Deliverable of WG5

Deliverable 21

A list of highly hazardous conditions commonly met in wastewater and which represent a high risk for wastewater reuse

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1. Introduction

Increasing water scarcity, driven by expanding populations, urbanisation and climate change, is a growing challenge for Member States (MS) throughout the EU. At a policy level, water reuse is a priority objective as seen by its inclusion within:

- the EU Water Framework Directive (EU WFD, 2000) as a key supplementary measure to be considered within river basin management planning,
- the EU's 'A Blueprint to Safeguard Europe's Water Resources' as a specific action (EC, 2012)
- the EU's Action plan for delivering a circular economy (EU, 2015)
- UN SDG Goal (water and sanitation) which requires a substantial increase in current levels of water reuse by 2030

However, whilst interest in treated wastewater reuse remains high at both international and European policy levels, its adoption into practice lags far behind with, on average, only 2% of Europe's treated wastewaters (TWW) reused. Barriers to the routine use of alternative water sources in a variety of applications include concerns over possible acute and chronic risks to human and environmental receptors.

A recent policy response to addressing this concern with regard to the use of TWW within agricultural irrigation is a proposal for a regulation on minimum water quality requirements for reuse by the European Commission (EC, 2018). Developed as a mechanism to ensure the safety of environmental and human health, the proposed regulation sets out a series of minimum water quality requirements (MQR) with regard to ensuring the safety of agricultural products following irrigation with TWW. Development of the MQR involved an extensive drafting process, with the Scientific Committee on Health, Environment and Emerging Risks (SCHEER) and the European Food Safety Authority (EFSA) asked to provide a scientific advice on Draft Version 3.3 (JRC, 2017). In its response to this draft, SCHEER concluded that the proposed MQR would provide insufficient protection to environmental or human health, highlighting they did not adequately address:

- contaminants of emerging concern (CECs)
- the role of TWW effluents as a pathway to spread antibiotic resistance
- possible risks associated with the formation and toxicity of disinfection by-products (Rizzo et al., 2018).

However, despite this early criticism, the current EC (2018) proposal for a regulation on MQR has not expanded its scope to robustly address these issues. The current proposal is also

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limited in scope in terms of its focus on assessing MQR on a 'substance by substance' basis as opposed to integrating the use of effect-based bioassays to enable the impact of mixture effects to be considered. Nor does the regulation – as currently proposed - consider risks to aquatic or terrestrial ecosystem health (Fatta-Kassinos et al., 2016; SCHEER, 2017; NEREUS D19).

MQR have been proposed for six 'traditional' parameters (*Escherichia coli*, BOD_5, total suspended solids, turbidity, *Legionella* spp. and intestinal nematodes), with specific values established for a series of reclaimed water classes which are linked to crop categories, irrigation technologies and monitoring frequencies. Whilst to-date a negative impact on, for example, human health as a result of consuming crops irrigated with TWW has not been reported in the scientific literature, a number of complete source-pathway-receptor chains have been theoretically identified (NEREUS D20). However, a recent study by Paltiel, (2016) reported a complete source-pathway-receptor chain for carbamazepine, documenting its transfer from TWW applied to crops to consumers as a result of the consumption of TWW-irrigated crops (Chefetz, 2018). Several studies have also reported the range of CECs occurring in TWW (NEREUS D2), their transfer to crops and soil as a result of irrigation with TWW (NEREUS D6) and their subsequent accumulation within crops (NEREUS D7). The findings of the NEREUS COST ACTION cite these (and many other) studies as sufficient evidence to include selected CECs within the MQR, under use of the precautionary principle (NEREUS D20).

It is within this context that this report considers 'highly hazardous conditions commonly met in wastewater and which represent a high risk for wastewater reuse' as a contribution to the debate on the need for emerging TWW quality requirements to encompass a broader range of parameters. It builds directly on NEREUS D19 (List of quality criteria concerning ARB&Gs and biological risks related to contaminants of emerging concern) and D20 (A list of parameters for consideration within a qualitative risk assessment framework). It integrates findings from WGs 1, 3 and 5 and is informed by discussions undertaken at the 7th NEREUS meeting (held in Sofia, Bulgaria, 22-23rd March, 2018).

