 1 Introduction to the project SERPIC

The project *Sustainable Electrochemical Reduction of contaminants of emerging concern and Pathogens in WWTP effluent for Irrigation of Crops – SERPIC* will develop an integral technology, based on a multi-barrier approach, to treat the effluents of wastewater treatment plants (WWTPs) to maximise the reduction of contaminants of emerging concern (CECs). The eight partners of the SERPIC consortium are funded by the European Commission and by six national funding agencies from Norway, Germany, Italy, Spain, Portugal and South Africa. The official starting date of the SERPIC project is 1. September 2021. The project has a duration of 36 months and will end 31 August 2024.

The overall aim of the SERPIC project is to investigate and minimise the spread of CECs and antimicrobial resistant bacteria/antibiotic resistance genes (ARB/ARG) within the water cycle from households and industries to WWTPs effluents, and afterwards via irrigation into the food chain, into soil and groundwater and into river basins, estuaries, coastal areas, and oceans with a focus on additional water sources for food production.

A membrane nanofiltration (NF) technology will be applied to reduce CECs in its permeate stream by at least 90 % while retaining the nutrients. A residual disinfection using chlorine dioxide produced electrochemically will be added to the stream used for crops irrigation (Route A). The CECs in the polluted concentrate (retentate) stream will be reduced by at least 80 % by light driven electro-chemical oxidation. When discharged into the aquatic system (route B), it will contribute to the quality improvement of the surface water body.

A prototype treatment plant will be set-up and evaluated for irrigation in long-term tests with the help of agricultural test pots. A review investigation of CECs spread will be performed at four regional showcases in Europe and Africa. It will include a detailed assessment of the individual situation and surrounding condition. Transfer concepts will be developed to transfer the results of the treatment technology to other regions, especially in low- and middle-income countries.

# 2 Report summary

Contaminants of emerging concern have been selected for assessing the capacity of the proposed technology to remove them and reduce their spread in the environment and in the food chain. The selection is the result of an in-depth survey among literature data in the four showcase regions relevant for the project (Spain, Portugal, Italy and South Africa). The selection is combined with an analysis of their persistence, bioaccumulation and toxicity. At the same time, the same classes of contaminants selected in the other projects funded by the same call JPI Aquatic Pollutants were included as well as the categories recommended by recent European Reports on strategic approaches on environmental quality and sustainability. Lastly, an analysis of the potential of the selected compounds to be removed by solar irradiation and a comparison with the CECs monitored in other projects of reuse of reclaimed water validate the list of compounds selected within SERPIC.

# 3 Deliverable description as stated in the Project Description

The deliverable contains the list of selected CECs (including ARGs and ARB) from task *T1.1 Selecting target CECs* to be monitored during the planned investigations at bench scale and with the prototype, the reasons for their inclusion and the expected ranges of concentrations in the water to be treated with respect to the levels of detection and levels of quantitation.

# 4 Introduction

It is well known that CECs include a wide spectrum of compounds from pharmaceuticals active ingredients to hormones, personal care products, synthetic musk, pesticides, PFOS, microplastics and nanoplastics. Their properties may vary largely. Recently, antibiotic resistant bacteria and genes have gained an increasing attention due to their ubiquitous occurrence in the aquatic environment (Pina et al., 2020) and their negative impact on the human health, also via the food chain. In this context, the EU has recently included them among the CECs.

CECs may be grouped in classes according to their use (e.g. personal care products), or function (e.g. antimicrobials, analgesics, lipid regulators, contrast media…). But if we analyse the behaviour of compounds when they undergo wastewater treatments, it emerges that compounds of the same class present different behaviour, different removal rates, and once in the environment they may have a different effect on the aquatic life. For this reason, in evaluating the efficacy of a new treatment technology for water reclamation with regard to CECs, it is more useful to identify *target* compounds, that are compounds which may be considered representative of a wide group of diverse substances in terms of occurrence, persistence against their degradation and removal, bioaccumulation, and ecotoxicity, irrespective to consider the class of usage they belong to.