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2. Scope, definitions and methodology

2.1 Scope and definitions

TWW may contain a range of hazardous substances which could – individually or in combination - pose a risk to human and environmental health (Gardner et al., 2013; Carere et al., 2018; Revitt and Lundy, 2018). Addressing these concerns points to the need for the assessment of the sources, behaviour and fate of a diverse range of hazardous pollutants within TWW. Whilst chemical analytical approaches are essential in identifying sources of pollutants, scientific consensus is emerging that it is no longer possible or perhaps even useful to monitor/determine dose responses to every individual known pollutant (NEREUS D13 and D14). Recent developments in the field of ecotoxicity testing support the use of bioassays as a viable complimentary approach for identifying and mitigating human and environmental health risks of pollutant mixtures. An assessment of how standardised chemical analytical approaches and emerging ecotoxicity methodologies can be best utilised, integrated and deployed within a broader water reuse risk assessment and mitigation framework is now sought.

Within this broad framework, the specific scope for this report was discussed at the 7th NEREUS meeting (held in Sofia, Bulgaria, 22-23rd March, 2018). Considerable discussion took place between members of all five NEREUS WGs focused around the meaning of 'highly hazardous conditions' from various WG and disciplinary perspectives. This session concluded with agreement that – within this deliverable – **highly hazardous conditions were to be defined as "hazardous conditions that would stop a wastewater treatment plant (WWTP) from operating" i.e. what conditions (in terms of detected substances or effects) would result in a WWTP no longing supplying TWW for reuse applications**. Hence, within this report, the context in which this evaluation takes place is the WWTP itself.

2.2 Methodology

Having agreed a focus on the WWTP, NEREUS deliverables from WG1, WG3, WG4 and WG5 were reviewed to identify how different disciplinary areas defined and addressed 'highly hazardous conditions'. This review was complimented by further online discussion between WG leaders. The results of the review and discussions are integrated within the following sections. Whilst representatives from WG2 participated within the above discussions, its scope of activities relate to the uptake of CECs by crops (i.e. outside the identified focus of the WWTP activities) and so its deliverables were not considered.

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3. Results and discussions

Outputs from WG1 define persistent ARB as antibiotic resistant bacteria that survive the wastewater treatment process and can therefore reach the receiving environment and potentially be disseminated to humans (NEREUS D2). Hazardous ARB were defined as a subset of those above, which belong to the taxonomic groups and/or harbour antibiotic resistance genes considered of high clinical relevance. Examples of these are the third-generation cephalosporin- or carbapenemase-resistant Gram-negative bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp.). Therefore, the hazardous ARGs were defined as genes that can be significant epidemiological burdens if they reach pathogens. At the time of writing, examples of these genes are the *mcr-1*, $bla_{\text{ndm-1}}$, bla_{KPC}, $bla_{\text{OXA-48}}$, $bla_{\text{OXA-58}}$, etc. However, the fast evolution of antibiotic resistance may require this list to be updated on an annual basis. Hazardous ARB may present a direct – although as yet unquantified - risk to human health. Although ARGs alone (i.e. outside a bacterial host), do not represent a human health risk, they are important indicators of evolutionary potential, in other words the 'resistance evolution' risk given their association and mode of dispersal via mobile genetic elements (MGEs; e.g. plasmids and transposons).

In terms of human health risk assessment, a list of hazardous ARB and ARGs in WWTPs have been established (see Table 1; NEREUS D2), where the use of hazardous refers to those considered relevant clinical threats. In contrast, the classification of 'resistance evolution' risks, is much more complex because

- the horizontal gene transfer dynamics of these genes in WWTPs is not well understood
- hazardous ARGs are constantly evolving with the discovery of new ARGs (*i.e.* recently discovered $bla_{\text{NDM-1}}$ and *mcr*-1 that are strongly associated with multidrug resistance)
- the distribution of hazardous ARGs may significantly vary as a function of geographic and temporal parameters.