No specific quality standards have been set for CECs in the case of water reuse, but additional requirements for water quality and monitoring may be defined according to Annex II of the recent EU regulation (EU 2020/741), in order to ensure adequate protection of the environment, and human and animal health. They may concern micropollutants, microplastics and antimicrobial resistance. In this context, the EU watch list, defined by the Directive 2008/105/EC and recently updated by the Commission Implementing Decision (EU) 2020/1161, provides a list of CECs for which further studies are necessary in order to acquire data on their occurrence, removal and toxicity.

As reported in the literature, the occurrence of CECs in the aquatic environment is strictly related to their specific local consumption and existing wastewater treatment steps (Wilkinson et al. 2022).

Spain, Portugal and Italy may be representative of South Europe area, characterized by a high density of population, presence of diffuse anthropic activities and a climate with high temperatures in summer. In addition, they present arid zones and also areas with scarcity of water resources. Most of the population is served by wastewater treatment plants.

South Africa, on the other hand is characterized by a diverse distribution of population, with many small agglomerates scattered in the country, where the new technology under study in this project could be implemented in order to satisfy the water demand for agricultural needs.

The aim of this report is to address the selection of the most representative compounds to optimize the novel treatment processes. In addition, they will be monitored in order to validate the effectiveness of the development of the prototype treatment plant, set-up on-site at UCLM in Ciudad Real, Spain, and evaluated for irrigation in long-term field tests in agricultural test pots.

The selection will lead to the definition of six CECs, one antibiotic-resistant bacterium (ARB), one antibiotic resistance gene (ARG) and four chemical compounds belonging to different classes which will be very frequently analysed. In addition, 24 further target compounds and ARG and ARB will be defined for the final assessment of the technology.

This document describes the main steps in the selection of the target CECs and in the validation of the defined list.

# 5 Results

## 5.1 CECs selection

### 5.1.1 The four chemical compounds

Many CECs can be still present in WWTP secondary effluents (Verlicchi et al., 2012) and thus the selection must refer to compounds of different characteristics and representative of the behaviour of the multitude of substances potentially present.

Different aspects can be considered for their selection. Among them, based on some literature suggestions in CECs ranking and prioritization (Daouk et al., 2015; Mendoza et al., 2015), the following criteria suit well with the SERPIC scope:

- CEC **occurrence** in secondary effluent
- CEC **persistence** during biological treatment
- CEC **bioaccumulation** in aquatic organism tissues
- CEC **toxicity** for the aquatic life

They are briefly summarized in Table 1.

**Table 1**: Criteria for selection of representative CECs.

| Criteria for CECs selection | Parameter | Unit | Description |
|---|---|---|---|
| Occurrence | Concen–tration | ng/L | Measured concentrations of CECs in the secondary effluent found in literature for the four regional showcases (Portugal, Italy, Spain and South Africa) and measured concentrations in real samples of the WWTP effluent in Ciudad Real (Spain). The higher the concentration of CECs, the higher the expected environmental impact. |
| Persistence | Removal efficiency | % | The persistence of a CEC is considered with respect to its resistance to be removed during wastewater treatments. The lower the removal efficiency of a CEC, the higher its persistence.<br>The removal efficiency of CEC in wastewater treatment plants (WWTPs) is evaluated as the ratio between the variation of CEC concentration after the biological treatment (effluent concentration minus influent concentration) and the concentration before the treatment (influent concentration). Removal efficiencies are collected from literature. |
| Bioaccumulation | $K_{ow}$ | - | Bioaccumulation refers to a compound potential to accumulate in the adipose tissue of aquatic organisms and it is related to compound lipophilicity. This property may be expressed by the octanol–water partition coefficient ($K_{ow}$), that is the ratio between the concentration of the CEC in n-octanol and the concentration in water. $K_{ow}$ values are obtained from literature. |
| Toxicity | PNEC | μg/L | The toxicity refers to aquatic organisms of any trophic level. It is correlated to the predicted no-effect concentration (PNEC), that is the lowest concentration of CEC below which no toxicity effects on aquatic organisms are measured. Data are collected from literature. |

An exhaustive compilation of potential CECs was defined on the basis of:

- an in-depth literature overview referring to CEC concentrations in secondary effluent of the four showcase regions of the project (Spain, Italy, Portugal and South Africa),
- analysis of real samples of the municipal WWTP effluent in Ciudad Real (Spain),
- the EU watch list (2008/105/EC, EU 2020/1161),
- the document *European Union Strategic Approach to Pharmaceuticals in the Environment COM/2019/128* which strongly recommends to consider of priority relevance **cytotoxic pharmaceuticals and X-ray contrast media**[1],
- the document *Chemicals Strategy for Sustainability Towards a Toxic-Free Environment COM/2020/667*, which strongly recommends considering of priority relevance per- and polyfluoroalkyl substances (**PFAS**)[2],
- classes of compounds under study in the other 17 funded projects within the JPI Aquatic Pollutants 2020 Call, (**antimicrobials**),
- hints and suggestions provided during the 3rd Water JPI conference (November 17, 2021) (**antimicrobials**).

By applying the four criteria reported in Table 1, with the specific scores defined in Table 2, four chemical compounds were identified as the most representative for the SERPIC project (Table 3).

**Table 2**: Assigned scores for persistence (P), bioaccumulation (B), toxicity (T) in accordance with Daouk et al. 2015; for occurrence (O) the scores are defined on the base of our proposal.

| **Rank** | **Occurrence (O)** | **Persistence (P)** | **Bioaccumulation (B)** | **Toxicity (T)** |
|---|---|---|---|---|
| **Criteria →<br>Score ↓** | *Concentration c (ng/L)* | *Removal in CAS R (%)* | *Log $K_{ow}$* | *PNEC (μg/L)* |
| **1** | $c < 50$ | $R > 80$ | Log $K_{ow} < 1$ | PNEC > 100 |
| **2** | $50 \leq c < 100$ | $60 < R \leq 80$ | $1 \leq$ Log $K_{ow} < 2$ | 10 < PNEC ≤ 100 |
| **3** | $100 \leq c < 500$ | $40 < R \leq 60$ | $2 \leq$ Log $K_{ow} < 3$ | 1 < PNEC ≤ 10 |
| **4** | $500 \leq c < 1000$ | $20 < R \leq 40$ | $3 \leq$ Log $K_{ow} < 4.5$ | 0.1< PNEC ≤ 1 |
| **5** | $c \geq 1000$ | $R \leq 20$ | Log $K_{ow} \geq 4.5$ | PNEC ≤ 0.1 |

**Table 3:** The four chemical compounds selected for the SERPIC project.

| **Class** | **Selected compound** |
|---|---|
| Anti-inflammatory | Diclofenac |
| X-ray contrast media | Iopromide |
| Antibiotic | Sulfamethoxazole |
| Psychiatric drug | Venlafaxine |

[1] https://ec.europa.eu/environment/water/water-dangersub/pdf/strategic_approach_pharmaceuticals_env.PDF
[2] https://ec.europa.eu/environment/pdf/chemicals/2020/10/Strategy.pdf

### 5.1.2 ARB and ARG selection

One antibiotic resistant bacterium and one antibiotic resistance gene were selected according to the following sources:

- Lessons learned from literature (Berendok et al., 2015; Hiller et al. 2019; Keenun et al., 2022, Alygizakis et al., 2020),
- suggestions from the Nereus COST action (Cost WG1-WG meeting 2016),
- Recommendations by European Commissions reports such as:
  *European Union Strategic Approach to Pharmaceuticals in the Environment COM/2019/128*[3]
  *A European One Health Action Plan against Antimicrobial Resistance (AMR) COM/2017/339*[4]
- The other 17 funded projects within the JPI Aquatic Pollutants Call,
- Hints and suggestions provided during the 3rd Water JPI conference (November 17, 2021),
- Experience of project partner NIVA in the field,
- Experience of project partner Stellenbosch University in the field.

The most representative ARB and ARG were found to be*:* *E. coli* and sul1. The reasons are reported in Table 4.