Within NEREUS D1, standardised methodologies to identify and quantify ARGs have been compiled. However, it should be noted that the challenge is not only limited to setting standard methods for detecting the occurrence of known ARB and ARGs; due to the fact that the behaviour of both ARB and ARGs is highly dynamic, with emerging pathogens, and different combinations of resistance genes and multidrug-resistant MGEs frequently reported (NEREUS D2 and D3).

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Table 1 - Potential indicators of hazardous ARB: list of clinically relevant ARB and ARGs frequently harboured.

Hazardous ARB	**Prevalent associated ARGs**
Escherichia coli	*bla*$_{KPC}$, *bla*$_{NDM-1}$, *bla*$_{OXA}$, *bla*$_{CTX-M}$, *bla*$_{OXA-48}$, *mcr1*
Klebsiella pneumoniae	
Enterobacter spp.	
Enterococcus faecalis	*vanA*
Enterococcus faecium	*vanA*
Staphylococcus aureus	*mecA*
Pseudomonas aeruginosa	*bla*$_{CTX-M}$, *bla*$_{GES}$, *bla*$_{OXA}$, *bla*$_{SHV}$
Aeromonas spp.	*bla*$_{CTX-M}$
Acinetobacter baumanii	*bla*$_{TEM}$

Activities within WG3 (Effect-based bioassays required for wastewater reuse schemes) have focused around the use of effects-based bioassays to monitor both WWTP efficiencies and TWW quality. The use of bioassays as screening tests in routine monitoring is gaining increasing attention as a complementary approach to chemical analysis which enables the identification of biological effects associated with treated effluents (NEREUS D13 and 14). Their inclusion is seen as a way to address the key limitations of traditional physico-chemical standards approach i.e. only substances analysed for can be detected and it does not address the occurrence of possible synergistic/antagonistic effects within complex mixtures. Whilst it is not yet possible to systematically and robustly correlate WWTP efficiencies or specific effluent qualities with biotest results (NEREUS D14), biotests which address a range of pertinent human health and environmental receptors via pertinent modes of action (MoA) are now available (NEREUS D14) and a protocol for their harmonized use is proposed (NEREUS D15). Within this context, NEREUS D14 identifies the following as protection targets to be considered when selecting biotests for use within an evaluation of TWW:

- Human health (e.g. drinking water consumption, vegetable/fruit consumption, soil children ingestion, fishery product consumption-interaction surface/groundwaters)
- Aquatic benthic community
- Aquatic pelagic community
- Top predators (aquatic birds, fish at higher trophic levels)
- Soil terrestrial organisms (including amphibians, worms, arthropods)

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Specific relevant MoAs to be considered include endocrine disruption, genotoxicity / mutagenicity, embryo toxicity, adaptative stress responses, cytotoxicity, neurotoxicity, systemic response/general toxicity and population/biodiversity responses. The use of a battery of bioassays which address these protection targets through assessment of the above listed MoAs, in combination with targeted chemical analysis of a short list of pollutants, is seen as an integrated approach to offering a higher level of protection at a lower economic cost than would be provided by chemical analysis alone.

As a step towards operationalizing this approach within a regulatory context, researchers have looked to associate bioassays with trigger values which indicate activity towards a selected endpoint (DEMEAU, 2015). NEREUS D14 cites examples of specific trigger values for estrogenic (ERα), androgenic (AR), progestagenic (PR), and glucocorticoid (GR) activities in TWW intended for the recharge of potable aquifers (see Table 2).

Table 2 - Examples of trigger values for estrogenic (ERα), androgenic (AR), progestagenic (PR), and glucocorticoid (GR) activities in reclaimed water used for recharge of potable aquifers.

Activity	Trigger value[a]
ERα	3.8 ng E2-eq/L
AR	11 ng DHT-eq/L
GR	21 ng DEX-eq/L
PR	333 ng Org2058-eq/L

[a]Based on Acceptable Daily Intake (ADI) values reported by the JECFA (FAO/WHO, 1995, 2000). E2 = 17β-estradiol; eq = equivalent; DHT = dihydrotestosterone; DEX = dexamethasone; Org2058 = 16a-ethyl-21-hydroxy-19nor-4-pregnene-3,20-dione (Brand et al., 2013).

Discussions within WG3 around recent developments within the field indicated that, from an effects-based biotest perspective, circumstances that would require TWW operators to stop supplying TWW for irrigation proposes could be expressed as the exceedance of a defined trigger value by a factor of 10. Whilst the concept was broadly supported, the lack of similar trigger values for all pertinent MoA / biotests currently prevents the approach from being implemented in any meaningful way.

The focus of WG4 (Technologies efficient/economically viable to meet the current wastewater reuse challenges) was to further our understanding of the role of wastewater treatment processes in contributing to and opportunities for mitigating the release of ARB and ARG. Within this context, NEREUS D16 identifies pathogenic strains of *E. coli* as a key hazard for consideration with a WWTP environment. Pathogenic *E. coli* are highly adapted for carrying

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ARGs from external environments such as WWTP back into the human gut as they are ubiquitous organisms which are able to live both intra- and extra-intestinally. These pathogenic bacteria have a high potential for hosting ARGs which, as they survive exposure to antibiotics, increases the likelihood of ARG transmission to the rest of a given microbial community, being this within the human gut or a natural aquatic environment. Their reported virulence provides a competitive advantage for intestinal colonization, increasing both their likelihood of occurrence and persistence within a WWTP environment. Initial studies on the impact of conventional activated sludge (CAS) processing as opposed to physico-chemical processes on ARGs prevalence within *E. coli* isolates indicated that the use of CAS led to an increase in multi-drug resistant *E. coli* while the same multi-drug *E. coli* decreased in prevalence after the use of physico-chemical processes (Biswal et al., 2014). NEREUS D17 considers the use of a range of best available technologies to minimize the release of a range of CECs (including ARB and ARGs). To make wastewater reuse safe, a multi-barrier approach to wastewater treatment is necessary and, within NEREUS WG4, different options of treatment trains for urban wastewater reuse were discussed and proposed. These barriers should include typical processes for urban wastewater treatment namely, primary mechanical pre-treatment (such as, grill treatment and grit removal), possible primary settling, biological treatment (e.g., either activated sludge, moving bed biofilm reactor (MBBR), membrane biological reactor (MBR) etc.) and advanced treatments. Whilst a diverse range of advanced treatment technologies (including ozonation, activated carbon adsorption, chemical oxidants / disinfectants, UV-C radiation, advanced oxidation processes and membrane technologies) are considered, key conclusions are that (i) removal performances tend to be WWTP specific in relation to a host of factors including the source of the effluent and (ii) the best available advanced treatment technology should be selected by taking into account different end points (namely, disinfection efficiency, CECs removal, formation of disinfection/oxidation by products and intermediates, effluent toxicity).

Within WG5 (Risk assessment and policy development), a hazard is defined as a biological, chemical, physical or radiological agent that has the potential to cause harm (NEREUS D20). NEREUS D19 notes that several WHO and JRC reports identify that TWW may contain a range of such hazards and that their presence can be a risk to human health. This knowledge has underpinned the development of TWW use guidelines and regulations in many countries, primarily focused on the use of TWW in agricultural irrigation (see WG3 Legislation on Quality Requirements, 2016) for a review of legislation pertaining to agricultural irrigation using TWW. The variations within and limited coverage of (in terms of parameters for which standards are developed) is identified, with a particular emphasis on the fact that possible risks deriving from

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CECs have yet to be considered in relation to either human health or receiving environmental compartments (either as single substances or as mixtures). As a contribution to addressing this gap, NEREUS D19 reviews the various methods available for short-listing CECs for inclusion within the development of monitoring programmes and includes an example list of substances for inclusion within monitoring programmes pertaining to TWW reuse in aquifer recharge schemes selected using identified approaches (see Table 3).