**Table 4:** The selected ARB and ARG and the main reasons according to Nereus COST Action WG1 – WG meeting 2016.

| Class | Selected | Main reasons |
|---|---|---|
| ARB | ***E. coli*** | Human commensal and/or opportunistic and nosocomial pathogens;<br>Recognized carriers of acquired ARG in aquatic environments;<br>Indicators of fecal contamination in aquatic environments;<br>Existence of well-established methods for selective isolation from the environment and further identification; |
| ARG | **sul1** | Clinically relevant, widespread and highly prevalent in treated wastewater worldwide;<br>Harboured by ubiquitous bacteria and/or indicators of fecal contamination in aquatic environments;<br>Associated with mobile genetic elements;<br>Existence of well-established methods for detection and quantification |

### 5.1.3 Complete list of 30 CECs

The complete list in Table 5 includes other chemical compounds and ARB and ARGs belonging to the starting compilation defined on the basis of the same criteria.

[3] https://ec.europa.eu/environment/water/water-dangersub/pdf/strategic_approach_pharmaceuticals_env.PDF
[4] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52017DC0339&from=EN

**Table 5**: Complete list of 30 CECs and ARBs and ARGs, in **bold** the four CECs and the ARB and ARG selected as targets for SERPIC.

| | Class | Compound |
|---|---|---|
| 1 | **Analgesics/ anti-inflammatories** | **Diclofenac** |
| 2 | **Psychiatric drug** | **Venlafaxine** |
| 3 | **X-Ray contrast media** | **Iopromide** |
| 4 | **Antibiotics** | **Sulfamethoxazole** |
| 5 | **ARB** | **Escherichia coli** |
| 6 | **ARG** | **sul1** |
| 7 | Psychiatric drug | Carbamazepine |
| 8 | Analgesics/anti-inflammatories | Ibuprofen |
| 9 | Antibiotics | Ciprofloxacin |
| 10 | Antibiotics | Clarithromycin |
| 11 | Antibiotics | Erythromycin |
| 12 | Surfactants | Perfluorooctane sulfonic acid (PFOS) |
| 13 | Lipid regulators | Gemfibrozil |
| 14 | Antihypertensives | Valsartan |
| 15 | Psychiatric drug | Oxazepam |
| 16 | Surfactants | Nonylphenol |
| 17 | Plastic additives | Bisphenol A |
| 18 | Analgesics/anti-inflammatories | Tramadol |
| 19 | Antibiotics | Azithromycin |
| 20 | Lipid regulators | Bezafibrate |
| 21 | Beta-blockers | Bisoprolol |
| 22 | Diuretics | Furosemide |
| 23 | Antihypertensives | Irbesartan |
| 24 | Antibiotics | Amoxicillin |
| 25 | Antibiotics | Tetracycline |
| 26 | Antibiotics | Trimethoprim |
| 27 | Psychiatric drug | Carbamazepine 10,11 epoxide (metabolite) |
| 28 | ARB | Fecal coliform |
| 29 | ARG | sul2 |
| 30 | ARG | 16s rRNA |

### 5.2 Validation of the defined list

The assessment of the adequacy of the selected CECs has been carried out on the basis of the following checks:

- Availability of analytical methods for the selected compounds,
- Further contribution to CEC removal due to solar exposure, miming the effect of the photoelectric reactor included in Route B of the SERPIC process chain. The analysis is done on the basis of the CEC photodegradability and the functional groups present in the molecule, according to Mathon et al., 2016, 2021,
- CECs selected in other projects of reuse of reclaimed water, with a specific focus on the bioaccumulation in the investigated crops (Ben Mordechay et al. 2021, Delli Compagni et al., 2020; Shahriar et al., 2021, Liu et al. 2020).

On this basis, the list of selected compounds is confirmed to be representative of the behaviour of a multitude of CECs expected to be present in the secondary effluent of WWTPs.

Lastly, the long-term monitoring of the selected CECs in the final effluents of route A and B of the SERPIC prototype will allow an assessment of the removal capacity of the proposed technologies and the reduction in their spread in the environment. This analysis will be combined with the development of toxicity assays *in vivo* and *in vitro,* according to the CEC analysis done at UCLM, as reported in the description of task *T1.3 Analysis of compounds in value chain*.

## 6 Publications and other dissemination activities

An open-access publication for these results is in preparation. It will also include the full data.

## 7 Literature

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