Table 3 - List of CECs to be included in monitoring programmes for aquifer recharge (the selection criteria are provided and explained in NEREUS Deliverable 14).

Indicator chemical	Human health relevant level (HRL) (ng/L)	Frequency	References - analytical method
Biodegradable[1]			
Diclofenac	100	Every 6 months	Loos et al., 2013
Gabapentin	1,000	Every 6 months	Kasprzyk-Hordern et al., 2008
Sulfamethoxazole	150	Every 6 months	Göbel et al,
Valsartanic acid	300	Every 6 months	Schultz et al., 2010
Not biodegradable, but oxidizable[2]			
Carbamazepine	500	Every 6 months	Loos et al., 2013
Difficult to degrade biologically; not amendable to chemical oxidation[3]			
Sucralose	tba	Every 6 months	Loos et al., 2013

[1] Biodegradable during biofiltration or soil-aquifer treatment.
[2] Not degradable during conventional activated sludge treatment, biofiltration or soil-aquifer treatment, but amendable to chemical oxidation.
[3] Not degradable during conventional activated sludge treatment, biofiltration or soil-aquifer treatment, not amendable to chemical oxidation.
tba: to be added

With specific regard to the issue of AR, domestic wastewater is identified as a major source of ARB and the need to consider this – both in terms of TWW reuse and wastewater treatment in general – is highlighted (NEREUS D19). In the absence of a requirement to include ARB in routine monitoring, it is suggested that *E. coli* resistant to cefotaxime (an antibiotic used to

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treat a range of bacterial infections) is used as an indicator of a wider range of ARB (see NEREUS D4). Justification for this recommendation is that *E. coli* is a harbour for ARGs and cefotaxime resistance is a good indicator of human sources of AR and is associated with a diversity of ARGs which are of clinical concern. As disinfection is an essential treatment process to ensure safe reuse of treated wastewater (NEREUS D17), the formation of toxic disinfection by products should be taken into account by monitoring specific substances depending on the type of chemical disinfection process used. For example, during chlorination relevant by-products are trihalomethanes (THMs) and haloacetic acids, bromate and nitrosodimethylamine may result from ozonation treatment, while chlorite and chlorate may occur following chlorine dioxide disinfection-.

The second NEREUS WG5 deliverable identifies a list of parameters to be considered within a qualitative risk assessment framework (NEREUS D20). Within this deliverable, hazards are defined as CECs and the parameters which enable an assessment of the likelihood of a particular CEC reaching soil following the use of TWW in agricultural irrigation are identified. The magnitude of its impact is considered in relation to an assessment of the bioavailability of the target CEC within receiving soils. Of note is the fact that the risk assessment framework is qualitative i.e. there is an implicit understanding that currently available data sets pertaining to this field are insufficient to support a fully quantitative approach. The NEREUS risk assessment framework identifies a range of parameters to be considered from source (i.e. source and characteristics of raw wastewater) to the protection goal or receiving compartment of interest. However, due to the lack of field data, dose response models and understanding of cumulative exposures it is currently only possible to apply the approach as far as the initial receiving soil. As such, rather than identifying the level of risk posed by specific CECs, the output of its application is a ranking of hazards to humans and/or environmental receiving compartments due to the reuse of TWW containing CECs in selected applications. As such, and within a WWTP context, it is possible to rank sources of wastewater in relation to an escalating likelihood of containing a specific CEC (e.g. see Table 4 for an example of a qualitative approach). Likewise, it is possible to rank (from lowest to highest) the likelihood of a specific CEC being removed by alternative types of wastewater treatment systems (see Table 5). However, it is not yet possible to make any quantitative judgements of these aspects i.e. a score of, for example, 4 is interpreted as 'likely to occur' but gives no further information in terms of predicted concentration.

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Table 4 - Example of an approach for allocating rank scores in relation to the influence of sources of wastewater on the likelihood of CECs occurring in soil.

<table>
<tr><th colspan="4">Sources of wastewater</th></tr>
<tr><td>Rural WW</td><td colspan="3">Urban/Municipal WW</td></tr>
<tr><td rowspan="4"></td><td>Residential sources</td><td>Industrial/hospital sources with on-site treatment</td><td>Industrial/hospital sources with NO on-site treatment</td></tr>
<tr><td colspan="3">4</td></tr>
<tr><td colspan="2">3</td><td></td></tr>
<tr><td>2</td><td colspan="2"></td></tr>
<tr><td>1</td><td colspan="3"></td></tr>
</table>

Key to qualitative grading system used: 4 = likely (expected to occur); 3 = possible (may occur sometimes); 2 = Unlikely (uncommon but known to occur); 1 = Rare (lack of evidence but not impossible (NEREUS D5.2, 2018).

Table 5 - Example of an approach for allocating rank scores in relation to the influence of the level of wastewater treatment on the likelihood of CECs occurring in soil.

<table>
<tr><th colspan="4">Characteristics of WW treatment</th></tr>
<tr><td>Secondary treatment (employing filter beds/activated sludge aeration)</td><td>Enhanced secondary treatment (e.g. employing membrane bioreactors)</td><td>Tertiary/Advanced treatment (where oxidation processes may lead to the presence of toxic by-products)</td><td>Tertiary/Advanced treatment (where there is NO possibility of toxic by-products</td></tr>
<tr><td>4</td><td></td><td></td><td></td></tr>
<tr><td colspan="2">3</td><td></td><td></td></tr>
<tr><td colspan="3">2</td><td></td></tr>
<tr><td colspan="4">1</td></tr>
</table>

Key to qualitative grading system used: 4 = likely (expected to occur); 3 = possible (may occur sometimes); 2 = Unlikely (uncommon but known to occur); 1 = Rare (lack of evidence but not impossible (NEREUS D5.2, 2018).

4. Conclusions

The aim of this report is to identify 'a list of highly hazardous conditions commonly met in wastewater and which represent a high risk for wastewater reuse'. During WG discussions, the question was more specifically interpreted as 'what conditions (in terms of detected substances or effects) would result in a WWTP no longing supplying TWW for reuse applications?' In looking to address this, the outputs of WGs1, 3, 4 and 5 have been reviewed to specifically identify how the term 'hazard' has been conceptualised from each disciplinary

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perspective. Whilst the outputs from each WG have directly (e.g. WG1 and 5) or indirectly (e.g. WG3 and 4) addressed the issue of hazard identification, this has, by necessity, been primarily a qualitative exercise. The occurrence, behaviour and fate of CECs in TWW is an emerging research topic and the evidence base is at an early stage of development. As a result, findings reported within the NEREUS WG deliverables contribute several major steps forward in proposing, debating and generating a consensus on new concepts, methodological approaches and frameworks to support and inform the next stage of research activities in this field of critical importance.

In terms of identifying the conditions which would result in a WWTP no longing supplying TWW for reuse applications, the following findings are put forward as particularly pertinent:

- A list of the top five most hazardous ARB and ARGs in TWW and methods for their analysis (NEREUS D1)
- A proposal for the harmonized use of bioassays for the routine analysis of treatment efficiency re: the removal of the toxicological hazards of water for reuse (NEREUS D15)
- A better understanding of the biological processes on the spread of ARB in the receiving environment (NEREUS D16)
- Possible treatment trains to minimize the release of CECs and AR determinants (NEREUS D17)
- A framework for the qualitative assessment of risks associated with the occurrence of CECs in TWW (NEREUS D20)

Whilst the evidence base is not yet sufficient to support the allocation of specific threshold values or discrete quantification of concepts such as absolute risks, the international NEREUS scientific community (representing 380 researchers from 43 countries) have reached a broad consensus on the current state of knowledge, research priorities going forward and the need to apply the precautionary principle when developing standards to safeguard human health and environmental compartments impacted by TWW.

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